The Effects of Varenicline on Working Memory and Long-Term Potentiation in Non-Smokers with Schizophrenia

by

Alanna Christina Bridgman

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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2015

Background: Deficits in the nicotinic acetylcholine receptor (nAChR) system are proposed to underlie vulnerability to nicotine dependence and cognitive impairment in schizophrenia. Varenicline is a smoking cessation medication that enhances cognition in schizophrenia while treating nicotine dependence, possibly through the modulation of neuroplasticity.

Methods: This study assessed the effects of varenicline (1 mg/day) versus placebo on neuroplasticity and working memory in 11 schizophrenia non-smokers and 11 non-smoker healthy subjects, to eliminate the confounding effects of tobacco smoking.

Results: Patients with schizophrenia performed worse on the 3-back task compared to healthy subjects. Varenicline enhanced working memory in low-performers only, regardless of diagnosis. Deficits in neuroplasticity were found in patients with schizophrenia compared to healthy subjects, which were enhanced with varenicline compared to placebo.

Conclusions: Varenicline may be a potential therapeutic option for the remediation of cognitive and neuroplasticity deficits in patients with schizophrenia regardless of smoking status.
Acknowledgments

This work is dedicated to Thomas Joseph Mullin and Patricia Rose Gates.

First and foremost, I’d like to acknowledge the participants that made this work possible. Thank you for your time, honesty, and genuine enthusiasm.

I’d like to express the utmost gratitude to my supervisor, Dr. Tony P. George, for the opportunity to take this study on as my own. Through your guidance, I have learned how to think critically and independently, and have grown as a scientist and an individual. I thoroughly enjoyed my 4 years in this lab, and will miss the close-knit community that you’ve built. I sincerely thank you for patience and support, and I hope I have made you proud.

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To Plucky, from Peanut: I don’t think we would have gotten through these past two years without each other. You’ve helped me through the toughest times, and I hope you can say the same about me. There are too many moments to reminisce about here, but maybe we can blog about it some time.

Rachel Rabin, you were the first friend I made here at CAMH, and I know our friendship will continue outside of these walls. Thank you for being a constant source of emotional and academic support for me, and I look forward to doing the same for you in the upcoming years!

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Contributions

The study protocol from which this thesis was derived was developed by Dr. Tony George (PI), Dr. Jeff Daskalakis, Dr. Mera Barr, and Dr. Tarek Rajji, and was entitled “The Effects of Varenicline on Cortical Neuroplasticity in Patients with Schizophrenia Compared to Non-Psychiatric Controls”.

The candidate, Alanna C. Bridgman, was responsible for recruitment, retention, and conduct of all study sessions.

Emily Simpkin and Matthew Tracey were responsible for conducting the structured clinical interviews and physical examinations performed during the screening session.

Dr. Mera Barr trained the candidate on all neurophysiological and neurocognitive measures, including working memory and paired associative stimulation.

The candidate, Alanna C. Bridgman, conducted all data analysis, interpretation of research data, and drafting of the thesis. Dr. Mera Barr and Dr. Tony George assisted with analysis and interpretation of data.

The Program Advisory Committee (PAC) members, Dr. Bernard Le Foll and Dr. Robert Chen, assisted with the interpretation of the data.

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List of Abbreviations

APB  Abductor Pollicis Brevis
AIMS  Abnormal Involuntary Movement Scale
ACh  Acetylcholine
AChR  Acetylcholine Receptor
AMPA  α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA  Analysis of Variance
BARS  Barnes Akathisia Rating Scale
BACDRL  Biobehavioural Addictions and Concurrent Disorders Research Laboratory
Ca²⁺  Calcium Ion
CaMKII  Calcium/Calmodulin-Protein Kinase II
CaMKIV  Calcium/Calmodulin-Protein Kinase IV
CO  Carbon Monoxide
CNS  Central Nervous System
CAMH  Centre for Addiction and Mental Health
CPZ  Chlorpromazine
COGS-2  Consortium on the Genetics of Schizophrenia
CSP  Cortical Silent Period
DSM-IV  Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSM-V  Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DTI  Diffusion Tensor Imaging
DLPFC  Dorsolateral Prefrontal Cortex
EKG  Electrocardiogram
EEG  Electroencephalography
EMG  Electromyography
ERK2  Extracellular Signal-Regulated Kinase 2
ERK/MAPK  Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase
FSIQ  Full Scale Intelligence Quotient
fMRI  Functional Magnetic Resonance Imaging
GABA  Gamma-aminobutyric Acid
HS  Healthy Subject
IQ  Intelligence Quotient
ISI  Interstimulus Interval
ICF  Intracortical Facilitation
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Chapter 1
Literature Review

1.1 Schizophrenia

1.1.1 Phenomenology, Epidemiology, and Etiology

Schizophrenia is a neuropsychiatric illness that affects approximately 1% of individuals in all cultures (Saha, Chant, Welham, & McGrath, 2005). In conjunction with positive and negative symptoms of the illness, patients with schizophrenia also experience deficits in social and cognitive functioning (Goff et al., 2005). While schizophrenia is equally common among males and females, the average age of onset differs. Males typically develop symptoms during the late teen years to early 20’s, while females typically present symptoms in their mid to late 20’s (American Psychiatric Association, 2013). Many patients with schizophrenia are unable to obtain or maintain employment; employment-related instability contributes to loss of societal productivity, especially since the illness usually presents at such a young age. Schizophrenia represents a huge burden to society through medical costs, years of lost productivity, and inpatient care. Even though schizophrenia has a relatively low prevalence compared to depression or alcohol use disorder, the 2010 Global Disease Burden reported schizophrenia as the highest disability weight out of all mental health and addictive disorders (Whiteford et al., 2013).

Symptoms of schizophrenia fall into several emotional and cognitive domains: perception (hallucinations), inferential thinking (delusions), motivation, thought, and speech (Schultz & Andreasen, 1999). Symptoms are typically classified into positive, negative, and cognitive symptoms. Positive symptoms include hallucinations, delusions, and disorganized speech. Fortunately, positive symptoms generally respond well to antipsychotic medication.
Negative symptoms are associated with deficits in behaviour, and include characteristics such as anhedonia, lack of motivation, and blunted affect, and are less likely to be effectively treated with antipsychotic medications. Cognitive deficits are one of the best predictors of functional outcome in schizophrenia (Green, 2006); symptoms typically appear before the onset of psychosis and persist stably throughout the lifetime (Bora, Yucel, & Pantelis, 2010). Moreover, patients with schizophrenia typically perform approximately one to two standard deviations below the mean on cognitive tasks compared to the general population (Bilder et al., 2000). Negative symptoms and cognitive impairment are not sufficiently treated by current medications, and both types of symptoms seriously impact quality of life for patients with schizophrenia.

For a diagnosis of schizophrenia, the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV TR) requires two of the following symptoms to have lasted in duration for a significant portion for at least one month: (1) delusions; (2) hallucinations; (3) disorganized speech; (4) grossly disorganized or catatonic behaviour; (5) negative symptoms (i.e., affective flattening or avolition, as well as major dysfunction in one or more areas of daily living) (American Psychiatric Association, 2000). According to the DSM-IV TR, these symptoms must be present for a minimum of 6 months without being induced by external influences.

The course of schizophrenia over the lifetime is fairly consistent among the vast majority of individuals (Lewis & Lieberman, 2000). Prodromal symptoms typically appear 2-3 years before illness onset, and may consist of mood symptoms, cognitive disturbances, and mild positive symptoms. Environmental stressors or triggers, such as military service or
exposure to drug abuse, may trigger a full-fledged psychotic episode in a prodromal individual (Lieberman, Sheitman, & Kinon, 1997).

In addition to phenotypic heterogeneity, the risk factors for schizophrenia are also poorly understood. The most significant and well-accepted risk factor remains a family history of schizophrenia (Mortensen et al., 1999), with heritability between 60-90% (Lichtenstein et al., 2009). Other hypotheses include maternal infections, socioeconomic status, and season of birth, yet none of these hypotheses have been conclusively proven (Bromet & Fennig, 1999; Mortensen et al., 1999). Neurodevelopmental hypotheses are also quite common in the literature; schizophrenia may be a manifestation of abnormal brain development stemming from childhood or even in utero (Deidda, Bozarth, & Cancedda, 2014). The consensus in the literature is that a combination of both biological and environmental factors is the most likely etiology of schizophrenia. For example, the two-hit model of schizophrenia suggests that disruption of early central nervous system (CNS) development by genetic and/or environmental insults are ‘hits’ that make an individual more vulnerable to the onset of schizophrenia symptoms later in life, usually after a second ‘hit’ or trigger (Maynard, Sikich, Lieberman, & LaMantia, 2001).

1.1.2 Pathophysiology of Schizophrenia

A high degree of variability exists surrounding both the diagnosis and manifestation of schizophrenia (Andreasen, 1995). The DSM-V has moved away from subtyping the diagnosis of schizophrenia found in the DSM-IV TR (paranoid, catatonic, disorganized, undifferentiated, and residual) to using clinical symptomatology as markers of the disease. This method recognizes schizophrenia as an extremely heterogeneous disease, while permitting clinicians more freedom when making diagnoses and treatment decisions. Thus,
due to an absence of concrete pathological markers of schizophrenia, researchers have tried to identify more definitive neurobiological disruptions that may shed light on the underlying pathophysiology of this complex disorder.

There are several important neurotransmitters that are hypothesized to be pathological in schizophrenia, including dopamine, glutamate, gamma-aminobutyric acid (GABA), and acetylcholine (ACh). These neurotransmitters regulate mood, cognition, and behaviour, and are also important for neuroplasticity and learning.

Dopamine is found throughout the brain is involved in several behaviours such as information processing, sleep, mood, pleasure, and working memory (Jones, Kilpatrick, & Phillipson, 1986). The dopamine hypothesis of schizophrenia was initially developed based on indirect findings and post-mortem studies, but soon developed into an intense area of in vivo research with the advent of new imaging technology such as positron emission tomography (PET). Early support for the role of dopamine in schizophrenia was shown via amphetamine administration in healthy individuals that increases extracellular dopamine to produce schizophrenia-like psychotic symptoms (Lieberman, Kane, & Alvir, 1987). These findings were supported by research showing that dopamine depletion reduces psychotic symptoms, supporting the hyperactive model of dopamine in schizophrenia (Arnold & Freeman, 1956; Campden-Main & Wegielski, 1955). From this research, a more recent formulation of the dopamine hypothesis of schizophrenia claims that dopaminergic activity is hyperactive in subcortical regions and hypoactive in prefrontal cortical regions. The fact that all antipsychotic medications block dopamine receptors to some degree (Volk et al., 1994) adds credence to this hypothesis.
Excitatory neurotransmission in the brain is largely glutamatergic. Glutamate is involved in several key functions, including synaptic plasticity, working memory, and executive function (Johnson, 1972). Glutamatergic neurotransmission occurs through ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors are subdivided into three types: N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainite receptors. Glutamatergic hypotheses of schizophrenia have been in circulation for many decades; the first known treatment of schizophrenia with glutamic acid was in 1949 (Kitzinger, Arnold, & et al., 1949). The original glutamate hypothesis simply stated that glutamate neurotransmission is reduced in schizophrenia; early post-mortem studies showed decreased NMDA receptor subtype densities in the superior frontal (Sokolov, 1998) and temporal cortices in patients with schizophrenia (Humphries, Mortimer, Hirsch, & de Belleroche, 1996). Over many years, this theory has been modified to now support NMDA receptor hypofunction is the primary component of glutamatergic dysfunction in schizophrenia (Stone, Morrison, & Pilowsky, 2007). This hypothesis arose in response to observations of psychotic-like symptoms in healthy subjects subsequent to NMDA receptor antagonist administration (Javitt, 2007; Lahti, Holcomb, Gao, & Tamminga, 1999; Lahti et al., 1997). More recent studies have also used imaging techniques after or during ketamine administration in healthy subjects and have found disrupted neuronal activation and functional connectivity in conjunction with psychotic symptoms (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). NMDA receptor
antagonism leads to downstream changes in cortical excitability, likely due to modulation of GABAergic interneurons (Homayoun & Moghaddam, 2007).

Dysfunctional GABAergic transmission has been implicated in the pathophysiology of schizophrenia (Roberts & Frankel, 1950). Inhibitory neurotransmission in the brain involves the suppression of cortical activity, and is predominantly mediated by GABA (Jones, 1993). GABA is mostly synthesized from glutamate, and the effects of GABA are generally mediated by ionotropic GABA$_A$ and metabotropic GABA$_B$ receptors. GABA$_A$ receptor activity is dependent on the concentration of chloride cations, while GABA$_B$ receptor activity depends on the presence of both calcium and magnesium. GABA$_A$ receptors are mostly found post-synaptically, while GABA$_B$ receptors are typically found presynaptically. When GABA or GABA agonists bind to their receptors, the release of dopamine, noradrenaline, and serotonin is inhibited. The GABAergic hypothesis of schizophrenia was introduced subsequent to the discovery of GABA’s inhibitory role in the central nervous system (Roberts & Frankel, 1950). Post-mortem analyses have repeatedly shown alterations in GABAergic interneurons in patients with schizophrenia (Tse, Piantadosi, & Floresco, 2014). Reductions in both the messenger RNA and protein for the GABA synthesizing enzyme GAD67 have been reported in various regions of the cortex in patients with schizophrenia (Guidotti et al., 2000; Hashimoto et al., 2003). This deficiency is most prominent in GABAergic interneurons containing parvalbumin (Hashimoto et al., 2003). Accordingly, parvalbumin expression is also reduced in multiple layers of the cortex in patients with schizophrenia (Lewis & Gonzalez-Burgos, 2008). Because parvalbumin-positive interneurons are fast spiking and well connected, they play a critical role in the generation of gamma oscillations in the cortex (Sohal, Zhang, Yizhar, & Deisseroth, 2009),
and are integral to cognitive function (Carlen et al., 2012). It follows that dysfunctional gamma oscillations are associated with cognitive deficits among patients with schizophrenia (Sun et al., 2011). Although post-mortem studies are important, evaluating GABA dysfunction in vivo allows researchers to study cortical inhibition under more controlled conditions. Studies examining GABA using transcranial magnetic stimulation (TMS) have consistently shown deficits in both GABA\textsubscript{A} and GABA\textsubscript{B} receptor function in schizophrenia (Daskalakis et al., 2002; Farzan et al., 2010; Takahashi et al., 2013). Alterations in GABA inhibitory neurotransmission are proposed to be due to excessive subcortical activation of dopamine D2 receptors, leading to heightened cortical excitability and potentially to psychotic symptoms (Benes, 1997).

ACh is involved in higher order cognitive processing, such as working memory, attention, and executive function (Pepeu & Giovannini, 2004). ACh primarily binds to muscarinic and nicotinic ACh receptors (AChRs) that are densely and widely distributed throughout the brain. Studying the cholinergic system in schizophrenia is partly motivated by the observation of significantly higher smoking rates in this population compared to the general public, suggesting that the metabolism and response to nicotine in schizophrenia differs from non-psychiatric individuals (Lewis & Picciotto, 2013). Patients with schizophrenia perform poorly on tasks requiring attention (Ahlers et al., 2014; Smucny, Olincy, Eichman, Lyons, & Tregellas, 2013), and studies have linked high anticholinergic activity to low performance on attentional tasks (Tracy, Monaco, Giovannetti, Abraham, & Josiassen, 2001). Nicotinic AChRs (nAChRs) are pathologically downregulated in patients with schizophrenia (Breese et al., 2000; Esterlis et al., 2014; Leonard et al., 2000) as shown by numerous post-mortem and imaging studies. Additionally, smokers with schizophrenia
show an upregulation of \( \alpha 4\beta 2 \) nAChRs compared to non-smokers with schizophrenia (Esterlis et al., 2014), although the availability of \( \beta 2 \)-containing nAChRs is still lower in smokers with schizophrenia compared to healthy subjects (D'Souza et al., 2012; Esterlis et al., 2014). In schizophrenia, decreased nAChR expression is also seen in the hippocampus and striatum, as shown by studies that demonstrate decreased binding of the nAChR partial agonist cytisine (Durany et al., 2000; Freedman, Hall, Adler, & Leonard, 1995). Together, these studies indicate disrupted AChR physiology in schizophrenia, which may contribute to the high smoking rates observed in this population.

Aberrant neural circuitry and cortical structure are a common finding in schizophrenia (Wheeler & Voineskos, 2014). One issue with neuroimaging involves parsing out causality – in other words, how can we be sure that neural circuitry deficits aren’t the result of factors such as chronic medication use or addiction? Studying first episode patients, unmedicated patients, and patients at ultra-high risk for psychosis is one technique that allows researchers to understand the neural circuitry of schizophrenia without added confounds. In chronic patients with schizophrenia, decreased connectivity is consistently found in the prefrontal cortex (PFC) and temporal lobes using diffusion tensor imaging (DTI) (Pettersson-Yeo, Allen, Benetti, McGuire, & Mechelli, 2011). Additionally, a recent meta-analysis determined that significant reductions are consistently found in the left frontal deep white matter and in the left temporal deep white matter in schizophrenia (Ellison-Wright & Bullmore, 2009). Although some studies have shown similar deficits in regions of the PFC and temporal lobes as in chronic patients with schizophrenia (Mendelsohn, Strous, Bleich, Assaf, & Hendler, 2006; Yao et al., 2013), abnormalities in white matter connectivity in first episode patients remains highly variable. Nonetheless, studies that show differences in
cortical connectivity between first episode patients and healthy subjects imply that structural abnormalities are present in the early phases of illness.

1.2 Tobacco Use in Schizophrenia

Tobacco use is one of the leading preventable causes of death in the world (Giovino et al., 2009). While approximately 20% of individuals in the general population smoke tobacco, a staggering 45-85% of patients with schizophrenia are nicotine dependent (Kalman, Morissette, & George, 2005; Lasser et al., 2000). Patients with schizophrenia smoke more cigarettes per day, inhale a greater puff volume, and are typically more nicotine dependent than non-psychiatric smokers (Tidey, Rohsenow, Kaplan, & Swift, 2005). Smokers with schizophrenia also have a greatly increased risk of mortality due to cardiovascular related illnesses compared to the general population (Gladigau, Fazio, Hannam, Dawson, & Jones, 2014; Ringen, Engh, Birkenaes, Dieset, & Andreassen, 2014). Deficits in nAChRs have been suggested to be involved in the pathophysiology of schizophrenia, thereby suggesting that the disproportionately high smoking rates in people with schizophrenia may be related to nicotine’s modulation of the ACh system (Chambers, Krystal, & Self, 2001; George, 2007).

1.3 The Nicotinic Acetylcholine Receptor System

In the central nervous system (CNS), nAChRs typically respond to endogenous ACh and modulate synaptic neurotransmission and excitability (Albuquerque, Pereira, Alkondon, Schrattenholz, & Maelicke, 1997; Dani, 2001). Presynaptic nAChRs modulate the probability of neurotransmitter release while postsynaptic nAChRs are involved in fast excitatory transmission and internal calcium (Ca²⁺) dependent second messenger systems (Dani & Bertrand, 2007).
Nicotine is the primary psychoactive ingredient in tobacco and preferentially binds $\alpha 4\beta 2$ nAChRs in the brain. Central nAChRs are pentameric ion channels composed of three $\alpha$ and two $\beta$ subunits in various combinations (Dani & Bertrand, 2007). There are seven isoforms of the $\alpha$ subunit ($\alpha 2 - \alpha 7$), and three isoforms of the $\beta$ subunit ($\beta 2 - \beta 4$) (Mineur & Picciotto, 2008). These receptors can be combined in either homomeric or heteromeric fashion (such as ($\alpha 7)_5$ or ($\alpha 4)_3(\beta 2)_2$, respectively). The most abundant nAChRs in the human CNS are $\alpha 4\beta 2$ receptors, which account for approximately 90% of nicotine binding in the brain (Benowitz, 2008).

When endogenous ACh is released into the synaptic cleft, postsynaptic nAChRs are activated almost instantaneously. After approximately one millisecond, ACh diffuses out of the synaptic cleft and is hydrolyzed by acetylcholinesterase, effectively precluding postsynaptic desensitization of nAChRs (Dani, Ji, & Zhou, 2001). In contrast, cigarette smoking delivers nicotine to the brain in a different fashion. Nicotine has a much larger effect on the whole brain (not just on pre/postsynaptic receptors), and remains in the synaptic cleft for longer periods of time since it is not hydrolyzed by acetylcholinesterase. In this way, chronic exposure to nicotine tends to desensitize nAChRs, and will thus have a much different effect on synaptic mechanisms than endogenous ACh alone (Dani & Bertrand, 2007; Dani & De Biasi, 2001). When nicotine binds nAChRs postsynaptically, cell depolarization occurs through rapid influx of sodium ($Na^+$) and $Ca^{2+}$. Nicotinic AChRs are found all on cell types, including GABAergic, glutamatergic, and dopaminergic neurons. Nicotine administration may therefore affect multiple brain regions and pathways common to both the pathophysiology of schizophrenia and addiction.
1.3.1 Nicotinic Acetylcholine Receptor Dysfunction in Schizophrenia

In schizophrenia, high-affinity nAChRs are downregulated (Breese et al., 2000) and receptor density at the level of the cortex is reduced (Durany et al., 2000). Adler and colleagues performed one of the first in vivo studies linking decreased cortical nAChR density to sensory function in schizophrenia (Adler et al., 1982). The authors used a paradigm called P50 sensory gating, where subjects hear two auditory clicks and an auditory evoked response to the second click is typically inhibited. Compared to healthy subjects, patients with schizophrenia do not elicit substantial inhibition to the second stimulus, suggesting a deficit in sensory gating, or the ability to filter out irrelevant information. This deficit was also observed in unmedicated first-degree relatives of patients with schizophrenia (Adler, Hoffer, Griffith, Waldo, & Freedman, 1992), which was normalized by nicotine in the patient group (Adler, Hoffer, Wiser, & Freedman, 1993).

Compared to healthy smokers, spatial working memory is more robustly impaired by overnight smoking abstinence in chronic smokers with schizophrenia, which is reversed upon reinstatement with nicotine (George et al., 2002). Administration of a nAChR antagonist blocks the ability of nicotine to restore cognitive impairment after overnight abstinence in schizophrenia smokers (Sacco et al., 2006), providing further evidence of the role of nAChRs in the pathophysiology of schizophrenia. However, since these studies were performed using an abstinence paradigm, the effect of nicotine on cognition may simply be due to nicotine’s ability to reduce withdrawal symptoms. For this reason, evaluating the effect of nicotine in non-smokers with schizophrenia is of utmost importance, and is discussed in section 1.5.2.2.
1.4 Varenicline

As an $\alpha_4\beta_2$ nAChR partial agonist, varenicline is one of the most successful smoking cessation medications currently available. The initial development of varenicline was based on the discovery that the nAChR partial agonist (−)-cytisine could be used as an efficacious smoking cessation drug. However, varenicline exhibits far greater specificity and efficacy as a smoking cessation medication than cytisine and other agonist/antagonist combinations that were used prior to its development (Glover et al., 2007), likely due to its primary action at $\alpha_4\beta_2$ nAChRs – the main receptor subtype involved in nicotine dependence.

The primary action of varenicline is to reduce subjective craving and mitigate withdrawal effects of smoking abstinence to promote successful cessation. A recent study showed that craving reduction typically seen with varenicline is associated with varenicline blood concentrations (Ravva, Gastonguay, Faessel, Lee, & Niaura, 2015), such that higher varenicline concentrations after 4 hour abstinence were associated with the greatest reduction in cravings. This data speaks to the importance of varenicline pharmacology, dose, and metabolism when evaluating its efficacy in promoting smoking cessation outcomes.

1.4.1 Pharmacology

Varenicline is a nAChR partial agonist with selectivity for the $\alpha_4\beta_2$ receptor. In fact, varenicline has a 20-fold greater binding affinity for the $\alpha_4\beta_2$ receptor than nicotine alone, with low affinity to other non-nicotinic receptors and other binding sites (Rollema et al., 2007). A large, single dose pharmacokinetic study examining the absorption of varenicline in 102 healthy volunteers found that 3 mg was well tolerated by smokers, while a lower 1 mg dose was tolerable in non-smokers (Faessel, Smith, et al., 2006). A subsequent multi-dose
pharmacokinetic study by the same group of researchers found that 2 mg repeated daily dosing was well tolerated in healthy smokers (Faessel, Gibbs, et al., 2006). This study also found that mean varenicline plasma concentrations were higher after repeated dosing compared to single oral administration, while maximum plasma concentration was achieved two to four hours post administration. After four days of repeated, twice daily dosing, steady state concentrations are reached (Faessel, Gibbs, et al., 2006). Together, these studies demonstrate that varenicline is safe to administer in both single and multi-dose regimens, and repeated dosing provides higher plasma concentrations and steady state levels.

Varenicline is excreted via the urine in a mostly unchanged state, with 81% of the drug eliminated as the ‘parent’ compound with 99% of the dose recovered in the urine (Obach et al., 2006). The half-life of varenicline varies depending on the dosing regimen. After a single oral dose, the half-life is between 16 to 27 hours (Faessel, Smith, et al., 2006), while after repeated dosing, the half-life is 18 to 43 hours (Faessel, Gibbs, et al., 2006). Varenicline is eliminated mainly through renal pathways and glomerular filtration (Canadian Pharmaceutical Association).

1.4.2 Preclinical Studies

Varenicline also has a high binding affinity for the α4β2 nAChR in rats and produces a slightly lower maximal response of nicotine compared to cytisine administration (32% and 40%, respectively). Varenicline pretreatment in rats significantly reduces self-administered nicotine intake compared to those not pretreated with varenicline. Furthermore, this effect is specific to nicotine, and does not affect other behaviours such as locomotor activity (Rollema et al., 2007). Another study found that pretreatment with varenicline dose-dependently reduced nicotine intake without affecting food intake, and also reduced the ability of a
nicotine cue to reinstate previously extinguished nicotine-seeking behaviour (Le Foll et al., 2012). Varenicline also blocks nicotine and cigarette smoke self-administration in rats (Costello et al., 2014); in this study, the rats that self-reinstated with cigarette smoke were more sensitive to stress than the rats that self-reinstated with nicotine. This study highlights the reinforcing properties of cigarette smoke, and the fact that nicotine seeking and reinstatement are mediated through nAChRs. Furthermore, varenicline also blocks the reinforcing properties of chronic nicotinic use in rats in a dose-dependent fashion, whereby low dose nicotine does not respond to varenicline treatment but high, chronic dosing does respond to treatment (Mello, Fivel, Kohut, & Carroll, 2014). These studies indicate that varenicline specifically affects nicotine-related behaviours in animal models of nicotine dependence, and may be used in a dose-dependent fashion to reduce the likelihood of nicotine reinstatement after the behaviour is extinguished. This is clinically relevant to human studies, as varenicline may be used to prevent or reduce smoking relapse in people who have successfully quit smoking.

1.4.3 Clinical Studies

1.4.3.1 Phase II Clinical Trials

Phase II clinical trials assess whether the drug has any biological activity or effect, and to determine the safety of the new medication in volunteer populations. Nides and colleagues examined three different doses of varenicline (0.3 mg once daily, 1.0 mg once daily, or 1.0 mg twice daily) compared to sustained-release bupropion hydrochloride (150 mg twice daily) and placebo in (N=638) healthy smokers (Nides et al., 2006). During the treatment, the continuous abstinence rates for varenicline (1.0 mg daily, 37.3%, and 1.0 mg twice daily, 48%) were greater than bupropion (33%) and placebo (17%). Confirmed
abstinence rates via carbon monoxide readings during 4 to 52 weeks of abstinence were also greater in the 1.0 mg twice-daily group compared to placebo (14.4% and 4.9%, respectively) (Nides et al., 2006). An additional study by the same group of researchers showed that a one-week titration of varenicline significantly reduced incidences of nausea (Oncken et al., 2006). In support of these results, a phase II clinical trial evaluated the dose-dependent effects of varenicline on smoking cessation in 618 healthy smokers (Nakamura et al., 2007). For all doses of varenicline (0.25 mg, 0.5 mg, or 1.0 mg daily for twelve weeks), the continuous abstinence rate at 9-12 weeks was higher than that of the placebo group. The highest abstinence rate at 9-12 weeks, 65.4%, was achieved with 1.0 mg varenicline. This dose of varenicline also had the highest abstinence rates during weeks 9-52 (Nakamura et al., 2007). An additional phase II trial assessed the combination of twice-daily 1.0 mg varenicline, twice-daily 150 mg sustained release bupropion hydrochloride, and behavioural therapy in a 12 week design (Ebbert, Croghan, et al., 2009). Abstinence rates were 71% at 3 months and 58% at 6 months. This study suggests that combination therapy may be more effective than monotherapy at achieving high abstinence rates among smokers. Additionally, varenicline in combination with nicotine replacement therapy (NRT) may be more effective than varenicline alone at achieving smoking abstinence at the end of three-month treatment (Koegelenberg et al., 2014). These clinical trials demonstrate that varenicline alone and varenicline in combination with NRT is more effective than placebo or bupropion at achieving sustained abstinence past the end of treatment in healthy smokers.

1.4.3.2 Phase III Clinical Trials

Phase III trials are designed to test a drug’s effectiveness after screening for safety and dosing regimens. Several phase III trials have been conducted to determine the effect of
varenicline on smoking cessation. Two of these trials examined the effect of 12-week varenicline treatment on smoking cessation outcomes compared to placebo and bupropion in a double blind and randomized fashion (Gonzales et al., 2006; Jorenby et al., 2006). Both of these studies observed a significant improvement in cessation rates with varenicline compared to bupropion and placebo, which persisted during the last 4 weeks of treatment as well as up to 52 weeks following treatment. A third phase III trial was a 12-week open-label varenicline study, followed by a randomized, double-blind, placebo controlled 12-week trial of either varenicline or placebo to those who had successfully achieved abstinence during the open-label trial (Tonstad et al., 2006). The objective of this trial was to assess the viability of varenicline as a maintenance therapy after abstinence has been achieved. Indeed, continuous abstinence rates were higher in the varenicline group (43.6%) compared to placebo (36.9%) between weeks 13-52 (Tonstad et al., 2006). Another phase III trial compared 1.0 mg twice-daily varenicline for 12 weeks to 21.0 mg daily (reducing to 7.0 mg daily) transdermal NRT for 10 weeks (Aubin et al., 2008). Non-treatment follow-up continued to week 52. This study found that varenicline was significantly more efficacious for achieving end-of-treatment abstinence (55.9% compared to 43.2% with NRT) as well as trending toward better abstinence rates at 52-week follow-up (26.1% compared to 20.3% with NRT) (Aubin et al., 2008). The most recent phase III clinical trial evaluated the effect of 5 mg of varenicline per day titrated over 3 weeks for smokers who did not respond to the standard dose used in previous phase III trials (Hajek et al., 2015). The authors did not find that a higher dose of varenicline improved smoking cessation outcomes in these smokers, suggesting that 2 mg per day may be an optimal dose and that varenicline at higher doses may have an antagonistic effect on nAChRs.
1.4.3.3 Phase IV Clinical Trials

Two important phase IV trials of varenicline have been conducted. The first study examined the effect of 2 mg per day of varenicline on smoking cessation outcomes in smokers who were not yet ready to quit but who wanted to reduce their cigarette consumption (Ebbert et al., 2015). Among these smokers, varenicline was found to improve smoking cessation outcomes at 24 weeks of treatment as well as one year after treatment. The second study evaluated the efficacy of retreatment with varenicline for smokers who have previously been treated with varenicline but were unsuccessful (Gonzales et al., 2014). Abstinence rates were similar to that of varenicline-naïve smokers: 45.0% with varenicline compared to 11.8% in the placebo condition.

These clinical trials demonstrate that varenicline is a more successful smoking cessation medication than both sustained-release bupropion and NRT, but that combination therapy may yield more effective results than therapy with varenicline alone (Ebbert, Croghan, et al., 2009). Additionally, varenicline may be safely and effectively used in smokers who are not yet ready to quit (Ebbert et al., 2015), but is not effective at doses higher than 2 mg per day (Hajek et al., 2015).

1.4.4 Safety and Efficacy of Varenicline in Non-Psychiatric Individuals and Patients with Schizophrenia

1.4.4.1 Safety and Efficacy of Varenicline

Although there have not been any randomized controlled trials explicitly evaluating the safety of varenicline in non-smokers, many experimental studies have administered varenicline to non-smokers and have reported safe and efficacious outcomes. Some reports have claimed varenicline to have depressogenic effects in non-psychiatric individuals.
(Moore, Furberg, Glenmullen, Maltsberger, & Singh, 2011), yet others have proposed varenicline to be used as an anti-depressant (Philip, Carpenter, Tyrka, & Price, 2010). Because smoking and smoking cessation can lead to depression itself, it is unclear whether varenicline plays a role in alleviating depression by modulating withdrawal symptoms during cessation or if varenicline contributes to already present depressive symptoms (Mocking et al., 2014). One group of researchers attempted to address these inconsistencies in the literature through a series of studies that examined the potential of varenicline to produce depressogenic effects. Their first study evaluated the effect of a week of varenicline treatment (1 mg/day) on suicidal and depressive symptoms in non-smoking healthy subjects through a sensitive neurocognitive battery (Mocking et al., 2013). Compared to placebo, varenicline did not influence negative biases or emotional processing. Since there are other factors involved in depression that may not manifest through cognitive testing, a second study evaluated the effects of the same dosing regimen of varenicline on cortisol levels in healthy non-smokers (Mocking et al., 2014). Varenicline did not alter cortisol levels compared to placebo, suggesting that varenicline does not have an effect on depression through modulation of the hypothalamic-pituitary axis. These studies, along with others that have administered varenicline to non-smoking healthy subjects during laboratory experiments and did not find any major adverse effects (Ari et al., 2011; Batsikadze, Paulus, Grundey, Kuo, & Nitsche, 2014; Ebert & Tetrault, 2014; Faessel, Smith, et al., 2006; Roh et al., 2014), suggest that varenicline is safe and well tolerated in the non-smoking population.

1.4.4.2 Safety and Efficacy of Varenicline in Schizophrenia

Very few studies have assessed the safety and efficacy of varenicline for smoking cessation in patients with schizophrenia. More broadly, Stapleton and colleagues found that
varenicline was as well tolerated and as effective in mentally ill individuals as in non-psychiatric participants, and that varenicline did not exacerbate psychiatric symptoms (Stapleton et al., 2008). In 2011, the first randomized, double-blind, placebo controlled pilot study examining the efficacy of varenicline on smoking cessation in schizophrenia found that varenicline significantly reduced carbon monoxide levels compared to the placebo group over a 12 week period, and that 75% of patients in the varenicline group were considered abstinent at 12 weeks compared to 0% in the control group (Weiner et al., 2011). A larger clinical trial evaluating varenicline on smoking cessation in patients with schizophrenia or schizoaffective disorder found that varenicline was associated with significantly higher cessation rates, was well tolerated, and did not exacerbate psychiatric symptomatology (Williams et al., 2012). Finally, Evins and colleagues conducted a large clinical trial of N=203 smokers with schizophrenia or bipolar disorder in an open-label varenicline study (Evins et al., 2014). At week 52, point-prevalence rates were 60% in the varenicline group versus 19% in the placebo group. From weeks 12-64, 45% of those in the varenicline group were continuously abstinent compared to just 15% in the placebo group (Evins et al., 2014). These results indicate that varenicline may be safely used to help patients with schizophrenia achieve smoking abstinence.

Overall, varenicline is a successful smoking cessation aid that has been extensively tested in animal models and human populations. Varenicline can be safely and effectively administered in both healthy smokers (Faessel, Gibbs, et al., 2006; Faessel, Smith, et al., 2006) and non-smokers (Faessel, Smith, et al., 2006), as well as in patients with schizophrenia without significantly exacerbating psychiatric symptomatology (Williams et al., 2012). Moreover, varenicline is more effective than other methods of smoking cessation
at achieving sustained abstinence (Aubin et al., 2008; Ebbert, Burke, Hays, & Hurt, 2009; Ebbert, Croghan, et al., 2009; Gonzales et al., 2006; Jorenby et al., 2006; Nakamura et al., 2007; Nides et al., 2006; Oncken et al., 2006; Tonstad, 2006; Tonstad et al., 2006).

1.5 Working Memory

Working memory refers to the online maintenance and manipulation of information over short periods of time (Baddeley, Logie, Bressi, Della Sala, & Spinnler, 1986), and as such, is necessary for functional day-to-day living. Compared to short and long-term memory, working memory is transient and does not induce physiological or structural synaptic changes that are necessary for the transition of memory items to longer-term storage (Barak & Tsodyks, 2014).

In addition to the hippocampus, the dorsolateral prefrontal cortex (DLPFC) is the most important region of the brain for working memory. Non-psychiatric individuals with lesions to the DLPFC show deficits in working memory (Barbey, Koenigs, & Grafman, 2013), and DLPFC activity can be modulated based on the difficulty of a working memory task such as the N-back (Barr et al., 2013). Additionally, low frequency non-invasive brain stimulation over the DLPFC that induces a virtual lesion interrupts working memory performance in healthy subjects (Mottaghy, Gangitano, Sparing, Krause, & Pascual-Leone, 2002; Schicktanz et al., 2015). The number of items held in working memory may preferentially activate different regions of the prefrontal cortex. For example, a recent functional magnetic resonance imaging (fMRI) study showed that manipulation of one item in working memory was associated with activity in the frontopolar cortex, while two items selectively increased activity in the DLPFC (Kim, Kroger, Calhoun, & Clark, 2015). This
may be why some studies find an inverted U-shaped distribution of DLPFC activity that corresponds with increased working memory load (Barr et al., 2010); under simple conditions with one memory item, DLPFC activity is minimal and increases with a task load of two items, followed by a decrease with a working memory load of three items (Barr et al., 2010; Callicott et al., 1999).

1.5.1 Working Memory Deficits in Schizophrenia

In schizophrenia, working memory deficits are one of the most well-established and replicated findings. Numerous studies have found poorer working memory performance in schizophrenia compared to healthy subjects (Anticevic, Repovs, & Barch, 2013; Barr et al., 2013; Barr et al., 2010; Basar-Eroglu et al., 2007; Chen et al., 2014; Forbes, Carrick, McIntosh, & Lawrie, 2009; Lee & Park, 2005). The most recent meta-analysis evaluating working memory across its subdomains (including visuospatial, verbal, and executive working memory) in schizophrenia was in 2009. The authors found an overall deficit in working memory in schizophrenia compared to healthy subjects, but no selective differences were seen between subdomains in patients (Forbes et al., 2009), perhaps due to the heterogeneity observed among high and low performers with schizophrenia (Karlsgodt et al., 2009; Larrison-Faucher, Matorin, & Sereno, 2004).

Logically, dysfunctional DLPFC activity is observed in conjunction with working memory impairment in schizophrenia, although the direction and magnitude of activity during such tasks has remained an inconsistent finding. For example, one study grouped schizophrenia patients into high and low performers on a working memory task (Karlsgodt et al., 2009). In high performers, DLPFC activity was hyperactive relative to controls, while in low performers DLPFC activity was hypoactive, reflective of the vast heterogeneity
associated with the illness. Further evidence for the link between working memory and prefrontal cortical function in schizophrenia was recently published using an intensive cognitive training program (Subramaniam et al., 2014). Patients with schizophrenia and healthy subjects performed a working memory task while in an fMRI scanner. At baseline, patients had poorer working memory performance and hypoactivity in the left frontal gyrus relative to healthy subjects (Subramaniam et al., 2014). After a two-week working memory training program, patients with schizophrenia significantly improved performance while also normalizing deficient activity in the left frontal gyrus to levels seen in healthy subjects. Another recent study used repetitive transcranial magnetic stimulation (rTMS), a form of non-invasive brain stimulation, to enhance working memory performance in schizophrenia (Barr et al., 2013). At baseline, patients with schizophrenia performed worse on a verbal working memory task compared to healthy subjects. After 20 sessions of high frequency rTMS to the DLPFC, patients displayed a substantial improvement in working memory scores that were similar to the scores of healthy subjects at baseline (Barr et al., 2013). In addition to replicating impairments in working memory performance and prefrontal cortical hypoactivity in schizophrenia, these studies demonstrated that cognition is a dynamic process that may be subject to alteration by cognitive training and non-invasive brain stimulation. However, the majority of studies evaluating working memory in schizophrenia do not control for antipsychotic medications, which have been shown to affect cognitive performance in schizophrenia (Nielsen et al., 2015). Therefore, it is unclear whether these deficits are present prior to illness onset or whether they are secondary to schizophrenia. In this regard, prefrontal cortical deficits are still observed in medication naïve patients during tasks that elicit normal DLPFC activation in non-psychiatric controls (Barch et al., 2001),
suggesting that deficits in the DLPFC may not be a function of antipsychotic medication or chronicity, but are inherent to the disease and present throughout the course of illness.

1.5.2 Nicotinic Modulation of Working Memory in Non-Psychiatric Individuals and Patients with Schizophrenia

1.5.2.1 Nicotinic Modulation of Working Memory

Many studies have evaluated the effect of nicotine on cognition in non-psychiatric individuals, but most of these studies did not control for acute withdrawal (Heishman, Taylor, & Henningfield, 1994). Studies noting that non-psychiatric smokers improve on measures of cognition, including working memory, after smoking may in fact be reporting the effects of smoking reinstatement after a period of withdrawal. To account for the confounding effects of withdrawal, some studies have examined the effect of nicotine on non-smokers or satiated smokers. Overall, these studies showed a small beneficial effect of nicotine on a limited number of cognitive domains (Heishman, 1998; Heishman et al., 1994; Sherwood, 1993), yet results were extremely variable. Other studies have demonstrated an improvement in areas of attention and response inhibition in healthy non-smokers following nicotine administration (Barr et al., 2008). In tasks of facial affect recognition, nicotine did not have an effect in healthy smokers, suggesting that nicotine may only affect task-specific domains of cognition (Drusch et al., 2013). In the most recent meta-analysis evaluating the effects of nicotine on cognition in only non-smokers or satiated smokers, nicotine had beneficial effects on attention, working memory, and motor abilities (Heishman, Kleykamp, & Singleton, 2010). These effects likely demonstrate true cognitive enhancement by nicotine, rather than an effect of withdrawal reversal. However, some studies have shown that healthy smokers who quit smoking overnight show improvements in working memory
suggesting that smoking may be detrimental to this specific domain in healthy individuals.

The most recent study on the effect of nicotine on working memory evaluated 16 non-smokers and 16 smokers on working memory performance following nicotine patch administration (Grundey et al., 2015). Performance was compromised in abstinent smokers yet returned to baseline upon reinstatement with nicotine. Non-smokers showed a decrease in performance and error rates with nicotine, suggesting an improvement with nicotine only on reversing abstinence-induced deficits in cognition (Grundey et al., 2015). Although these results are in contrast to those previously described, disparate findings may be due to differences in methodology.

1.5.2.2 Nicotinic Modulation of Working Memory in Schizophrenia

Nicotine has been shown to modulate cognition in patients with schizophrenia, but it is dependent on current smoking status. For instance, smokers with schizophrenia perform better on some cognitive tasks compared to non-smokers with schizophrenia (Wing, Bacher, Sacco, & George, 2011). Additionally, visuospatial working memory in schizophrenia smokers tends to be impaired after overnight smoking abstinence (George et al., 2002), but reinstatement of smoking reverses these impairments (Sacco et al., 2005). Administration of a nicotinic receptor antagonist prevents this reversal of impairment by nicotine reinstatement (Sacco et al., 2005), implicating nicotine and nAChRs in cognition in patients with schizophrenia. Other studies have supported this reversal of abstinence-induced impairment in cognition in smokers with schizophrenia (AhnAllen, Nestor, Shenton, McCarley, & Niznikiewicz, 2008; Smith, Singh, Infante, Khandat, & Kloos, 2002; Smith et al., 2006). Additionally, nicotine improves pre-existing deficits in working memory and attention in
patients with schizophrenia (Barr et al., 2008; Depatie et al., 2002; Harris et al., 2004; Jacobsen et al., 2004). In contrast to the above-mentioned studies, one recent study identified current smokers with schizophrenia as having worse cognitive functioning than past or never smokers (Depp et al., 2015), challenging some previous studies showing the opposite relationship (Wing et al., 2011). However, it is important to note that methodological variability among studies may account for these discrepancies.

In regards to working memory in particular, one recent study using the Consortium on the Genetics of Schizophrenia (COGS-2) identified smoking status to be an important modulator of working memory in schizophrenia. In this study, smokers with schizophrenia performed worse than non-smokers with schizophrenia on the letter-number span task (Lee et al., 2014). Another study showed that nicotine patch administration improved working memory performance during the N-back task in smokers with schizophrenia, while worsening performance in non-psychiatric smokers (Jacobsen et al., 2004), suggesting that patients with schizophrenia may experience a specific pro-cognitive effect of nicotine compared to non-psychiatric smokers.

In summary, placebo-controlled, crossover, double-blinded studies where acute doses of nicotine are administered to either non-smokers with schizophrenia (Avila, Sherr, Hong, Myers, & Thaker, 2003; Barr et al., 2008; Harris et al., 2004; Sherr et al., 2002) and/or to abstinent smokers with schizophrenia (Adler et al., 1993; AhnAllen et al., 2008; Avila et al., 2003; Depatie et al., 2002; George et al., 2006; Harris et al., 2004; Jacobsen et al., 2004; Levin, Wilson, Rose, & McEvoy, 1996; Sacco et al., 2005; Sherr et al., 2002; Smith et al., 2002; Smith et al., 2006) show that nicotine administration improves neurophysiological
impairments specific to schizophrenia, such as sensory gating and saccadic tracking, as well as cognitive processes including attention and working memory.

1.5.3 Effects of Varenicline on Working Memory in Non-Psychiatric Individuals and Patients with Schizophrenia

1.5.3.1 Effects of Varenicline on Working Memory

Although relatively few studies have examined the effects of varenicline on working memory in healthy non-smokers, some demonstrate an improvement in cognition with varenicline. One study administered varenicline in a titrated manner over 7 days (0.5 mg/day for the first 3 days, and 1 mg/day for the next 4 days) (Mocking et al., 2013). The subjects randomized to varenicline did not differ from the placebo group in measures of emotional processing or mood, but varenicline did significantly improve working memory performance and declarative memory. However, another study evaluating a single dose of 1.0 mg varenicline did not find any improvement on cognition or working memory compared to placebo (Roh et al., 2014). Since this study used a single dose of varenicline, steady state levels would not have been achieved (Faessel, Smith, et al., 2006); it is possible that varenicline only enhances working memory in non-smokers under repeated dosing conditions.

1.5.3.2 Effects of Varenicline on Working Memory in Schizophrenia

Studies examining the influence of varenicline on working memory in schizophrenia report mixed results. One study administered varenicline at 0.5 mg/day for one week, and 1.0 mg/day for a second week and examined its effects on P50 sensory gating and cognition in both smokers and non-smokers with schizophrenia. Varenicline significantly reduced the P50 suppression deficit in non-smokers, but not for smokers, and also improved executive
functioning by reducing anti-saccadic errors (Hong et al., 2011). However, varenicline was not found to be effective on tasks of visuospatial working memory. These results of this study suggest that the effect of chronic nicotine in long-term smokers may normalize sensory gating, such that additional treatment with varenicline renders little to no effect. Another study found that treatment with pretreatment with varenicline prior to overnight abstinence in smokers with schizophrenia prevented any abstinence-induced deficits in visuospatial working memory compared to control smokers (Wing, Wass, Bacher, Rabin, & George, 2013). In contrast to these results, a recent study found no effect of varenicline compared to placebo on a test of sustained attention in smokers with schizophrenia, yet found a worsening of performance after a single dose of mecamylamine, a nAChR antagonist (Roh et al., 2014). These results suggest that specific α4β2 agonism may not exert cognitive benefit in patients with schizophrenia, although there are too few studies concerning this matter to definitively answer that question. Additional studies are needed to identify the contributions of various receptor subtypes to working memory and cognitive deficits in schizophrenia.

1.6 Neuroplasticity

Neuroplasticity can be described as the large-scale capacity of the brain to alter its internal experiences and behaviour in response to stimulation. Synaptic plasticity, on the other hand, refers to the specific modification of synapses in response to a stimulus that changes the way the synapse responds to future stimulation. This alteration can either be to strengthen synaptic connections to increase the likelihood of firing to additional stimulation, or it could be to reduce the likelihood of firing by weakening synaptic connections. Synaptic plasticity is the basis of the brain’s ability to change in response to experience, and forms the biological basis of learning and memory by facilitating the transition of temporary
information to long-term memory (Citri & Malenka, 2008). Synaptic plasticity may be involved in CNS development via synaptic pruning, and therefore may play a key role in the pathophysiology of many psychiatric and neurodegenerative disorders such as schizophrenia (Daskalakis, Christensen, Fitzgerald, & Chen, 2008).

1.6.1 Long-Term Potentiation and Long-Term Depression

In the late 1940’s, Donald Hebb proposed a theory of memory formation claiming that when presynaptic activity correlates with postsynaptic firing, associative memories can form through strengthening of synaptic connections (Hebb, 1949). Experimental support for Hebb’s findings came a couple of decades later, when it was discovered that high-frequency repetitive stimulation to excitatory cells of the hippocampus can lead to synaptic strengthening for hours or even days afterward (Bliss & Gardner-Medwin, 1973). This phenomenon was eventually termed ‘long-term potentiation’ (LTP), a cellular mechanism that enhances the likelihood of the post-synaptic neuron firing an action potential in the future, effectively strengthening the targeted synapse.

LTP has been an exciting area of investigation over the past few decades, as it is thought to be crucial for understanding the cellular and molecular mechanisms of memory formation (Martin, Grimwood, & Morris, 2000; Pastalkova et al., 2006). Like memory, LTP can be induced rapidly and is strengthened by repetition. Consequently, when these phenomena are better understood, they may be used for therapeutic purposes such as rehabilitation and cognitive enhancement. The main principles of LTP are rapid onset and persistence of potentiation (Abraham, Logan, Greenwood, & Dragunow, 2002), cooperativity, associativity, and input specificity (Nicoll, Kauer, & Malenka, 1988). Rapid
potentiation within 30 minutes is a key characteristic of LTP, as well as the persistence of this potentiation for more than one hour (Abraham et al., 2002). Cooperativity refers to coincident activation of a critical number of synapses required for LTP induction. Associativity is the capacity for a weak input to be potentiated when it is activated in relation to a stronger input. Finally, input specificity refers to the topographic influence of LTP; it is only induced at targeted synapses even within the same cell (Nicoll et al., 1988).

LTP relies on two types of ionotropic glutamate receptors on the postsynaptic cell membrane: NMDA and AMPA receptors. AMPA receptors are permeable to Na\(^+\) and potassium (K\(^+\)), and are responsible for the generation of most of the excitatory response when the cell is near its baseline resting potential. Unlike AMPA receptors, NMDA receptors are voltage-gated and are blocked by magnesium (Mg\(^{2+}\)) during negative membrane potential states (Mayer, Westbrook, & Guthrie, 1984). Depolarization of the postsynaptic cell dislodges Mg\(^{2+}\) from the channel, allowing an influx of Na\(^+\) and Ca\(^{2+}\) into the cell. During postsynaptic depolarization, inward Ca\(^{2+}\) concentrations likely must reach a certain threshold before the cascade of intracellular mechanisms involved in the induction of LTP can occur (Malenka, 1991). Many of these intracellular mechanisms are still being parsed out, but the main steps in the pathway of LTP induction have been deduced. Calcium/calmodulin-dependent kinase II (CaMKII) is a key protein kinase that undergoes autophosphorylation following the induction of LTP in the postsynaptic cell (Barria, Muller, Derkach, Griffith, & Soderling, 1997) (Fukunaga, Muller, & Miyamoto, 1995). Activated CaMKII then causes calcium-independent phosphorylation and autophosphorylation of AMPA receptors. Ultimately, this series of intracellular mechanisms results in upregulation of AMPA receptors.
at the postsynaptic cell surface, driven by activity-dependent changes in AMPA receptor trafficking.

During the induction of LTP, AMPA receptors are upregulated at the cell surface, which facilitates the depolarization of the postsynaptic neuron following glutamate release from the presynaptic cell. This ‘trafficking’ is thought to occur via endosomes located in dendrites that contain reserves of AMPA receptors that are mobilized during LTP to facilitate rapid response to subsequent stimuli (Park, Penick, Edwards, Kauer, & Ehlers, 2004). Surprisingly, AMPA receptors are not actually inserted into the cell membrane internally; they are exocytosed from perisynaptic sites, and diffuse laterally into the plasma membrane (Park et al., 2004).

Due to limitations surrounding the extent of stable electrical stimulation, much of the focus on the duration of LTP has been between its initial 30 and 60 minutes. However, late-phase LTP is responsible for synaptic changes that persist for longer periods of time after stimulation. The persistence of late-phase LTP is dependent on new protein synthesis (Reymann & Frey, 2007), triggered by a number of key proteins including calcium/calmodulin-dependent kinase IV (CaMKIV), extracellular signal-regulated kinase mitogen-activated protein kinase (ERK-MAPK), and protein kinase A (PKA) (Thomas & Huganir, 2004). These proteins activate crucial transcription factors that signal for the production of effector proteins that promote synaptic enhancement. The ability of LTP to persist for long periods of time may be due to structural remodeling of the potentiated synapses (Luscher, Nicoll, Malenka, & Muller, 2000). In addition to upregulation of AMPA receptors via endosomes, lipids may also be supplied to the postsynaptic membrane to enlarge the synapse (Luscher et al., 2000; Park et al., 2004).
Experimental triggering of LTP can occur in multiple ways; some examples include tetanic stimulation of postsynaptic synapses and the induction of ‘spike-timing dependent plasticity’ (STDP), where LTP is induced if presynaptic afferent stimulation generates a response in a fixed time window before depolarization of the postsynaptic cell (Dan & Poo, 2006). In other words, pairing presynaptic and postsynaptic stimulations enhances or depresses synaptic strength depending on the time between inputs.

Synaptic plasticity can be induced bidirectionally; that is, while LTP facilitates the strengthening of synaptic connections, long-term depression (LTD) weakens synaptic connections. Both forms of neuroplasticity play important roles in the brain in terms of experience-dependent learning, synaptic pruning, and addiction (Malenka & Bear, 2004). LTD is also NMDA receptor dependent, and can typically be induced experimentally via low-frequency tetanic stimulation (Mulkey & Malenka, 1992). The dominant hypothesis regarding the underlying mechanisms of LTD induction is that it requires a modest increase in intracellular Ca\(^{2+}\) (Cummings, Mulkey, Nicoll, & Malenka, 1996), compared to the relatively high levels of Ca\(^{2+}\) needed to induce LTP (Malenka & Nicoll, 1993). In contrast to the role of protein kinases in LTP, LTD relies on the involvement of protein phosphatases that dephosphorylate cell surface proteins involved in AMPA expression (Lisman, 1989). Interestingly, the opposite process that occurs during LTP is present during LTD. Rather than the exocytosis of AMPA receptors and other surface proteins during LTP; AMPA receptors are endocytosed back into the intracellular compartment during LTD (Bredt & Nicoll, 2003; Malenka & Bear, 2004). There is evidence that shrinkage of dendritic spines may also accompany the loss of AMPA receptors from the plasma membrane, contributing to synaptic...
weakening (Hsieh et al., 2006; Nagerl, Eberhorn, Cambridge, & Bonhoeffer, 2004; Zhou, Homma, & Poo, 2004).

In summary, synaptic plasticity is a bidirectional process that relies on NMDA receptor function. If glutamate release from the presynaptic membrane causes sufficient increases in intracellular Ca\(^{2+}\) of the postsynaptic membrane, then LTP is induced via AMPA receptor trafficking to the cell surface. If, however, the intracellular calcium levels are only modestly increased, then LTD is induced via endocytosis of AMPA receptors from the cell membrane. These processes are thought to underlie learning and memory in the brain, and therefore may play an important role in cognition, addiction, and neurodevelopmental disorders such as schizophrenia.

1.6.2 Induction of Neuroplasticity Using Brain Stimulation

There are several ways in which neuroplasticity can be reliably indexed and induced in the human brain. Many non-invasive brain stimulation protocols have been developed and used to induce neuroplasticity with varying degrees of success, including transcranial direct current stimulation (tDCS), repetitive transcranial magnetic stimulation (rTMS), and paired associative stimulation (PAS). These protocols induce rapid changes in excitability in the cortex, and outputs can be measured through the use of TMS paired with electroencephalography (EEG) or through motor evoked potential (MEP) amplitude, if assessed in the motor cortex. Since changes in inhibition and excitation reflect changes in synaptic mechanisms that are also involved in neuroplasticity, TMS offers a useful tool to allow researchers to examine the effects of these protocols after stimulation has ended.
1.6.2.1 Transcranial Direct Current Stimulation

Transcranial DCS consists of the application of a low-intensity current – usually 1 to 2 mÅ – over the scalp between two electrodes that are soaked in a saline solution and powered by a battery-driven stimulator (Nitsche & Paulus, 2000). The two electrodes can be placed in a variety of locations, but the optimal transfiguration to induce excitability changes in the motor cortex is one electrode over the motor cortex and the other over the contralateral orbital bone. Although the majority of effects are intracortical, one study showed changes in corticospinal excitability (Ardolino, Bossi, Barbieri, & Priori, 2005), though this effect was not seen at lower intensities (Nitsche & Paulus, 2000).

During stimulation, the effects of anodal tDCS seem to be purely through changes in membrane potential; Ca\(^{2+}\) and Na\(^{+}\) channel blockers appear to abolish the effects of anodal tDCS in the brain (Nitsche et al., 2003). While the immediate effects of anodal and cathodal brain stimulation during treatment rely on changes in membrane potential, the after-effects are thought to be mediated by changes in synaptic plasticity – namely LTP and LTD (Nitsche & Paulus, 2000). Anodal stimulation increases intracortical facilitation (ICF), a measure of NMDA receptor function indexed through TMS. Dextromethorphan blocks this increase in NMDA receptor function if administered after stimulation (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2003), suggesting that the increase in ICF is specific to the after-effects of tDCS (Nitsche, Jaussi, et al., 2004; Nitsche et al., 2005). Following this, anodal stimulation is thought to increase excitability by depolarizing neurons, while cathodal stimulation is thought to decrease excitability (Nitsche & Paulus, 2000).

Recent studies have experimented with stimulation protocols to examine whether intermittent or repeated stimulation can induce longer lasting changes in excitability. One
study used tDCS in conjunction with a motor skills training task in healthy volunteers over 5 consecutive days (Reis et al., 2009). Subjects in the anodal tDCS condition had enhanced motor skill acquisition and maintained this level of improvement up to 3 months later, whereas those in the cathodal condition did not achieve these results. Most studies examining anodal tDCS in the motor cortex support the role of tDCS in enhancing LTP-like neuroplasticity (Stagg & Nitsche, 2011), although one study showed a suppression of neuroplasticity when tDCS was applied during training of a motor task. Since this was one of the only studies to evaluate tDCS during practice-dependent neuroplasticity, tDCS may not have effects on rapid synaptic plasticity (Rosenkranz, Nitsche, Tergau, & Paulus, 2000).

In summary, tDCS is a method used to induce LTP- or LTD-like neuroplasticity in the human cortex using weak direct current stimulation. Anodal stimulation produces depolarization of the underlying neurons through changes in membrane excitability, while cathodal stimulation produces hyperpolarization of neurons, thus increasing and decreasing synaptic plasticity respectively. Transcranial DCS has been reliably shown to enhance motor learning, which is thought to share similar LTP-like mechanisms. Transcranial DCS may therefore be a reliable tool to explore learning and memory in patients where such mechanisms are dysfunctional.

1.6.2.2 Repetitive Transcranial Magnetic Stimulation

Repetitive TMS is delivered via repetitive trains of magnetic pulses that, depending on intensity, frequency, and duration of stimulation, can be used to transiently inhibit or excite the activity of a cortical region. Repetitive TMS can be used to functionally map the human brain, to create temporary functional lesions, and is now commonly being used as treatments for psychiatric and neurological disorders including schizophrenia. Stimulation
intensity is usually set at a certain percentage of an individual’s resting motor threshold (RMT), defined as the lowest possible stimulator intensity that can elicit a MEP greater than 50 μV in 50% of trials. High frequency rTMS (≥1 Hz) is considered to be excitatory, while low frequency rTMS (≤1 Hz) is thought to be inhibitory (Hallett, 2000). Typical rTMS paradigms last for approximately 30 minutes, and can be applied using unilateral or bilateral stimulation to the regions of interest.

Although the use of rTMS is widespread both clinically and in the laboratory, its precise mechanism of action remains unclear. One recent study examined the effects of the NMDA receptor antagonist dextromethorphan, the GABA receptor agonist lorazepam, and the Na⁺ channel blocker carbamazepine to characterize the nature of the effects of 5 Hz rTMS to the motor cortex (Sommer et al., 2013). Motor cortex facilitation after rTMS was blocked by lorazepam and reduced by carbamazepine, with no effects of dextromethorphan. Carbamazepine also reduced MEP facilitation after rTMS application, likely due to its Na⁺ channel blocking properties at the cell membrane. The blockade of facilitation by lorazepam is similar to other studies, whereby lorazepam inhibits activity after application of anodal tDCS (Nitsche, Liebetanz, et al., 2004) and after paired pulse inhibition paradigms (Di Lazzaro et al., 2000). These data indicate that rTMS may not rely on LTP-like mechanisms, since administration of NMDA receptor antagonists such as dextromethorphan tend to block mechanisms involved in synaptic plasticity (Monte-Silva et al., 2013; Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Wankerl, Weise, Gentner, Rumpf, & Classen, 2010), and that it may instead rely on GABAergic neurotransmission. Nonetheless, rTMS does appear to enhance neuroplasticity through improvements in motor skill learning or cognition (Jung, Shin, Jeong, & Shin, 2008; Simis et al., 2013).
Since deficits in cognition may be due to dysfunction in the DLPFC (Paulman et al., 1990), many studies have used rTMS to target the DLPFC in order to remediate these cognitive impairments. One recent meta-analysis included 33 experiments that evaluated the effects of either tDCS or rTMS on working memory (Brunoni & Vanderhasselt, 2014). All studies included active and sham stimulation to the prefrontal cortex and some variation of the N-back task. They found that active versus sham stimulation produced decreased response time, increased correct responses, and lower error responses on the N-back task. However, upon performing a meta-regression analysis, the authors found that only rTMS was responsible for producing the effect sizes associated with increased performance, decreased response time, and decreased error rate (Brunoni & Vanderhasselt, 2014).

In summary, rTMS can be used to transiently inhibit or excite cortical brain regions, which allows it to be used for treating psychiatric disorders, neurological disorders, and to experimentally identify functional areas of the brain. Although the mechanisms of rTMS are not well known, it appears to enhance cognition through changes in GABAergic neurotransmission rather than through LTP or LTD-like processes.

1.6.2.3 Paired Associative Stimulation

PAS is a non-invasive brain stimulation technique that pairs an electrical pulse to the median nerve with TMS to the targeted contralateral brain region. Ensuing excitability changes are probed in the motor cortex by evaluating changes in MEP amplitudes using TMS. PAS has been used in the exploration and treatment of brain related conditions, including schizophrenia, major depression, and stroke (Carson & Kennedy, 2013). The mechanisms underlying PAS are thought to closely mimic Hebbian plasticity, due to a similar requirement for concomitant postsynaptic depolarization of the same cell by an
additional stimulus (Buonomano & Merzenich, 1998). From this, PAS may be used to augment rehabilitation or cognitive training by targeting the physiological changes upon which this enhancement is based (Harris-Love & Cohen, 2006). The motor cortex has been well studied with this technique due to the ease of using the MEP as an output variable, although recent advances have allowed neuroplasticity to be induced and measured in other regions of the brain via cortical evoked potentials from EEG (Rajji et al., 2013).

When stimulating nerves of the upper limb, such as the median nerve or ulnar nerve, the common interstimulus interval (ISI) is 25 ms. This interval was initially chosen due to peripheral conduction times to the primary somatosensory cortex (~20 ms) and from there to the primary motor cortex (~3 ms) (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). Although this protocol has been shown to generate robust and sustained increases in cortical excitability (Sale, Ridding, & Nordstrom, 2007; Stefan et al., 2000; Wolters et al., 2003), fixed intervals of 21.5 (Weise et al., 2006) and 35 ms are also effective (Stefan et al., 2000). Interestingly, PAS\textsubscript{21.5} and PAS\textsubscript{25} may have different mechanisms of action; one recent study showed that cathodal tDCS over the cerebellum abolished PAS\textsubscript{25} but not PAS\textsubscript{21.5}, suggesting that the cerebellum may control late-phase but not early-phase LTP (Hamada et al., 2012). On the other hand, an ISI of 10 ms induces LTD-like effects because the median nerve input arrives later than the TMS input (Stefan et al., 2000).

This temporally bidirectional effect of PAS induction is consistent with the hypothesis that PAS-induced LTP resembles STDP (Stefan et al., 2000). The effects of PAS can be either excitatory or inhibitory, and the direction of these effects is dependent on a critical time window of tens of milliseconds. STDP is a phenomenon that is traditionally studied at the cellular level, but recent advances in TMS technology have allowed researchers
to study this form of neuroplasticity using PAS. The premises of PAS and STDP are based on the notion that two inputs (one from median nerve stimulation and the other from TMS) converge on the same neuron and, depending on the timing between inputs, increase or decrease LTP-like neuroplasticity by modulating elements involved in LTP such as NMDA and AMPA receptors.

PAS shares many characteristics with STDP; as a result, their mechanisms of action may also be similar. These characteristics include its rapid induction (typically after 30 minutes), persistence, reversibility, topographical specificity, and reliance on NMDA receptor mechanisms (Stefan et al., 2000). Accordingly, MEP amplitudes are typically increased between 30 and 60 minutes following PAS induction, and are fully reversible within 24 hours (Stefan et al., 2000). PAS is also topographically specific – that is, application of the PAS paradigm using the median nerve will only result in potentiation of those muscles innervated by the median nerve that are also stimulated by TMS. For example, one study used TMS to stimulate both the abductor pollicis brevis muscle (innervated by the median nerve) and the first dorsal interosseous muscle (innervated by the ulnar nerve), yet only stimulating the median nerve during the PAS paradigm (Ridding & Taylor, 2001). In spite of large increases in excitability in the abductor pollicis brevis muscle, the first dorsal interosseous muscle amplitude was unchanged, suggesting a topographical specificity of PAS stimulation. Moreover, dextromethorphan, an NMDA receptor antagonist, fully blocks the facilitatory or inhibitory effects of PAS after induction (Stefan et al., 2002). Additionally, if subjects are pretreated with a voltage-gated Ca²⁺ channel blocker, PAS fails to induce a depression of MEP size (Stefan et al., 2000). These results indicate that PAS is likely dependent on synaptic mechanisms of neuroplasticity, namely STDP.
Several lines of evidence exist that support localized effects of PAS to the cortex. For example, the cortical silent period (CSP), which is a cortically generated phenomenon that indexes GABA<sub>B</sub> receptor function (Kukowski & Haug, 1992), is increased after PAS (Stefan et al., 2002). Additionally, the F-wave, a measure of spinal motoneuron excitability, remains unchanged after PAS (Stefan et al., 2000). These studies support the notion that the effects of PAS are input specific, and that the enhanced motor output is not simply a result of enhanced spinal motoneuron excitability.

Since PAS can induce LTP and LTD, is reproducible, and is topographically specific, it may be of therapeutic use for disorders in which neuroplasticity is compromised. One of the more common disorders where PAS has been used to investigate neuroplasticity is focal dystonia, which can occur in individuals who suffer from repetitive movements. Motor cortical neuroplasticity of the hand region is abnormal in patients with focal hand dystonia, such as those with writer’s cramp (Quartarone et al., 2003). PAS has also been used to show abnormal neuroplasticity in individuals with major depression (Player et al., 2013), nicotine dependence (Grundey et al., 2012a), and schizophrenia (Daskalakis, Christensen, Fitzgerald, & Chen, 2008), among many other neuropsychiatric illnesses.

### 1.6.3 Neuroplasticity Deficits in Schizophrenia

Schizophrenia is characterized by pervasive cognitive deficits, which is associated with changes in DLPFC activity (Karlsgodt, Shirinyan, van Erp, Cohen, & Cannon, 2005; Tan, Choo, Fones, & Chee, 2005) – the brain region most heavily involved in executive function and working memory (Berman et al., 1995; Levy & Goldman-Rakic, 2000). Due to the involvement of synaptic plasticity in memory and neural development, it has been
suggested that schizophrenia may be a disorder of pathological information flow (Abbott & Regehr, 2004). Both cognitive deficits and positive symptoms in schizophrenia suggest altered information processing (Mongillo, Barak, & Tsodyks, 2008), thus implicating aberrant synaptic plasticity in the pathophysiology of schizophrenia.

There is strong neurophysiological evidence to support deficits in neuroplasticity in schizophrenia. One of the most influential studies showing decreased neuroplasticity in the motor cortex of patients with schizophrenia was in 2008 by Frantseva and colleagues (Frantseva et al., 2008). This study examined LTP-like neuroplasticity and motor skill learning in 15 patients with schizophrenia and 15 healthy subjects using the PAS paradigm, with post-PAS intervals of 0, 15, 30, and 60 minutes. Patients with schizophrenia were found to have decreased MEP facilitation compared to healthy subjects, and also showed deficits in motor skill learning that positively correlated with MEP facilitation post-PAS (Frantseva et al., 2008). Another study used rTMS to increase cortical excitability and therefore neuroplasticity in the premotor cortex of patients with schizophrenia compared to healthy subjects (Oxley et al., 2004). In contrast to healthy subjects, patients with schizophrenia did not modulate excitability in response to rTMS. Furthermore, one study used tDCS to induce LTD in the motor cortex of patients with schizophrenia, their unaffected first-degree relatives, and healthy subjects (Hasan, Misewitsch, et al., 2013). LTD induction was significantly reduced in both schizophrenia patients and their relatives, suggesting that deficits in neuroplasticity may constitute a putative endophenotype of the illness. Similar to these results, the same group of researchers found that patients with schizophrenia did not modulate neuroplasticity in response to unilateral or bilateral tDCS to the motor cortex compared to healthy subjects (Hasan, Bergener, et al., 2013). From these studies, it is clear
that patients with schizophrenia display reduced neuroplasticity compared to healthy subjects; however, many studies that have assessed neuroplasticity in schizophrenia have not controlled for smoking status, which is an important modulator of excitability and neuroplasticity.

1.6.4 Nicotinic Modulation of Neuroplasticity in Non-Psychiatric Individuals and Patients with Schizophrenia

1.6.4.1 Nicotinic Modulation of Neuroplasticity

Nicotinic AChRs can influence synaptic plasticity in a multitude of ways. Presynaptically, nAChRs initiate an inward Ca\(^{2+}\) flux that increases the probability of neurotransmitter release into the synaptic cleft (Albuquerque et al., 1997; Jones, Sudweeks, & Yakel, 1999; McGehee & Role, 1995; Wonnacott, 1997). When this occurs at presynaptic glutamatergic neurons, studies have shown the induction of synaptic plasticity to be enhanced at the postsynaptic cell (Ji, Lape, & Dani, 2001; Mansvelder & McGehee, 2000). Postsynaptically, if nAChR activation coincides with presynaptic glutamate release then synaptic plasticity is also potentiated. In sum, coincident activation of pre/postsynaptic nAChRs can effectively enhance synaptic plasticity on the postsynaptic cell membrane by allowing additional Ca\(^{2+}\) influx (Ji et al., 2001), as well as modification of NMDA and AMPA receptor densities on the cell surface (Levy & Aoki, 2002).

Animal models have been popular in recent decades for evaluating the effect of nicotine on neuroplasticity both in vivo and ex vivo. In the ventral tegmental area (VTA), an area critical for nicotine addiction (Dani & De Biasi, 2001), nicotine administration to rat brain slices enhances the release of glutamate onto postsynaptic dopamine neurons, potentiating synaptic plasticity and likely playing a role in the plastic responses involved in
nicotine dependence (Mansvelder & McGehee, 2000). Another study showed that nAChR activation induces LTP in the mouse dentate gyrus, in vivo (Matsuyama, Matsumoto, Enomoto, & Nishizaki, 2000). Additionally, nicotine reverses the age-related decline of LTP induction in the rat hippocampus (Fujii & Sumikawa, 2001a), reverses GABAergic inhibition of LTP (Fujii, Jia, Yang, & Sumikawa, 2000), and facilitates LTD induction (Fujii & Sumikawa, 2001a, 2001b).

The PFC receives its glutamatergic inputs from the medial dorsal thalamus (Groenewegen & Uylings, 2000). These thalamo-cortical glutamatergic projections are excited in the presence of nicotine (Couey et al., 2007; Kassam, Herman, Goodfellow, Alves, & Lambe, 2008; Lambe, Picciotto, & Aghajanian, 2003), effectively increasing glutamatergic release onto the postsynaptic neuron and potentiating synaptic plasticity. In the PFC, α4β2 and α7 nAChRs are the most common, and are mostly located on the presynaptic cell membrane (Dickinson, Kew, & Wonnacott, 2008). Both receptor types modulate synaptic plasticity in different ways. For example, α7 nAChR activation enhances presynaptic influx of Ca^{2+} currents via mutual activation of pre-synaptic extracellular signal-regulated kinase 2 (ERK2). In contrast, activation of α4β2 nAChRs presynaptically increases Ca^{2+} influx through the recruitment of voltage-gated Ca^{2+} channels (Dickinson et al., 2008). In summary, although nicotine activates both α4β2 and α7 nAChRs in the PFC and results in facilitation of the postsynaptic cell, the mechanisms through which synaptic plasticity is potentiated differs depending on the density of these receptors.

Recent research has revealed lasting effects of nicotine on synaptic plasticity in the PFC, outlasting the duration of stimulation or state of desensitization (Jiang & Role, 2008; Kawai, Lazar, & Metherate, 2007; Zhong et al., 2008). Importantly, synaptic plasticity in the
PFC is associated with learning and memory (Laroche, Davis, & Jay, 2000), and modulation by nicotine may consequently alter the cellular processes of LTP/LTD and downstream cognition. If nAChR activation occurs on GABAergic neurons, then the observed effect will likely be LTD of the synapse in question, and if on glutamatergic neurons, then the effect will likely be an enhancement of LTP. For example, in rodent models, nicotine excites GABAergic interneurons and thus enhances inhibitory output to pyramidal cells in the cortex and has been shown to induce LTD rather than LTP in the cortex (Couey et al., 2007). This may seem counterintuitive considering that nAChR activation is usually excitatory and conducive to LTP induction (Mansvelder, Mertz, & Role, 2009), but due to the high density of nAChRs on GABAergic interneurons compared to glutamatergic neurons in the PFC, inhibitory output likely dominates excitatory output subsequent to cortical nAChR activation in rodents.

In non-smokers, application of a rivastigmine, an acetylcholinesterase inhibitor, enhances focal PAS-induced neuroplasticity while abolishing non-focal neuroplasticity induced by tDCS (Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007). Nicotine acts in a similar fashion, enhancing focal neuroplasticity in the cortex and abolishing non-focal neuroplasticity (Thirugnanasambandam, Grundey, Adam, et al., 2011). This ‘focusing’ effect of ACh and nicotine on LTP may be related to its effect on specific cognitive domains rather than on global cognitive functioning in healthy individuals.

Very little is known about the specific nAChR subtypes involved in mediating synaptic plasticity, although given that both tDCS and PAS induce Ca\(^{2+}\) dependent neuroplasticity, nAChRs with Ca\(^{2+}\) channel properties are likely involved (Batsikadze et al., 2014). For this reason, Batsikadze and colleagues administered varenicline, a common
smoking cessation medication that acts as an $\alpha_4\beta_2$ nAChR partial agonist, to 25 non-psychiatric non-smokers to evaluate its effects on focal and non-focal neuroplasticity (Batsikadze et al., 2014). Similar to the effects of nicotine (Thirugnanasambandam, Grundey, Adam, et al., 2011) and rivastigmine (Kuo et al., 2007), varenicline preserved focal LTP-like neuroplasticity and abolished non-focal LTP-like neuroplasticity. The similar focusing effects of global cholinergic activation, varenicline, and nicotine suggest that the specific effect of nicotine on cognition and neuroplasticity may be dependent on $\alpha_4\beta_2$ nAChR activation. In contrast to these results, one study administered nicotine nasal spray on 48 healthy non-smokers during 4 paradigms: anodal and cathodal tDCS, and PAS$_{25}$ and PAS$_{10}$ (Grundey et al., 2012b). Nicotine nasal spray abolished all facilitatory LTP-like neuroplasticity regardless of focality, and weakened all LTD-like neuroplasticity. Although in opposition to the above-mentioned studies, the use of rapid-intake nasal spray may be the cause of these discrepancies, as its mechanisms of action differ from that of an oral medication or a cigarette. Regardless, these different methodologies speak to the complexity of evaluating the effect of nicotine on neuroplasticity when studies use multiple methods of administration. Another study used tDCS and PAS to evaluate the effects of smoking abstinence and reinstatement by nicotine patch in 12 non-psychiatric smokers (Grundey et al., 2012a). LTP-like neuroplasticity was abolished during abstinence, but was restituted by nicotine administration. In contrast, LTD-like neuroplasticity was not affected by abstinence. However, under the influence of nicotine reinstatement, LTD-like neuroplasticity using tDCS was abolished, yet the LTD-like effects seen with PAS were prolonged.

In summary, nicotine has a wide range of effects on neuroplasticity in the human brain. While activation of pre and postsynaptic nAChRs results in similar downstream effects
on synaptic plasticity, the mechanisms by which they drive LTP- and LTD-like effects are remarkably different. Presynaptic activation of nAChRs enhances the release of glutamate into the synaptic cleft, while postsynaptic activation of nAChRs modulates Ca\textsuperscript{2+} influx into the cell, thus increasing the probability of depolarization. While research on nicotinic modulation of neuroplasticity in humans is a relatively new field, the evidence points to a focal effect of nicotine on neuroplasticity, implicating \(\alpha_4\beta_2\) nAChRs in both synaptic plasticity and its downstream effects on cognition and learning.

1.6.4.2 Nicotinic Modulation of Neuroplasticity in Schizophrenia

Patients with schizophrenia experience aberrations in nAChR density, form, and function (Breese et al., 2000; Durany et al., 2000; Esterlis et al., 2014; Freedman et al., 1995; Leonard et al., 2000). Since nAChRs play both direct and indirect roles in synaptic plasticity, patients with schizophrenia may smoke to ameliorate deficits associated with nAChR dysfunction, such as sensory gating and working memory impairment (Winterer, 2010). Few studies have examined the effect of nicotine on neuroplasticity in humans, although some – albeit indirect – research in rodent models has accumulated over the past decade. One recent study examined the interaction between nicotine and clozapine on synaptic plasticity in rats pre-treated with these drugs (Rajkumar, Suri, Deng, & Dawe, 2013). This interaction is of particular interest since clozapine is a frequently used antipsychotic in the treatment of schizophrenia. This study showed a priming effect of pre-treatment with both nicotine and clozapine after administration of a nicotine/clozapine challenge, such that rats pre-treated with either nicotine or clozapine showed a significant enhancement of synaptic plasticity compared to vehicle treated rats (Rajkumar et al., 2013). This data suggests that early exposure to nicotine or clozapine may modify the brain’s response to subsequent treatment.
Another study used a rodent model of schizophrenia to investigate the effects of nicotine on NMDA disruption in mice pretreated with NMDA receptor antagonists (Andre, Leach, & Gould, 2011). Nicotine reversed the cognitive impairment induced by NMDA receptor antagonists, broadly suggesting that high smoking rates in schizophrenia could be a result of nAChR modulation of NMDA receptor dysfunction.

Only one study has been done in patients with schizophrenia evaluating the effect of nicotine on neuroplasticity induced by non-invasive brain stimulation. Using the tDCS paradigm, these researchers found that LTD-like neuroplasticity was abolished in non-smokers with schizophrenia, whereas normal LTD-like neuroplasticity was seen in patient smokers (Strube et al., 2015). The authors propose that nicotine may stabilize cortical inhibition/excitation imbalances in patients with schizophrenia, which is why these deficits in LTD appear in non-smokers with schizophrenia yet they remain intact in smokers. Unfortunately, most of the evidence surrounding nicotine and schizophrenia focuses on phenotypic manifestations of the illness, such as cognitive performance and sensory gating. Since neuroplasticity is the basis of learning and memory, it can only be inferred that nicotine, having a large role in cognition, may also have a large role in modulating synaptic plasticity in schizophrenia.

1.6.5 Effects of Varenicline on Neuroplasticity in Non-Psychiatric Individuals and Patients with Schizophrenia

No studies have yet evaluated the effect of varenicline on neuroplasticity in schizophrenia. Only one such study has evaluated varenicline on neuroplasticity in non-smoking healthy subjects, using anodal and cathodal tDCS as well as PAS\textsubscript{25} and PAS\textsubscript{10} to examine non-focal and focal neuroplasticity, respectively (Batsikadze et al., 2014).
Varenicline preserved focal neuroplasticity using \( \text{PAS}_{25} \) but abolished tDCS induced facilitatory, non-focal neuroplasticity. It is possible that varenicline did not have a beneficial effect on neuroplasticity in non-psychiatric healthy subjects due to optimal pre-existing nAChR functioning.

1.7 Overall Summary

Schizophrenia is a devastating neuropsychiatric illness that, in addition to positive and negative symptoms, is also characterized by cognitive deficits that are unresponsive to available antipsychotic treatments. Patients with schizophrenia also experience deficits in working memory, which may be due to underlying aberrations in neuroplasticity. Dysfunctional nAChR function has been proposed to be the basis of both cognitive deficits as well as vulnerability to nicotine dependence in schizophrenia, as the prevalence of smoking in schizophrenia is extremely high and nicotine appears to benefit cognition in these patients.

The neurophysiological basis of learning, memory, and cognition is neuroplasticity, or LTP. Nicotine and nAChRs modify neuroplasticity by modulating glutamate release presynaptically and \( \text{Ca}^{2+} \) flux postsynaptically. In this regard, patients with schizophrenia who smoke may in fact experience improvements in cognition due to the influence of nicotine on neuroplastic processes. Consequently, deficits in neuroplasticity may constitute a shared vulnerability for both cognitive impairment and nicotine dependence. However, it is difficult to infer whether deficits in neuroplasticity are due to dysfunctional nAChRs in schizophrenia, or whether dysfunctional nAChRs are due to disrupted neuroplasticity. Varenicline is a common smoking cessation medication that acts as a partial agonist at the \( \alpha_{4}\beta_{2} \) nAChR, and is safe and tolerable in both smokers and non-smokers with and without schizophrenia (Faessel, Smith, et al., 2006; Williams et al., 2012). As varenicline has already
been shown to improve cognition in patients with schizophrenia, it is pertinent to identify the mechanisms by which this improvement occurs. It is possible that varenicline modifies neuroplasticity and downstream cognitive processes by enhancing glutamate release from the presynaptic cell (Dickinson et al., 2008). Since nicotine has been shown to enhance neuroplasticity in patients with schizophrenia (Strube et al., 2015), varenicline, acting similarly to nicotine at the α4β2 receptor, may also enhance neuroplasticity in these patients.

1.8 Study Objectives and Hypotheses

The primary objective of the current study is to evaluate the effects of subchronic dosing of the α4β2 nAChR partial agonist, varenicline, on LTP-like neuroplasticity in non-smokers with schizophrenia compared to non-psychiatric non-smokers using the PAS25 paradigm. We hypothesize that patients with schizophrenia will display deficits in motor cortical LTP induction compared to healthy subjects, similar to other published studies (Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Frantseva et al., 2008), during the placebo condition. We hypothesize that varenicline will improve deficits in PAS-induced LTP in patients with schizophrenia. As single dose varenicline has been shown to preserve LTP in healthy non-smokers (Batsikadze et al., 2014), we similarly do not expect to see an effect of varenicline on LTP in healthy non-smokers compared to placebo.

A secondary objective of this study is to examine working memory performance in non-smokers with schizophrenia compared to non-psychiatric non-smokers, as well as the effects of varenicline on these measures. We evaluated working memory performance during a baseline session, and hypothesized that patients with schizophrenia would perform worse on the 3-back task compared to healthy subjects (Barr et al., 2010). We also expect to
replicate these findings during the placebo condition. With varenicline, we do not expect to find any change in working memory performance in healthy subjects, but we do hypothesize that 3-back performance (Hong et al., 2011) will be enhanced in schizophrenia with varenicline.
Chapter 2
Methods

2.1 Study Design

This study is a randomized, crossover, double blind, placebo-controlled human laboratory study investigating the effects of varenicline on LTP-like neuroplasticity and working memory in non-smokers with schizophrenia compared to non-psychiatric non-smokers. A total of 22 participants completed the entire study, while an additional 2 participants (N=24) completed the baseline component of the study evaluating working memory. The study duration was approximately 4 weeks and included a screening visit, a baseline visit, two medication pick-up days, and two testing days subsequent to the medication pick-up days. Both testing days were separated by a minimum washout period of two weeks (see figure 2.1). Testing day details are provided in section 2.4 (study procedures). This study was approved by the Centre for Addiction and Mental Health (CAMH) Research Ethics Board (REB #214/2012), and was conducted in accordance with the Declaration of Helsinki. The study was registered on www.clinicaltrials.gov.

2.2 Participants

2.2.1 Total Sample

Over a 15-month period, 43 subjects completed an in-person screening session, and 19 of these subjects were ineligible, leaving 24 eligible participants (see figure 2.3). Two healthy subjects dropped out after the baseline visit. Twenty-two subjects (11 schizophrenia patients and 11 healthy subjects) 18-55 years of age completed this study. All subjects provided written informed consent (see Appendix A). Schizophrenia patients were recruited through CAMH via flyers, word-of-mouth, visiting outpatient clinics, and referrals. Healthy
subjects were recruited through flyers, posting ads on Craigslist, and referrals from other studies.

Schizophrenia participants met diagnostic criteria for either schizophrenia or schizoaffective disorder based on the criteria in the Structured Clinical Interview of the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (SCID for DSM-IV TR) (American Psychiatric Association, 2000). Healthy subjects did not meet SCID criteria.
for DSM-IV TR criteria for any current or past Axis I psychiatric disorder except for past major depression if in remission for over a year, and could also not be taking any psychotropic medications or have a first-degree relative with psychosis. Schizophrenia patients were required to be psychiatrically stable during participation, which required a score <70 on the Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein, & Opler, 1987), as well as a stable dose of antipsychotics for a least one month prior to study participation. Participants were also excluded if they were hospitalized within the last 3 months. All patients were medicated and their chlorpromazine (CPZ) equivalents of antipsychotics were calculated (American Psychiatric Association, 2000; Woods, 2003).

All subjects were excluded if they met DSM-IV criteria for abuse or dependence of alcohol or illicit substances within the past 3 months prior to study enrolment (except for caffeine dependence). Current drug use was assessed with Medtox urine toxicology screens (7-panel; THC, Opiates, Amphetamine, Cocaine, Phencyclidine, Barbiturates, and Benzodiazepines). Use of any nicotine, tobacco, or nicotine replacement products in the past year was exclusionary. Concomitant medical illnesses (e.g., renal insufficiency, hypothyroidism) or a personal or family history of seizures or syncope was also exclusionary. Premorbid intelligence was assessed using the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001), and any participant with a Full Scale IQ (FSIQ) score <80 was excluded from the study. The Research Nurse and the study principle investigator medically cleared all participants for participation by physical examination and blood work.

2.3 Measures

2.3.1 Clinical interview measures

Structured Clinical Interview for the DSM-IV-TR (SCID-IV)
The SCID-IV (First, Spitzer, Gibbon, & Williams, 2002) is a semi-structured interview that assesses current and lifetime Axis I psychiatric disorders. The SCID was used to both rule-out psychiatric diagnoses in healthy subjects and to confirm a diagnosis of schizophrenia or schizoaffective disorder in patients.

Positive and Negative Syndrome Scale (PANSS)

The PANSS measures positive, negative, and general psychopathology severity in patients with schizophrenia. The PANSS is a clinical interview using 7-point scale ratings (1=absent, 7=extreme) for each of the 30 items. The PANSS was administered only during the screening session as an eligibility measure. The PANSS has high inter-rater and internal reliability (Kay, Opler, & Lindenmayer, 1988), as well as adequate construct validity (Kay et al., 1987).

Mini Mental State Examination (MMSE)

The MMSE (Folstein, Folstein, & McHugh, 1975) is a brief questionnaire used to assess cognitive impairment. The MMSE was used to identify participants with cognitive impairment that could affect study participation. The minimum score required for eligibility on the MMSE was ≥ 20 out of 30.

2.3.2 Extrapyramidal side effects

Abnormal Involuntary Movement Scale (AIMS)

The AIMS (NIMH, 1974) is a scale designed to assess symptoms of dyskinesia in those with schizophrenia on neuroleptic regimens. The AIMS is a 12-item scale with items that rate involuntary movements of the patient’s body. Each of these items are rated on a 5-
point scale that ranges from 0 (none) to 4 (severe), except for items 11 and 12 (assess dental care) which are answered with either a “yes” or a “no”. A rating of 2 or higher is evidence of tardive dyskinesia.

Barnes Akathisia Rating Scale (BARS)

The BARS (Barnes, 1989) is used to measure both subjective and objective neuroleptic-induced akathisia in patients with schizophrenia. The BARS uses a 4-point scale to assess objective akathisia, subjective awareness of restlessness, and distress related to restlessness. A global clinical assessment is rated using a 5-point scale.

Simpson Angus Rating Scale for Extrapyramidal Symptoms (SARS)

The SARS (Simpson & Angus, 1970) assesses neuroleptic-induced Parkinsonian symptoms. The SARS includes 10 items that are rated on a 4-point scale. The items measure gait, rigidity, tremor, glabella tap, and salivation.

2.3.3 Physical examination

The Research Nurse conducted the physical examination during the screening visit. The physical examination consisted of vitals, expired carbon monoxide, electrocardiogram (EKG), blood work (to assess medical eligibility), medical history, current and past substance use, and a brief physical examination of the head, neck, and abdomen for medical clearance of the study medication. Particular emphasis was placed on assessing hepatic or renal insufficiencies, as approximately 90% of varenicline remains unprocessed and is cleared both passively and actively through the kidneys (Obach et al., 2006). The principle investigator
verified that the participant was medically eligible after the screening session by reviewing the physical examination package.

**Carbon Monoxide (CO)**

Carbon monoxide (CO) was used to biochemically verify non-smoking status (SRNT, 2002). CO was measured in parts per million (ppm) and a CO value of <10ppm was used to define current non-smoking status. Expired breath CO monitors measure the rate of conversion of CO to CO$_2$ as the participant’s breath passes over an active electrode.

### 2.3.4 Working Memory

**N-back task**

The N-back was administered according to our previous published protocols (Barr et al 2013). Capital letters were presented in a continuous sequence on a computer screen in front of the participant. Each letter is present on the screen for 250 ms, followed by a 3000 ms time frame to respond (see figure 2.2). Subjects were asked to complete the N-back task at two working loads (1 and 3) during the baseline session and testing sessions. The 3-back condition has an increased cognitive demand compared to the 1-back. The subject was asked to respond to each letter by pressing a ‘2’ button (target) if the letter was identical to the letter ‘N’ (1 or 3) letters back, or a ‘1’ (non-target) if it was not identical. The number of target letters in each condition was: 46 of 198 (23.2%) 1-back and 59 of 400 trials (14.6%) in the 3-back condition. The N-back took 45 minutes to complete with the order of conditions randomized within subjects and counterbalanced across subjects to prevent order effects. The primary outcome measure was task accuracy.
2.3.5 Paired Associative Stimulation

Electromyography (EMG) Recording

EMG was recorded from the right abductor pollicis brevis (APB) muscle using disposable disc 9 mm diameter electrodes (de Jesus et al., 2011). Subjects were asked to stay as relaxed as possible throughout the experiments, and the EMG was monitored on a computer screen. Two electrodes were placed on the hand: one electrode (active) was placed on the muscle belly of the APB, which was found by asking subjects to contract their muscle by squeezing the thumb and forefinger together, and one electrode (reference) on the proximal phalange of the thumb. An additional ground electrode was placed on the inside of the forearm distal to the wrist with a metal electrode and conducting gel. 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.

**Transcranial Magnetic Stimulation (TMS)**

Monophasic TMS pulses were administered using a 7 cm figure-of-eight coil, and two Magstim 200 stimulations (Magstim Company Ltd, UK) connected via a Bistim module. TMS was delivered over the left motor cortex corresponding to the right APB muscle. The coil was held with the handle pointing backward and at 45 degrees from the midline of the head. The direction of the induced current was from posterior to anterior and has previously been determined to be optimal for transsynaptic activation of corticospinal neurons (Kaneko, Kawai, Fuchigami, Morita, & Ofuji, 1996).

**Resting Motor Threshold (RMT)**

The RMT was defined as the output intensity that produced a MEP of ≥ 50 μV in 5 of 10 trials conducted in the relaxed APB muscle (Rossini et al., 1994). The 1 mV peak to peak was evaluated as the output intensity that elicits a peak-to-peak MEP between 0.5 and 1.5 mV in the relaxed APB muscle, and was the intensity used for the testing paradigms (Rossini et al., 1994).

**Sensory threshold**

Sensory threshold of the right peripheral nerve was evaluated as the lowest possible intensity that the participant could detect a sensation. Sensory threshold was conducted using the peripheral nerve stimulator (PNS) with the cathode pointing toward the wrist and the anode pointing toward the elbow on the inside of the wrist. Participants were asked to close
their eyes and verbally announce if they could feel the stimulus as the intensity of the output stimulator was lowered incrementally. When threshold had been found, the intensity was tripled as per the PAS paradigm (Stefan et al., 2000).

Pre-PAS

Baseline MEP was measured before the PAS paradigm was administered. Twenty pulses were delivered to the left motor cortex at the 1 mV peak-to-peak value for each subject (see figure 2.3). The averages of these twenty pulses were calculated as the baseline MEP size before LTP-like induction of neuroplasticity. Each pulse was separated by an ISI of 10 seconds, for a total of three minutes.

PAS

PAS consisted of 180 pairings of PNS with TMS to the left motor cortex. PNS was used as the conditioning stimulus with an ISI of 25 ms between the PNS pulse and the TMS pulse (see figure 2.3). This ISI allows both inputs to arrive in the motor cortex simultaneously. Ten seconds elapsed between each pair of pulses, for a total of 30 minutes of testing. To account for the effects of attention on neuroplasticity (Stefan et al., 2000), subjects were randomly asked to report the number of paired pulses they felt every 30 minutes.

Post-PAS intervals

The MEP size was measured post-PAS at 0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes using TMS (see figure 2.3). The post-PAS interval was the same as pre-PAS: 20 pulses with an ISI of 10 s, for a total of three minutes. The 20 pulses in each post-PAS
interval were averaged to assess the extent of LTP-like induction measured peripherally from the APB muscle at that time point.

**Figure 2.3:** Representation of the paired associative stimulation (PAS) paradigm. Peripheral nerve stimulation (PNS) to the right abductor pollicis brevis muscle is paired with transcranial magnetic stimulation (TMS) over the left motor cortex for 180 stimulations, thought to elicit LTP-like neuroplasticity. This change in neuroplasticity is assessed via the motor evoked potential every 15 minutes for two hours post-PAS, compared to the MEP amplitude pre-PAS (-30 minutes).

### 2.4 Study Medication

Varenicline tartrate (Pfizer Pharmaceuticals) tablets were encapsulated in a plain generic capsule that was indistinguishable from the placebo capsules. Placebo capsules were composed of only lactose monohydrate. Subjects received a dose of 0.5 mg BID of varenicline for 5 doses over 3 days (5th dose delivered on morning of 3rd day) during the active condition week.

### 2.5 Study Procedure
### 2.5.1 Screening

Once potential participants were identified or had called in response to ad postings, a telephone screen was completed. 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 (see figure 2.4).

![Study Recruitment Diagram](image)

**Figure 2.4: Study Recruitment Diagram**
The screening assessment took place on one occasion. The candidate, Alanna Bridgman (AB), with the exception of the SCID, PANSS, and physical examination, conducted all screening measures. Matthew Tracey (MT, Research Analyst) and Emily Simpkin (ES, Research Nurse) conducted the SCID, PANSS, and physical examination. Upon arrival for a screening assessment, subjects were asked to complete the informed consent procedure. Once consent had been granted, participants then completed a post-consent quiz to determine understanding of the procedures, in which a score of 80% was required. If this score was not achieved, the information was re-explained to participants. Demographic information forms followed the post-consent quiz. IQ testing was then completed via the WTAR, and clinical assessments were conducted with the SCID-IV, PANSS, and MMSE, with the AIMS, SARS, and BARS for schizophrenia patients only. Urine was tested for any recent drug use with the Medtox™ urine toxicology screening. The Research Nurse conducted the physical examination, consisting of an electrocardiogram, vitals, blood work, and detailed questioning about health and medical history. The PI approved all screening documents.

2.5.2 Randomization

The CAMH research pharmacist prepared the randomization table. Randomizations were conducted using a block design, and participants were randomized within their respective groups. The randomization code was kept within a secure location at the CAMH Queen Street site research pharmacy. Should an adverse event occur after hours, the on-call pharmacist had access to the randomization codes should a treating physician require that the blind be broken.
2.5.3 Varenicline Administration

Varenicline was dispensed from the CAMH Research Pharmacy. Participants came into the lab two days prior to the testing day at 9 am. AB administered and observed the first medication dose, and safety evaluations followed (see Appendix A). Subjects were instructed to take an additional dose that same evening at 6 pm, the next morning at 9 am, and the next evening at 6 pm. AB administered the fifth and final dose at 9 am the morning of the testing day. Subjects were asked to fill out a medication compliance log, which asked for time of medication, any adverse events, and participant initials. This form was brought back to AB on the morning of the testing day and was signed by AB.

2.5.4 Assessment of Compliance

Subjects were asked to return their empty blister pack as well as their medication compliance log. Compliance was calculated as the percentage of pills taken during the 2-day dosing period.

2.5.5 Testing Sessions

Once the screening visit was successfully completed and the PI deemed the participant eligible, the baseline study visit was scheduled. All testing sessions were completed by AB at the Temerty Centre for Therapeutic Intervention, CAMH, located at 1001 Queen Street West, Toronto, Canada.

Testing days were separated by a minimum of two weeks to ensure proper varenicline washout (Faessel et al., 2010). Varenicline or placebo was administered according to the procedure outlined in section 2.5.3 (see figure 2.5). These medication pick-ups were
approximately 30 minutes in duration. During the baseline visit, only working memory using the N-back task was evaluated. Both testing days were identical. First, the 5\textsuperscript{th} medication dose was administered, along with safety questionnaires and vitals. If any adverse events were reported, they were immediately relayed to the study PI via email. N-back was then assessed using the 1- and 3-Back conditions. Following N-back, pre-PAS measurements were assessed, followed by the PAS intervention lasting 30 minutes. Post-PAS measurements were assessed at 0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes post-PAS. Subjects were compensated for their time at a rate of $15 per hour, which they received at the end of every testing session. The total compensation for the study ranges from approximately $300 - $350 depending on time spent in the lab.

<table>
<thead>
<tr>
<th>Dose #1</th>
<th>Dose #5</th>
<th>Vitals/Safety</th>
<th>Break</th>
<th>Pre-PAS</th>
<th>Post-PAS</th>
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<td>9:00 am</td>
<td>9:30 am</td>
<td>12:00 pm</td>
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<td>2:00 pm</td>
</tr>
</tbody>
</table>

**Figure 2.5:** Example of a testing week. A minimum washout of two weeks separates the two testing days. Participants take the first dose of medication at 9:00 am the morning of the medication pick up day. The 2\textsuperscript{nd} dose is taken at 6:00 pm, the 3\textsuperscript{rd} dose at 9:00 am the following morning, and the 4\textsuperscript{th} dose at 6:00 pm that same day. The 5\textsuperscript{th} dose is administered the morning of the testing day. Dashes lines indicate time not spent in the lab, where the 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} doses are taken at home.

### 2.6 Data Analysis

All statistical calculations were completed using the Statistical Package for the Social Sciences (SPSS) for Windows version 21.0. Baseline demographic and clinical data were analyzed using independent t-tests for continuous variables and Chi-square ($\chi^2$) tests for categorical variables. All analyses were set to a significance of $p < 0.05$. All data are expressed as mean ± standard deviation. AB was unblinded after data collection and analysis.
2.6.1 Working Memory Analysis

Baseline working memory performance was assessed using a repeated measures analysis of variance (rANOVA) with N-back load (1- and 3-back) as the within-subjects factor and diagnosis (schizophrenia vs. healthy subject) as the between-subjects factor. For the effect of varenicline on working memory, a rANOVA with N-back load as the within-subjects factor and diagnosis and medication (varenicline vs. placebo) as the between-subjects factor was performed. The Mauchly test of sphericity was performed for all ANOVAs, and the Greenhouse-Geisser correction applied when necessary. Post-hoc independent samples t-tests were performed if significance was found in the ANOVAs. Correlation analyses were separately performed on performance in the 1-back and 3-back condition and demographic variables, and were analyzed within groups and medication conditions when the overall correlation was significant.

2.6.2 Long-Term Potentiation Analysis

For PAS-induced potentiation, the individual means of 20 MEP amplitudes were calculated for each time point (pre-pas, 0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes post-PAS) for every subject, and the mean MEP amplitudes during the post-PAS timepoints were normalized to the mean baseline MEP amplitude before PAS (quotient of post-PAS MEPs vs. pre-PAS MEP). The effect of varenicline on LTP-like neuroplasticity was assessed using a rANOVA with time as the within-subjects factor and medication condition and diagnosis as the between subjects factors.
The peak MEP across all time points was also calculated for each participant, due to large expected variability over 9 time points. The effect of varenicline on peak MEP was analyzed using a two-factor ANOVA with peak MEP as the dependent variable and diagnosis and medication as the fixed factors. The Mauchly test of sphericity was performed for all ANOVAs, and the Greenhouse-Geisser correction applied when necessary. In case of significant ANOVA results, exploratory post-hoc t-tests were applied to assess differences between groups and medication conditions. Correlations were analyzed within groups and medication conditions when the overall correlation was significant.
3.1 Baseline Results

3.1.1 Demographics

A total sample of 24 individuals completed this baseline portion of this study: 11 patients with schizophrenia, and 13 non-psychiatric healthy subjects. There were no differences in age or years of education, or in gender ($\chi^2 = 0.509, p = 0.476$) or race ($\chi^2 = 4.178, p = 0.124$). There were differences in IQ score as measured by the WTAR ($t_{(22)} = -2.63, p = 0.015$) and the MMSE score ($t_{(22)} = -2.24, p = 0.035$) (see table 1).

<table>
<thead>
<tr>
<th></th>
<th>HS (n=13)</th>
<th>SZ (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>35.5 ± 10.5</td>
<td>38.5 ± 9.0</td>
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</tr>
<tr>
<td>Sex (M:F)</td>
<td>10:3</td>
<td>7:4</td>
<td>0.476</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.124</td>
</tr>
<tr>
<td>Caucasian</td>
<td>12 (92%)</td>
<td>7 (64%)</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>1 (8%)</td>
<td>1 (9%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>110.8 ± 4.2</td>
<td>104.3 ± 7.8</td>
<td>0.015*</td>
</tr>
<tr>
<td>Years of Education</td>
<td>16.5 ± 2.9</td>
<td>15.4 ± 2.8</td>
<td>0.345</td>
</tr>
<tr>
<td>CPZ Equivalents (mg/day)</td>
<td>-</td>
<td>343.9 ± 176.4</td>
<td>-</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>-</td>
<td>14.1 ± 6.4</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>12.1 ± 3.2</td>
<td>-</td>
</tr>
<tr>
<td>General</td>
<td>-</td>
<td>24.3 ± 5.3</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>50.5 ± 11.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation
Results that are significant at the 0.05 level are marked with an (*)

All schizophrenia patients were on atypical antipsychotic medications: 37% (4) on olanzapine, 18% (2) on clozapine, 18% (2) on quetiapine, 18% (2) on risperidone, and 9%
(1) on paliperidone. CPZ equivalents were 343.9 ± 176.4. In addition to antipsychotic medications, 37% (4) were on clonazepam, 18% (2) on lithium, 9% (1) on paroxetine, and 9% (1) on citalopram. In all figures and tables, healthy subjects are labeled as HS, and schizophrenia subjects are labeled as SZ.

3.1.2 Baseline Working Memory Performance

A boxplot analysis (outlier: more than two standard deviations from the group mean) identified one schizophrenia subject that was a significant outlier and so was removed from baseline analysis, resulting in 13 healthy subjects and 10 schizophrenia subjects.

Figure 3.1: Baseline N-back performance between groups. Performance is significantly lower across diagnoses in the 3-back condition compared to the 1-back, \( p < 0.001 \). Performance was significantly decreased in the 3-back condition in patients compared to healthy subjects \( p = 0.036 \). Bars represent standard deviations. Asterisks (*) represent significant findings.
A rANOVA revealed decreased N-back performance from the 1-back (91.04% ± 8.32) to the 3-back (57.15% ± 23.75) across all subjects, F(1, 21) = 55.28, p < 0.001; (t(22) = 6.51, p < 0.001). A significant N-back load x diagnosis interaction was also found (F(1, 21) = 5.78, p = 0.025). A planned independent samples t-test based on visual inspection was performed only on the 3-back condition. In line with our hypothesis, there were significant differences on the 3-back task between patients and healthy subjects, t(21) = -2.25, p = 0.036, with patients (45.5% ± 21.0) performing worse on the 3-back compared to healthy subjects (66.1% ± 22.4) (see figure 3.1). We also found a significant positive correlation between premorbid IQ (as assessed by the WTAR) and 3-back performance across all groups, r(23) = 0.621, p = 0.002. This correlation was trending to significance in patients (r(10) = 0.61, p = 0.061) and healthy subjects (r(13) = 0.49, p = 0.092 (see figure 3.2).
Figure 3.2: *Trending to significant correlations between premorbid IQ and 3-back accuracy in patients with schizophrenia compared to healthy subjects.*

3.2 Varenicline

3.2.1 Complete Sample Demographics

A total of 22 individuals completed the entire study: 11 patients with schizophrenia and 11 healthy subjects. There were no differences in race ($\chi^2 = 3.53, p = 0.171$) or gender ($\chi^2 = 0.209, p = 0.647$) between groups, nor were there any differences in age or years of education. There were differences between groups in IQ ($t_{(20)} = -2.36, p = 0.029$) and in MMSE score ($t_{(20)} = -2.12, p = 0.047$) (see Table 2).

Table 2
*Demographics, clinical means, and standard deviations of the completed sample by diagnosis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SZ (n=11)</th>
<th>HS (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.5 ± 9.0</td>
<td>37.4 ± 10.2</td>
<td>0.792</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>7:4</td>
<td>8:3</td>
<td>0.647</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.171</td>
</tr>
<tr>
<td>Caucasian</td>
<td>7 (64%)</td>
<td>10 (91%)</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>1 (9%)</td>
<td>1 (8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (27%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>104.3 ± 7.8</td>
<td>110.6 ± 4.4</td>
<td>0.029*</td>
</tr>
<tr>
<td>Years of Education</td>
<td>15.4 ± 2.8</td>
<td>17.1 ± 2.6</td>
<td>0.159</td>
</tr>
<tr>
<td>CPZ Equivalents (mg/day)</td>
<td>343.9 ± 176.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14.1 ± 6.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>12.1 ± 3.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>General</td>
<td>24.3 ± 5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>50.5 ± 11.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± standard deviation*

*Results that are significant at the 0.05 level are marked with an (*)*
The same schizophrenia patients completed baseline and the full study, and their antipsychotic medications and CPZ equivalents are listed in section 3.1.1. All reported side effects are summarized in table 3.

Table 3
Adverse events reported for placebo and varenicline conditions

<table>
<thead>
<tr>
<th>Symptom Type</th>
<th>Placebo</th>
<th>Varenicline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HS (n=11)</td>
<td>SZ (n=11)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Stomach Pain</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Loss of Appetite</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mood/Behaviour/Cognition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0 (0%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Vivid Dreams</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Nervous/Musculoskeletal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Muscle Aches</td>
<td>1 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Dysphoria</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic Taste</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Frequent Urination</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

3.2.2 Working Memory

As in the baseline working memory results, the same schizophrenia participant was removed from analysis due to an abnormally high non-target non-correct response percentage, leaving 11 healthy subjects and 10 schizophrenia patients for analysis. A rANOVA with N-back load as the within-subjects factor and medication condition and diagnosis as the between-subjects factors found a main effect of N-back load, $F_{1,38} = 79.49$, $p < 0.0001$ (see figure 3.3). Subsequent paired samples t-tests found a significant difference
between the 1-back and 3-back regardless of diagnosis or medication condition, $t_{(40)} = 7.77, p < 0.0001$. The rANOVA also found a significant load x diagnosis interaction, $F_{(1, 38)} = 12.70, p = 0.001$. Post-hoc independent samples t-tests were performed on the 1-back and 3-back with diagnosis as the between-subjects factor. There was no significant difference between subjects on the 1-back, $t_{(40)} = 0.28, p = 0.782$, but patients with schizophrenia ($53.34\% \pm 20.35$) performed worse on the 3-back compared to healthy subjects ($75.06\% \pm 19.93$), $t_{(40)} = -3.49, p = 0.001$. The rANOVA also revealed a between-subjects effect of diagnosis, $F_{(1, 38)} = 7.81, p = 0.008$ (see figure 3.3).

**Figure 3.3:** Impact of varenicline on working memory performance. As shown in baseline results, 3-back performance was significantly reduced in patients with schizophrenia compared to healthy subjects during the placebo condition, $p = 0.005$. However, varenicline did not affect 1-back or 3-back performance in either patients or healthy subjects. Bars represent standard deviations. Asterisks (*) represent significant findings.
 Additionally, we examined whether there were differences in change scores (defined as varenicline condition score – placebo condition score for each N-back condition) between patients and healthy subjects. A rANOVA did not find a main effect of N-back load change score, $F_{(1,19)} = 1.04, p = 0.321$ (see figure 3.4).

**Figure 3.4:** Change in N-back target correct performance (score during active varenicline condition – score during placebo condition) during the 1-back and 3-back. No differences were found in change scores between subjects. Bars represent standard deviations. Asterisks (*) represent significant findings.

Since we did not find a medication effect when examining change score, we decided to explore whether there were differences in N-back change score depending on performance level. We stratified subjects by diagnosis into high and low performers using a median split to determine if varenicline would have a different effect on N-back score depending on the type of performance (high vs. low) during placebo. The median score for schizophrenia
patients was 94% in the 1-back and 47% in the 3-back. The median score for healthy subjects was 95% in the 1-back and 83% in the 3-back. Individuals below the median split were classified as low performers, and those above the median split were classified as high performers.

**Figure 3.5:** Varenicline-related change in N-back target correct performance based on performance during the placebo condition (low vs. high). More robust change scores were found for low performers in the 1-back condition than for high performers, $p = 0.001$. No differences were found for the 3-back condition. Bars represent standard deviation. Asterisks (*) represent significant findings.
Separate two-way ANOVAs were calculated for each N-back condition, with the N-back (1- or 3-back) change score as the dependent variable, and diagnosis and performer (low or high) as the fixed factors. For the 1-back condition, there was a simple main effect of performer, $F_{(1, 17)} = 15.28, p = 0.001$, with low performers (10.78% ± 7.40) having a significantly larger change in performance during the varenicline condition than high performers (0.26% ± 4.17), $t_{(19)} = 4.07, p = 0.001$ (see figure 3.5). These results suggest that low performers may receive a selective benefit of varenicline on 1-back scores. In the 3-back condition, an ANOVA with performer and diagnosis as the fixed factors and 3-back change score as the dependent variable did not reveal any simple main effects or interaction effects, so post-hoc tests were not explored.

### 3.2.3 Long-Term Potentiation

We examined differences in RMT using a two-way ANOVA, with RMT as the dependent variable and diagnosis and medication as the fixed factors. A simple main effect of diagnosis was found, such that patients displayed higher RMT than healthy subjects regardless of medication condition, $F_{(1, 40)} = 11.66, p = 0.001$. Using a two-way ANOVA with sensory threshold as the dependent variable and diagnosis and medication as the fixed factors, $F_{(3, 34)} = 0.06, p = 0.981$, there were no differences in any groups or medication conditions (healthy subjects: placebo = 2.06 ± 0.54, active = 2.07 ± 0.49, schizophrenia subjects: placebo 1.98 ± 0.57, active = 2.06 ± 0.50).

Using a box-plot analysis for the LTP data, two schizophrenia participants and one healthy control were determined to be significant outliers in the peak MEP data (see figure
These participants were removed from analysis, leaving a total of 10 healthy subjects and 9 schizophrenia patients.

Figure 3.6. Three significant outliers are classified in orange, 1 healthy subject and 2 schizophrenia patients.

Additionally, one healthy subject did not complete post-PAS 105 or post-PAS 120 during both testing days; that data is regarded as missing. Baseline MEP amplitude before normalization was significantly different between diagnoses regardless of medication group, $F_{(3, 34)} = 12.25, p = 0.001$, with patients $(0.86 \pm 0.29)$ having lower baseline MEP before PAS compared to healthy subjects $(1.15 \pm 0.22), t_{(36)} = -3.52, p = 0.001$. A rANOVA using time as the within-subjects factor and diagnosis and medication as between-subjects factors did not find a main effect of time on MEP amplitude, $F_{(8, 256)} = 0.50, p = 0.77$ (see figure 3.7), nor were there any interaction effects, possibly due to the substantial inter-individual variability.
Figure 3.7: Average MEP at post-PAS intervals between placebo and varenicline. A) Healthy subjects. No differences were found in averaged MEP amplitude. B) Schizophrenia patients. No differences were found in averaged MEP amplitude. The dashed line represents the threshold for LTP induction (>1). Bars represent standard deviations. Asterisks (*) represent significant findings.

We also examined the peak potentiation per individual during each medication condition. The maximal MEP value was chosen per individual regardless of which time point it occurred. This allows us to minimize time-to-peak inter-individual variability, as the time at which individuals displayed peak potentiation was widely distributed. There was a significant between-subjects interaction between medication and diagnosis on peak MEP amplitude ($F_{(1, 34)} = 6.04, p = 0.019$), evaluated using a two-way ANOVA with medication and diagnosis as the fixed factors and peak MEP as the dependent variable (see figure 3.8).
Figure 3.8: Impact of varenicline on peak MEP between patients with schizophrenia and healthy subjects. Shown are baseline-normalized MEP amplitudes after neuroplasticity induction by the PAS protocol. There were no differences between patients and healthy subjects on peak MEP during the placebo condition. Varenicline does not alter peak MEP in healthy subjects, but does significantly enhance peak MEP in patients with schizophrenia, \( p = 0.016 \), with an effect size of \( d = 1.38 \). The dashed line indicates the threshold for LTP induction (> 1). Bars represent standard deviations. Asterisks (*) represent significant findings.

Among healthy subjects, there were no differences in peak MEP between the placebo (1.96 ± 0.92) and the active conditions (1.53 ± 0.40), \( t_{(18)} = 1.37, p = 0.19 \). Among patients with schizophrenia, varenicline (1.89 ± 0.48) significantly enhanced MEP amplitude compared to placebo (1.39 ± 0.28), \( t_{(16)} = -2.69, p = 0.016 \) (see figure 3.8), with an effect size of Cohen’s \( d = 1.38 \).

Since attention has been shown to modulate neuroplasticity (Stefan et al., 2000), we asked participants to count the number of pulses they felt throughout the 30 minute stimulation protocol. A two-way ANOVA with count as the dependent variable and
diagnosis and medication as the fixed factors found no differences, $F_{(3, 34)} = 0.93, p = 0.44$, suggesting that our results were not confounded by differences in attention between groups and medication conditions.

Finally, there were no significant relationships between long-term potentiation and working memory across groups or between groups determined through correlation analyses.
Chapter 4
Discussion

4.1 General Discussion

The high co-morbidity of nicotine dependence in schizophrenia remains a serious public health issue and prevents optimal treatment of schizophrenia. Furthermore, the mechanism by which nicotine modulates cognition in schizophrenia remains unclear and is further confounded by studying smokers in various stages of dependence and withdrawal. Therefore, the aim of this current study was to evaluate the effects of the nAChR partial agonist, varenicline, on working memory and LTP-like neuroplasticity in non-smokers with schizophrenia compared to non-smoker healthy subjects, to control for the confounding effects of nicotine on behaviour. Based on previously published literature, we hypothesized that patients with schizophrenia would display deficits working memory performance and in LTP-like neuroplasticity during the placebo condition. We also hypothesized that varenicline would selectively enhance working memory performance and deficits in LTP-like neuroplasticity in non-smokers with schizophrenia compared to healthy subjects. In addition to this novel paradigm, we expected to see a relationship between the extent of LTP induction and working memory performance during the placebo condition in both groups.

To date, only a single study has evaluated the effects of varenicline on LTP-like neuroplasticity in healthy non-smokers (Batsikadze et al., 2014). In line with the findings of that study, we also replicated the preservation of LTP-like neuroplasticity in healthy subjects under the influence of varenicline using the PAS$_{25}$ protocol. However, the abovementioned study has several limitations that bring to question its validity in assessing the effects of varenicline on LTP-like neuroplasticity. First, the authors used only a single dose of
varenicline administered 3 hours before the start of testing, at doses of 0.1 mg, 0.3 mg, or 1.0 mg. As varenicline has a half-life of approximately 20 hours (Faessel, Smith, et al., 2006), a single dose study paradigm would likely not be able to assess the effects of varenicline at its intended therapeutic dose. The current study improved upon this design by administering 5 doses of varenicline over a 3-day period, similar to our previously published protocols (Wing et al., 2013), although the Wing et al. study administered 6 doses of varenicline, and was therefore more likely to achieve steady state varenicline levels compared to this study. Similar to the present study, the Batsikadze et al. study also evaluated MEP amplitude up to post-PAS 120 minutes, but in intervals of 0, 5, 10, 15, 20, 25, 30, 60, 90, and 120 minutes. For their analysis, the authors took the grand average of the first 7 time points (0 minutes to 30 minutes) to evaluate the effects of varenicline, whereas we chose to evaluate the peak MEP value. Many PAS studies only evaluate 4 time points (Frantseva et al., 2008; Rajji et al., 2011; Wolters et al., 2005), which would reduce variability in MEP amplitude when using a rANOVA. In this study, we observed high inter-subject variability in peak potentiation that could explain why a rANOVA did not produce significant results. Taking the peak MEP from each subject eliminates this inter-subject variability, and allows us to compare the maximum potentiation during varenicline and placebo. Also, we were initially unsure if varenicline would alter the distribution of peak MEP between conditions, which could have resulted in a less pronounced effect using rANOVA.

4.1.1 Study Sample

In our sample, one of the most striking demographic findings is the relatively high IQ scores in both the healthy subjects as well as in patients. Typically, patients with schizophrenia score about half a standard deviation lower in IQ compared to healthy subjects
(Khandaker, Barnett, White, & Jones, 2011), which we did replicate in this study. However, the mean IQ score of patients with schizophrenia is generally around 90 - 95, whereas in healthy subjects it is around 100 (Woodberry, Giuliano, & Seidman, 2008). The mean IQ in this study for both groups was slightly higher compared to general population means. One possible explanation for the higher IQ scores is the IQ test that we used; the WTAR is a test of premorbid IQ (Wechsler, 2001). Premorbid IQ has been shown to be higher than IQ measured post-symptom onset in schizophrenia (Khandaker et al., 2011), so it is possible that non-smokers with schizophrenia have comparable levels of premorbid IQ to the general population. Another possible explanation is that smokers in general have lower IQs than their non-smoking counterparts, as assessed by a large cohort study that found adolescents who smoke have lower IQs than matched non-smokers (Weiser, Zarka, Werbeloff, Kravitz, & Lubin, 2010). Whether low IQ is due to a neurobiological impairment that predisposes individuals to smoking or whether smoking in adolescents lowers IQ is unknown. Another study showed that smoking individuals have lower neurocognitive performance than non-smoking individuals (Wagner et al., 2013) further supporting the increased IQ seen in the non-smoking healthy subjects in this sample.

In this sample, every schizophrenia participant was on only one atypical antipsychotic medication. This greatly reduces confounding effects of medication in our data. The individuals who volunteered to participate in this study were those who were motivated to come into the lab on 6 separate occasions and were compliant with 5 doses of varenicline. The requirements of this study alone may have necessarily selected individuals with higher motivation and cognitive functioning.
We chose to perform this study in non-smokers with schizophrenia to rule out the confounding effects of tobacco smoke on cognitive function and neuroplasticity. A moderate dose of 0.5 mg of varenicline BID per day was chosen to elicit the fewest side effects while producing partial agonism at α4β2 nAChRs (Faessel, Smith, et al., 2006).

4.1.2 Antipsychotic Medications

While atypical antipsychotic medications affect a broad range of neurotransmitters, the primary target of these medications is antagonism at the dopamine D2 receptor (Goyer et al., 1996; Ichikawa et al., 2001; Volk et al., 1994). Due to excessive subcortical dopaminergic activity in schizophrenia (Howes et al., 2012), antipsychotics are used to minimize dopamine binding and reduce positive symptoms. Atypical antipsychotics have varying effects on cognition compared to first-generation antipsychotics (Nielsen et al., 2015), and therefore may be a potential confounding factor for our results. Patients who take either typical or atypical antipsychotic medications tend to perform more poorly than medication naïve or medication free patients (Nuechterlein et al., 2015), perhaps due to greater illness severity or detrimental effects of medication on cognitive function. However, the patients recruited for this study had a higher IQ than the average schizophrenia population, suggesting that illness severity may not be a heavily contributing factor to these results.

Certain properties of common antipsychotic medications, such as receptor binding and occupancy, may interact with varenicline and could cause changes in cognitive performance (Levin & Rezvani, 2007). For example, some studies have demonstrated an interaction between nicotine administration, antipsychotic medication, and cognitive performance in patients with schizophrenia. Clozapine in particular, which exhibits
serotonergic 5HT2A receptor antagonism, blocks the procognitive effects of nicotine on working memory performance in rodents (Addy & Levin, 2002). Since varenicline acts through methods similar to nicotine, it is possible, yet unlikely, that clozapine interfered with our results given that only two of our participants were on clozapine. The patients in this study were also all on a single atypical antipsychotic medication, and since the study was performed as a cross over design, it is unlikely that antipsychotic medications would contribute to the intra-individual differences between testing days, yet it is possible that antipsychotic medications affected neuroplasticity during the placebo condition (discussed in section 4.1.4). Using a within-subjects design, we are able to isolate the effects of varenicline and placebo in each subject given that antipsychotic treatment and dose was unchanged during the study.

When studying smokers with schizophrenia, it is necessary to consider the interaction between cigarette smoking and antipsychotic metabolism since similar enzymes are responsible for their metabolism (Meyer, 2001; Seppala, Leinonen, Lehtonen, & Kivisto, 1999). Higher doses of antipsychotic medications are typically prescribed to counteract the effect of smoking, which may alter cognitive performance. For these reasons, this study was performed in non-smokers with schizophrenia.

### 4.1.3 Working Memory

In line with previous research (Barr et al., 2013; Jacobsen et al., 2004), we found decreased 3-back performance in patients with schizophrenia compared to healthy subjects during baseline, which we were able to replicate during the placebo condition. Patients with schizophrenia, regardless of smoking status, consistently perform worse than matched controls on domains of working memory, attention, and executive functioning (Green, 2006).
While relatively few studies have evaluated the same N-back task that we used in patients with schizophrenia, the ones that have generally show patients performing at around 40% (Barr et al., 2013), while we showed performance accuracy at 50% in the placebo condition in patients. Since smoking status in schizophrenia has never been examined before in the N-back task, one possible explanation for our finding of higher accuracy is that non-smokers with schizophrenia may perform better on tasks of verbal learning and memory than smokers with schizophrenia (Depp et al., 2015).

Many studies show that nicotine administration enhances cognition in schizophrenia (Ahlers et al., 2014; Harris et al., 2004; Jubelt et al., 2008; Sacco et al., 2005; Smith et al., 2006), and even in adolescents with ultra-high risk of psychosis (Gupta & Mittal, 2014). On the other hand, there are studies that do not show an association between cigarette smoking and cognition in schizophrenia (Drusch et al., 2013; Zhang et al., 2013), and one recent study shows that schizophrenia smokers have worse cognitive functioning that never- and ex-smokers with schizophrenia (Depp et al., 2015). Interestingly, prefrontal cognitive dysfunction is associated with a failure to quit smoking in schizophrenia (Moss et al., 2009), which could either speak to lower cognitive function in schizophrenia smokers to begin with, or a detrimental effect of smoking on cognitive function in schizophrenia.

We did not find an effect of varenicline on working memory accuracy in either patients or healthy subjects in contrast to previous studies showing an improvement of working memory with varenicline treatment (Hong et al., 2011; Wing et al., 2013). To date, only 4 studies have evaluated the effect of varenicline on indices of working memory in schizophrenia (Hong et al., 2011; Roh et al., 2014; Smith et al., 2009; Wing et al., 2013). Only two of these studies utilized the N-back task, but did not use the exact format that we
used in this study. Moreover, the studies that showed an improvement of working memory with varenicline treatment either used a different dosing paradigm than we did, evaluated a different domain of working memory, or studied smokers instead of non-smokers. For example, Wing et al. (2013) used a similar dosing regimen, but studied smokers with schizophrenia. Participants were randomized to 0.0 mg, 1.0 mg, or 2.0 mg over 3 days. Smokers underwent overnight abstinence on the second day of testing, and were tested under abstinence conditions and during reinstatement on the third day. Varenicline attenuated the abstinence-induced deficits in visuospatial working memory at the 1.0 mg condition, but during regular smoking on day 1, 1.0 mg varenicline did not have an effect on memory, and 2.0 mg worsened memory (Wing et al., 2013). Because this study was performed in smokers with schizophrenia, it is possible that there was a ceiling effect during regular smoking that prevented varenicline from having an effect, but a more likely explanation is that higher 2.0 mg doses of varenicline had an antagonist effect on nAChR function. This interpretation would align well with models of schizophrenia suggesting that patients smoke to normalize deficits in nAChR function and ensuing cognitive deficits (Chambers et al., 2001), and that treatment with varenicline after abstinence is simply reversing the abstinence-induced impairments in cognition. One of the larger studies using a moderate dose of varenicline on cognitive function in schizophrenia found no effect on working memory in either smokers or non-smokers with schizophrenia (Hong et al., 2011), although that study used a model of spatial working memory instead of N-back working memory as is studied in this sample. Taken together, it appears that the effects of varenicline on working memory in schizophrenia are extremely varied.
Although one might expect varenicline to have a larger effect on non-smokers than smokers due to reduced pre-exposure to nicotinic agents, we did not find an effect of varenicline on accuracy in either patients or healthy subjects, in line with another study that administered nicotine to healthy smokers and non-smokers before an N-back task, and found that nicotine worsened performance in the non-smokers but reversed abstinence-induced deficits in smokers (Grundey et al., 2015). Our results are also supplemented by a study that found an improvement in cognition with varenicline in smokers with schizophrenia, but not in non-smokers (Shim et al., 2012). It may be that non-smoking schizophrenia patients do not have pre-existing deficits in nAChRs to the same extent as smokers (Breese et al., 2000), and thus do not seek compensatory nicotinic stimulation. One study supporting this statement examined the effect of the nAChR antagonist mecamylamine on cognitive function in non-smokers with schizophrenia compared to healthy subjects (Sacco et al., 2006). Mecamylamine did not affect cognitive performance in either group, suggesting that non-smokers with schizophrenia may not have deficits in nAChR function to the same extent as smokers with schizophrenia. On the contrary, the effect of mecamylamine in smokers with schizophrenia is pronounced and detrimental (Roh et al., 2014; Sacco et al., 2005). Perhaps, regardless of smoking status, some patients with schizophrenia have deficits in nAChR function and some do not. This may explain why some studies find an effect of varenicline on cognition while others show more modest effects or no effects at all. Since schizophrenia is such a heterogeneous illness, it seems logical that not everyone would have the same pathological deficits, especially when there are patients with higher cognitive functioning (Heinrichs, Ammari, McDermid Vaz, Miles, & Muharib, 2011; Kremen, Seidman, Faraone, Toomey, & Tsuang, 2000; MacCabe et al., 2012). Thus, we propose that varenicline may
only show beneficial effects not based on current smoking status, but based on pre-existing deficits in nAChR function. This hypothesis is also in line with our finding of higher IQ in the patient group compared to the average IQ in schizophrenia.

While we did not find an effect of varenicline on working memory accuracy, we did find more of an improvement in 1-back scores in participants with the lowest placebo 1-back score regardless of diagnosis. Studies exist that support our findings: notably, one study in healthy subjects found that nicotine enhanced performance on anti-saccadic movements only in the low performers (Petrovsky et al., 2012), and another study showed an identical finding but in patients with schizophrenia (Larrison-Faucher et al., 2004). Because we found differences in the effect of varenicline on working memory after performance stratification, it is likely that analyzing high and low performers together washed out any potential effects of varenicline. The ability of varenicline to only enhance performance in low performers during the 1-back regardless of diagnosis suggests a global effect on attention. As noted throughout this thesis, many studies have found a selective improvement on tasks requiring attention compared to other cognitive domains in both healthy subjects and in schizophrenia (Hong et al., 2011; Roh et al., 2014; Smith et al., 2009).

Providing further evidence of the role of the cholinergic system in working memory, one study administered scopolamine (a muscarinic AChR antagonist), mecamylamine (a nAChR antagonist) and a combination of the two to healthy subjects before performing a spatial N-back task (Green et al., 2005). Scopolamine but not mecamylamine significantly worsened performance on the N-back, but a combination of the two worsened performance beyond that of scopolamine, supported by a subsequent study by the same group (Ellis et al., 2006). This suggests that nAChRs and muscarinic AChRs may act in synergy to modulate
working memory performance. From this, it is possible that muscarinic AChRs play more of a role in working memory performance impairment in schizophrenia than nAChRs. Interestingly, very little work has been done on muscarinic receptor modulation of working memory in schizophrenia since the publication of that research.

A final consideration is whether a different dosing paradigm would have altered our results. While the studies that have found a beneficial effect of varenicline have either shown this effect in smokers with schizophrenia (Wing et al., 2013), or during a longer (i.e. 8 week) paradigm (Hong et al., 2011), it is difficult to comment on whether we would have found similar results. Since the dosing paradigm we used has been shown to yield results in other studies (Wing et al., 2013), it is unlikely that changing the dosing regimen would result in different findings. Additionally, all patients were compliant with their medication (as assessed by compliance logs).

4.1.4 Long-Term Potentiation

In the present study, we hypothesized that schizophrenia patients would show deficits in neuroplasticity compared to healthy subjects during the placebo condition. In support of previous research (Frantseva et al., 2008), we did show impairment in LTP-like neuroplasticity in schizophrenia after the PAS\textsubscript{2s} paradigm when analyzing all time points (as seen in figure 3.5). However, we also did not show an effect of time on MEP amplitude in the healthy controls during placebo, but that could be due to the large variability in peak potentiation as well as the amount of time points tested.

To account for the inter- and intra-individual variation found in this study, we chose to analyze the point of peak potentiation for each subject as part of our secondary hypothesis.
In this way, we acknowledge the fact that individuals vary in the extent and time of potentiation after PAS, since many studies have shown high inter-individual variability in LTP response after PAS\textsubscript{25} (Fratello et al., 2006; Sale et al., 2007). One key study showed that healthy subjects vary in the magnitude and direction of potentiation based on RMT amplitude and age (Muller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008). In particular, this study showed that individuals who were PAS responders (exhibiting potentiation \(\geq 100\%\)) had lower RMTs than non-responders. Although we attempted to control for age by limiting enrollment to those between 18 – 55 years and matching between groups, it is possible that the non-responders in our study washout out our results. In the present study, we found higher RMT in patients with schizophrenia compared to healthy subjects, which may be attributed to factors such as cortical atrophy or increased cortical distance from the skull that are commonly seen in patients with schizophrenia (Fitzgerald, Brown, Daskalakis, & Kulkarni, 2002; Sasamoto et al., 2014; Witthaus et al., 2008). These factors may necessitate a higher RMT to elicit similar MEPs compared to individuals without cortical atrophy. However, we are not at liberty to remove PAS non-responders due to an already small sample size, as well as already having removed significant outliers from the data. We also did not wish to bias our results or limit generalizability by selecting or analyzing only PAS responders. Using a more conservative threshold of \(\geq 120\%\) peak potentiation during the placebo condition, around 20\% of our sample, including two healthy subjects and two schizophrenia subjects were non-responders, consistent with what has been shown in the literature (Fratello et al., 2006; Muller-Dahlhaus et al., 2008; Stefan, Wycislo, & Classen, 2004).

Variability in response to PAS can come from internal or external factors (Koski, Schrader, Wu, & Stern, 2005). Internally, variability can arise due to attentional effects,
hormonal influences, and other physiological or anatomical variability. For instance, attention has been shown to modulate neuroplasticity (Stefan et al., 2004), which is why participants were asked to count the number of pulses they felt during the PAS$_{25}$ protocol. No differences were found between groups or medication conditions in the final count, suggesting that attention is not a covariate we need to be concerned with for this study. Other possible sources of variation include cortical thickness (Conde et al., 2012) and the menstrual cycle (Smith et al., 1999). Seven of our subjects were female, and since experiments were conducted 2 weeks apart without taking the menstrual cycle into consideration, hormone levels would differ between testing days. There have not yet been any studies examining the influence of menstruation on PAS effectiveness, but since there were approximately equal numbers of females in both groups, it is unlikely that menstrual cycle-related changes in hormones would account for the differences we reported between groups.

External variability can arise through the use of different PAS parameters that elicit different responses in MEP facilitation (Sale et al., 2007), whether differences are in stimulation parameters (i.e. number and frequency of stimulation) or in time of day. For instance, PAS conducted during the afternoon is more reproducible and elicits more robust MEP facilitation (Sale et al., 2007), which is why in our study PAS was started no earlier than noon in all subjects. Many studies have reported significant variability in the range of potentiation as well as time to peak (Muller-Dahlhaus et al., 2008), similar to the results of this study.

An interesting study by Fratello and colleagues in 2006 tested the same group of subjects using the PAS$_{25}$ paradigm exactly one week apart to see if this paradigm would produce intra-individual reliability (Fratello et al., 2006). They found that there was
significant variability between testing days in participants, with some participants showing LTP one week and LTD the other week. This brings into question the role of homeostatic metaplasticity; the notion that the effect of brain stimulation on cortical excitability may depend on recent activity within the cortex (Wankerl et al., 2010). Perhaps a washout of one week was not enough to allow the prior effects of PAS to dissipate. On the other hand, the authors also showed similar facilitation in MEP amplitude on average during both testing days (Fratello et al., 2006), suggesting that although intra-individual variability may be common, it is unlikely to affect the overall effect of PAS\textsubscript{25}. This final piece of evidence supports the use of cross over study designs using PAS\textsubscript{25} protocol. In our study, while it is true that some individuals showed an LTD-like response one week and an LTP-like response the other week, we could not reliably determine whether this change in direction was due to the medication.

There were no differences in peak MEP during placebo between patients and healthy subjects. However, the peak potentiation in healthy subjects was around 196%, and 139% in patients. Although 139% potentiation seems high for a schizophrenia sample, this is likely because the only other study assessing LTP-like neuroplasticity in schizophrenia using the PAS\textsubscript{25} paradigm reported average MEP values for each time point rather than a peak value for each person (Frantseva et al., 2008). Additionally, the Frantseva and colleagues study only evaluated 4 time points (0, 15, 30, and 60), significantly reducing the amount of comparisons made. It is important to note that due to natural fluctuations in MEP over time, nearly all individuals would be expected to peak over 100% at some time point. However, the mean peak potentiation of nearly 200% in healthy subjects suggests that this peak is likely due to the induction of LTP-like neuroplasticity rather than natural fluctuation.
In line with our hypotheses, varenicline did not have an effect on peak potentiation in healthy subjects, but did enhance neuroplasticity by increasing the peak potentiation value in schizophrenia patients by close to 50%. The only study that has examined the effect of varenicline on PAS-induced LTP only included healthy non-smokers, and found that varenicline preserved MEP amplitude without increasing or decreasing neuroplasticity (Batsikadze et al., 2014). Our results corroborate these earlier findings, suggesting that varenicline does not have a therapeutic effect in healthy non-smokers perhaps due to a ceiling effect or to proper functional nAChRs.

It is important to consider how other neurotransmitters may have affected our results. Dopamine and GABA critically modulate synaptic plasticity dependent on the receptor subtype involved. For example, dopamine D1 agonism enhances LTP-like neuroplasticity (Huang & Kandel, 1995), while D2/D3 receptor activation tends to dampen both NMDA and GABA neurotransmission resulting in reduced LTP-like neuroplasticity (Chen et al., 1996). In rodent models of schizophrenia, administration of olanzapine, an atypical antipsychotic and a dopamine D2 antagonist, to adolescent rats causes an alteration in the levels of GABA and glutamate in the nucleus accumbens (Xu, Gullapalli, & Frost, 2015), supporting an indirect role of dopamine in neuroplasticity. More direct evidence shows dopamine D2 receptor regulation of structural neuroplasticity in the basal ganglia of rats (Cazorla et al., 2014). Dopamine D2 receptors modulate dendritic spine connections (Jia, Zhao, Hu, Lindberg, & Li, 2013), which are critical for structural neuroplasticity and connectivity. Dopamine antagonism also abolishes the effect of LTP-like neuroplasticity induced by high frequency stimulation in rat hippocampal slices (Frey, Schroeder, & Matthies, 1990). Dendritic spine connections are drastically reduced in schizophrenia mouse models, but are
normalized with D2 receptor blockade (Jia et al., 2013). Additionally, schizophrenia rodent models have an opposite pattern of neuroplasticity induction after high-frequency stimulation (i.e. LTD instead of the expected LTP), yet this reversal is restored after treatment with an antipsychotic medication (Belujon, Patton, & Grace, 2014). In non-psychiatric healthy subjects, dopamine administration modulates neuroplasticity induced by non-invasive brain stimulation techniques. For example, L-DOPA alters neuroplasticity in a dose-dependent manner using the PAS paradigm, whereby moderate doses facilitate LTP-like neuroplasticity yet low and high doses disrupt neuroplasticity induction (Thirugnanasambandam, Grundey, Paulus, & Nitsche, 2011). Another study using the tDCS paradigm showed that administration of sulpiride, an antipsychotic medication, abolished the facilitatory after-effects of tDCS, and D1 activation in the presence of the D2 blockade by sulpiride did not restore this effect (Nitsche et al., 2006). These results highlight the unique role of D2 receptors in neuroplasticity, which are hyperactive in schizophrenia. Since antipsychotic medications block D2 receptor activity, neuroplasticity may vary depending on the medication status of patients with schizophrenia.

In patients with schizophrenia, alteration of GABA neurotransmission by antipsychotics is a well-known phenomenon. Clozapine, an atypical antipsychotic, lengthens the CSP (Daskalakis, Christensen, Fitzgerald, Moller, et al., 2008; Liu, Fitzgerald, Daigle, Chen, & Daskalakis, 2009). Although GABA neurotransmission is an important modulator of neuroplasticity (Couey et al., 2007; Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Jones, 1993), in this study only two individuals of eleven were on clozapine, reducing the likelihood of a significant confounding effect of this particular medication on our results. However, since this study utilized a cross over design, variability in response to varenicline
versus placebo is likely a true effect of the study medication as all patients were required to be on stable doses of their antipsychotic medication for at least one month prior to participation.

Although the effect of antipsychotic medication on response to induced LTP-like neuroplasticity between testing days may be ruled out, deficits in GABAergic neurotransmission (Chen et al., 2014; Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Fitzgerald et al., 2003; Liu et al., 2009; Strube et al., 2014; Takahashi et al., 2013; Tse et al., 2014; Volk & Lewis, 2002; Wobrock et al., 2008; Wobrock et al., 2009) may also contribute to deficits in neuroplasticity seen during the placebo week in patients with schizophrenia. Ethanol disrupts and impairs synaptic plasticity via non-invasive brain stimulation techniques (Zorumski, Mennerick, & Izumi, 2014), suggesting a modulatory role of GABA on inhibitory and facilitatory synaptic plasticity. However, medication naïve patients with schizophrenia similarly display deficits in cortical inhibition (Hasan et al., 2012), suggesting that impaired GABAergic neurotransmission may be inherent to the illness rather than reliant on antipsychotic medication.

4.1.5 Varenicline: Proposed Mechanisms of Action and Therapeutic Implications

In this study, we found a more specific effect of varenicline in patients with schizophrenia, while in healthy subjects varenicline only enhanced performance on the 1-back in low performers. In schizophrenia, varenicline enhanced change in performance on the 1-back in low performers, and normalized deficits in LTP-like neuroplasticity to levels seen in the healthy subject placebo group. As this is the first study to examine the effect of varenicline on LTP-like neuroplasticity and working memory in non-smokers with
schizophrenia, our interpretation of results depends on our interpretation of similar studies using different populations and medications.

We originally hypothesized that working memory performance and LTP would be related. This assumption posits that the mechanisms underlying working memory and LTP are similar, based on the notion that LTP is the neurophysiological basis of learning and memory. From this, we expected that varenicline would similarly enhance working memory and LTP-like neuroplasticity deficits. Since we only found varenicline to affect the change score of low performers on the N-back task, we are led to believe that the actions of varenicline on working memory and LTP differ, or that working memory and LTP mechanisms differ between low and high performers. However, since this study is very preliminary, we are not at liberty to conclude whether the mechanisms of varenicline differ on LTP and working memory until future studies with larger sample sizes are done. Importantly, we are able to draw similarities between the effects of nicotine and varenicline on cognitive performance. As mentioned in the above discussion on working memory, nicotine preferentially enhances performance in low performers with and without schizophrenia (Larrison-Faucher et al., 2004; Petrovsky et al., 2012). This stratification in performance could be a result of true performance impairment or it could be due to differences in attention. If these differences are in attention, then our results are similar to many studies suggesting a beneficial effect on varenicline on attention in schizophrenia (Hong et al., 2011; Roh et al., 2014; Smith et al., 2009). However, if varenicline does have an effect on attention, we might expect subjects to differ in attention during the 30-minute PAS protocol, measured by their final count of how many pulses they received. We did not find
any such difference between groups or between medication conditions, further complicating our interpretation of these results.

The after-effects of PAS are dependent on NMDA receptor mechanisms and Ca\(^{2+}\) flux (Stefan et al., 2002; Wolters et al., 2003). Since \(\alpha_4\beta_2\) receptors are ligand-gated ion channels, they may influence synaptic plasticity by modulating Ca\(^{2+}\) flux or the resting membrane potential (Lisman, 2001). Studies show that nicotine enhances synaptic plasticity through activation of the \(\alpha_7\) receptor (Griguoli, Cellot, & Cherubini, 2013; Welsby, Rowan, & Anwyl, 2006), yet other studies have determined both the \(\alpha_4\beta_2\) and the \(\alpha_7\) receptor to be necessary for the induction of LTP (Matsuyama & Matsumoto, 2003). Although non-significant, the direction of change in peak LTP is opposite in patients and healthy subjects with varenicline pretreatment. This does not discount the role of nAChRs in neuroplasticity, due to the non-linear relationship of Ca\(^{2+}\) with synaptic plasticity. High levels of Ca\(^{2+}\) entering the cell activates pathways involved in the induction of LTP, while low levels activate pathways involved in LTD (Lisman, 2001). With this in mind, varenicline may act differently in patients and healthy subjects. Varenicline may induce more of a Ca\(^{2+}\) mediated inward current in patients with schizophrenia, resulting in an enhancement of LTP, while in healthy subjects this Ca\(^{2+}\) current may be too low to induce LTP. The differences between patients and healthy subjects may therefore lie in the state of nAChRs on the postsynaptic cell membrane. In healthy non-smokers with normal nAChR function, varenicline may have antagonistic properties that would reduce the amount of postsynaptic Ca\(^{2+}\) influx. The effects of varenicline on working memory and LTP like neuroplasticity may therefore fall on an inverted U-shaped curve, whereby varenicline alters performance and LTP in patients toward the top of the curve while either having no effect or altering these outcomes towards the
bottom of the curve in healthy subjects. Although very few studies exist on varenicline in healthy non-smokers, some research shows minimal influence of varenicline on cognition this population (Roh et al., 2014) supporting our finding that varenicline does not alter LTP-like neuroplasticity in healthy non-smokers.

In contrast, varenicline did significantly enhance peak LTP in schizophrenia, which is in support of prior studies showing an enhancement of cognition with varenicline treatment in non-smokers with schizophrenia (Hong et al., 2011). Together, this research suggests that deficits in neuroplasticity and nAChR function, not density, may be a core feature in schizophrenia regardless of smoking status. Because varenicline increased the peak MEP to levels seen in the healthy subject group during placebo, we can infer that varenicline may act to normalize deficits in LTP in the patient group. This may occur through stimulation of both α4β2 and α7 nAChRs, resulting in increased postsynaptic Ca^{2+} influx that activates CaMKII and other second messengers involved in the upregulation of AMPA receptors to the cell surface (as discussed in section 1.3.1). Another likely and perhaps concomitant mechanism that is congruent with the results of this study is that varenicline stimulates α4β2 nAChRs found on presynaptic neurons in the cortex, which enhances the release of glutamate into the synaptic cleft (Dickinson et al., 2008). Increased glutamate release into the synaptic cleft then increases the likelihood of subsequent depolarization of the postsynaptic neuron, potentiating synaptic plasticity.

Although the interaction between varenicline and other neurotransmitters cannot be ruled out, there is very little direct evidence supporting the modulation of GABA or dopamine by varenicline. However, there are some studies showing that varenicline may be a successful treatment for alcohol dependence (Fucito et al., 2011; McKee et al., 2009;
Nocente et al., 2013), perhaps due to interactions between GABAergic receptors and nAChRs. Both nicotine and alcohol stimulate α4β2 nAChRs in the VTA, leading to increased dopamine release in the nucleus accumbens/PFC pathway (Nocente et al., 2013). It is possible that varenicline, acting on α4β2 nAChRs located broadly throughout the brain, may activate these receptors on GABAergic and dopaminergic neurons in addition to glutamatergic neurons. As discussed in the above sections, GABA and dopamine modulate neuroplasticity. Thus, although the likely mechanism of varenicline in this study is through modulation of nAChRs on glutamatergic neurons, the contribution of other neurotransmitter systems cannot be ruled out. Additionally, it is possible that varenicline interacts with antipsychotic medications and that this could be a potential confound for this study. However, no studies have evaluated whether this interaction exists, so these results must be interpreted with caution.

Our results suggest that varenicline may be an effective treatment option for neuroplasticity deficits in schizophrenia, particularly low performers, although replication studies with a larger sample size will be needed to determine the effects of varenicline on absolute working memory performance and LTP like neuroplasticity. As neuroplasticity is the basis of learning and memory, varenicline may not only alter neuroplasticity, but could improve downstream deficits in cognition. Since deficits in neuroplasticity and in global cognition seem to be irrespective of smoking status in schizophrenia, the continued use of varenicline for smoking cessation and for cognitive improvement in smokers with schizophrenia as well as in non-smokers with schizophrenia is warranted. However, to achieve the most robust benefit of varenicline, it may be advisable to treat only the low cognitive performers.
Varenicline is relatively selective for α4β2 nAChRs; yet since it is a partial agonist, we are not likely to observe identical therapeutic effects compared with the full agonist effect of nicotine. Dysregulation of α4β2 nAChRs is a well-documented phenomenon in schizophrenia (Breese et al., 2000; Esterlis et al., 2014; Freedman et al., 1995) that is heavily involved in cognitive functioning (Grottick & Higgins, 2000; Papke, Webster, Lippiello, Bencherif, & Francis, 2000), and is not an after-effect of smoking (Breese et al., 2000). Varenicline offers a potential therapeutic avenue for reversing or improving α4β2-related cognitive impairment, potentially through the modulation of neuroplasticity as shown in this study.

In summary, we propose that varenicline primarily acts on presynaptic α4β2 nAChRs to increase glutamate release into the synaptic cleft, thereby enhancing synaptic plasticity. We propose that the differential effects of varenicline on neuroplasticity in patients compared to healthy subjects are due to pre-existing dysfunction in various neurotransmitter systems that are important for the modulation of neuroplasticity, and that are affected by varenicline. Additionally, varenicline may target nAChRs found on GABAergic neurons, and in schizophrenia, this activation may ameliorate pre-existing reduced GABAergic inhibition (Daskalakis et al., 2002), thus strengthening GABAergic tone to modify deficits in neuroplasticity in conjunction with enhanced presynaptic glutamate release.

4.2 Limitations

This study should be interpreted in the context of several limitations. While we carefully age and sex matched our sample, there were differences in IQ between the patient and healthy subject groups. While this is an expected difference, the IQ is slightly higher
than in the average general and schizophrenia population. As mentioned in section 4.1, the study sample may comprise a subset of patients with higher motivation and cognitive functioning, which possibly contributed to the relatively high 3-back scores seen in the patient group. Additionally, after removing outliers, only 11 healthy subjects and 10 patients were eligible for working memory analysis, and 10 healthy subjects and 9 patients were eligible for PAS analysis. Although this is a very small sample size, we were able to find significant results based on peak potentiation and working memory change scores. With a larger sample size, this study may have been able to detect differences between patients and healthy subjects using the rANOVA across all time points, as is seen in studies with more than 20 participants in each group (Batsikadze et al., 2014). The cross over study design has several advantages and disadvantages in this study. This design allows us to increase power and reduce variability by examining both the within-subjects and between-subjects effect of varenicline. However, the main issue with this design is the potential for carry-over effects, which we effectively controlled for by using a washout period of two weeks. As 5 doses of varenicline are eliminated from the body in approximately 60-72 hours (5 half-lives of varenicline, approximately 12-18 hours), a two-week washout period should have been more than sufficient to achieve this goal.

Varenicline was administered using a dosing regime that has proven effective in smokers with schizophrenia (Wing et al., 2013). Although this 5-dose schedule allows varenicline to reach steady state levels (Faessel, Gibbs, et al., 2006), it is not reflective of clinical practice. Additionally, we did not test for varenicline kinetics at the end of the testing days and were solely reliant on participants’ self-reported compliance.
Some researchers have advocated for selecting participants based on scoring a certain minimum on a cognitive task before continuing with the study (Honey, Bullmore, & Sharma, 2002). This way, the variability between subjects is reduced, and performance stratification is not necessary to see an effect. In the present study, we did not preselect subjects with higher working memory performance nor did we select PAS responders for the purpose of having generalizable results that can apply to the broader schizophrenia population. However, we did decide to train subjects on the N-back task prior to both testing days (baseline) in order to minimize practice effects. We also chose not to test the PAS protocol at baseline in order to reduce the number of testing sessions and thus potential carry over effects, and also because PAS does not require any cognitive demand that would be susceptible to training effects on the part of the subject. However, our lack of baseline PAS may be viewed as a potential limitation, as it is possible that being randomized to receive varenicline on the first testing day may prime the motor cortex for potentiation during the second testing day (although these effects should be minimized with a two week washout and a cross over design).

Another potential limitation of our PAS protocol was that we did not use the N-20 latency as our interstimulus interval (ISI). The N-20 latency provides a more tailored ISI; it refers to the amount of time it takes a peripheral nerve stimulus to reach the somatosensory cortex. This latency can vary based on height, skin thickness, or other factors (Stefan et al., 2000). Although our chosen ISI of 25 milliseconds is a common and effective interval, it is possible that using the N-20 latency may have provided different results.

4.3 Conclusions
To our knowledge, this study was the first to investigate the effects of varenicline on LTP-like neuroplasticity and working memory in non-smokers with schizophrenia. We were able to replicate deficits in both working memory performance (Barr et al., 2013; Jacobsen et al., 2004) and in LTP-like neuroplasticity in patients with schizophrenia (Frantseva et al., 2008), while showing that varenicline may selectively improve working memory performance in schizophrenia low performers and normalize deficits in LTP-like neuroplasticity to levels seen in healthy subjects. Since nAChRs are heavily involved in cognition, the low performers in our study may constitute a distinct subpopulation of schizophrenia, and could benefit from varenicline due to more severe deficits in nAChRs that are not seen in the high performers with schizophrenia. However, this study was conducted in a relatively small sample size with a number of significant outliers. In order to establish the maximal effect of varenicline on working memory and LTP-like neuroplasticity in schizophrenia, a larger sample size is needed to reduce variability.

4.4 Future Directions

Since this is the first study to establish a beneficial effect of varenicline on LTP-like neuroplasticity and change in working memory in schizophrenia non-smokers, we expect future studies to address the limitations we expressed above, including expanding the sample size and studying unmedicated patients with schizophrenia. Most importantly, however, this research needs to be conducted in smokers with schizophrenia. We decided to start with non-smokers to eliminate any cognitive and behavioural confounds associated with nicotine and nicotine withdrawal and to establish a core deficit in schizophrenia that can be inferred to be irrespective of smoking status. However, varenicline is primarily a smoking cessation medication, and thus has the potential to ease the smoking cessation process for smokers with
schizophrenia while reducing abstinence-induced cognitive impairment. One potential mechanism of varenicline, as elucidated in this study, is that it may improve cognition through remedying well-known deficits in neuroplasticity in schizophrenia. While we established that varenicline might be useful for non-smokers with schizophrenia, future studies need to first establish whether deficits in neuroplasticity are present in smokers with schizophrenia. It is possible that through smoking, patients with schizophrenia normalize deficits in neuroplasticity, and thus LTP-like neuroplasticity in smokers with schizophrenia might look similar to healthy individuals. Alternatively, smokers with schizophrenia might represent a subpopulation with more severe premorbid deficits in neuroplasticity that increases their vulnerability to becoming nicotine dependent. These questions can be addressed by assessing LTP-like neuroplasticity during smoking satiation, abstinence, and reinstatement, in order to first determine the state of LTP-like neuroplasticity during regular smoking, followed by the effects of nicotine withdrawal on neuroplasticity, and finally whether reinstatement with nicotine reverses abstinence-induced impairments in neuroplasticity.

Additionally, to further elucidate the mechanism of action of varenicline in this study, future research should co-administer varenicline with GABA/glutamate agonists or antagonists to a similar population of non-smokers to determine the effects on LTP-like neuroplasticity. If NMDA antagonism through glutamatergic stimulants alters LTP-like neuroplasticity in the presence of varenicline, then we may be able to reliably infer that varenicline acts through nAChRs located on glutamatergic neurons, and likewise with GABAergic stimulation. However, since many neurotransmitter systems are involved in neuroplasticity, it may be difficult to induce direct contributions of select neurotransmitters
without combining these types of studies with imaging techniques. These are all important questions that may allow researchers to uncover and treat the neurophysiological basis of both cognitive impairment and nicotine dependence in schizophrenia.


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Appendix A: Informed Consent Form

Effects of Varenicline on Cortical Neuroplasticity and Working Memory in Patients with Schizophrenia and Healthy Controls

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Summary of the Study:
You are asked to give consent to participate in a research study using Transcranial Magnetic Stimulation (TMS) combined with electroencephalography (EEG) and varenicline tartrate (Champix®) challenge to see if varenicline affects how the brain works. The idea is that varenicline may transiently improve the brain function of patients with schizophrenia and lead to better attention and memory. Normally, Varenicline is used as a smoking cessation aid in cigarette smokers, but we are using it here to understand its potential cognitive enhancing effects.

Who is being asked to take part in this research study?
You have been asked to participate in this study because you do not smoke cigarettes and have a diagnosis of schizophrenia or schizoaffective disorder. We expect to enroll 17 subjects with schizophrenia and 17 without schizophrenia.

What procedures will be performed for research purposes?
There are two parts to the study. The second part of the study will only occur after the first part has been completed, and will be separately consented if you wish to participate in part 2. For the purposes of the present study, part 1 will be explained and consented in this document. The first part entails 5 visits and the second 4 visits:

Part 1:
The first visit will involve an interview to assess your psychiatric status. You will also be taking some tests to measure your intellectual level (cognitive function). You will be asked to provide a urine test for drugs, a blood sample (14 mL in total) for markers of brain health, and a breath test to confirm smoking status. This visit will take about 3 hours.

The second visit will take about 3 hours. During this visit you will practice the cognitive test, and will have a baseline TMS session.

During this visit you will also be given 4 doses of varenicline or placebo (sugar pill) and will be instructed to start taking these pills two days before you come back for the third
visit. On the morning of the third visit, you will be given one more dose of the same medication to take in the lab. These pills should be taken at 9 am and 6 pm of each day together with food.

The third visit will include approximately 4 hours of tests to measure attention, memory and brain functioning using TMS, electroencephalography (EEG) and stimulation of the nerve of your hand. We will ask for a blood sample from you during this visit to analyze markers of brain health and nicotine levels.

On the fourth visit, three days before the fifth visit, you will pick up the study medication.

The fifth visit will be two weeks after the third visit and will be identical to the third visit.

The following methods will be used in the study:

- To record your brain activity using EEG, a cap will be placed over your head to record the electrical activity of the brain through the skull.

- TMS involves the use of an 8-shaped magnetic coil of about 10 x 5 cm in size. The coil will be placed over the skull for periods not exceeding 30 minutes at a time. During these periods the coil generates weak electric currents in the outer layer of your brain.

- We will also measure the activity of the muscles in the thumb using 2 soft electrodes on your right thumb.

- While the cap is on the skull, we will stimulate the brain using TMS. During one of these periods, we will also stimulate the nerve in your hand by placing electrodes on your skin, over the wrist area.

- We will measure your attention and memory using a computerized test.

What are the possible risks, side effects, and discomforts of this research study?

Varenicline tartrate (Champix®):

Varenicline (Champix®) is approved as a quit smoking treatment in adults in combination with quit smoking counseling. It is a drug that prevents nicotine from producing its effects in the brain, such as feeling pleasure after smoking a cigarette.

Common side effects from the study medication varenicline are: Nausea (30% of people experience this), constipation, gas, and vomiting. You may have trouble sleeping, or have vivid, unusual, or strange dreams while taking varenicline. You should use caution while driving or operating machinery until you know how varenicline may affect you.
There have been rare reports of serious neuropsychiatric symptoms with Champix® (varenicline), including depressed mood, agitation, hostility, changes in behavior, suicidal ideation and suicide, as well as worsening of pre-existing psychiatric illnesses (previously diagnosed or not) such as psychosis or hypomania during clinical treatment for smoking cessation (e.g. with 1 or more weeks of treatment). While such side effects are possible with even just a few doses of the varenicline study medication as is planned in this study, Dr. Tony George (the study’s principal investigator) thinks this is less likely, and we will have daily contact with you to monitor you closely for the development of these symptoms.

If either you or your family notice agitation, severe depressed mood, or if you have suicidal or homicidal thoughts or actions, stop taking the study medication and call Dr. Tony George immediately using the emergency wallet card number.

Transcranial Magnetic Stimulation (TMS):

There may be some mild side effects with TMS. At certain positions on the head, the stimulation may cause the eyes to blink, or can result in 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 within 24 hours. Acetaminophen (Tylenol) is effective in treating these side effects. If you have further concerns you may contact the study doctor at any time. The magnetic stimulator makes a clicking noise when it stimulates. If you find this distracting, you will be provided with ear-plugs which mute the sound. Magnetic brain stimulation has been used on thousands of individuals in the United States, Canada and Europe in the last several years and is usually well tolerated. However, in a few cases magnetic brain stimulation has caused brief epileptic seizures in patients with stroke and epilepsy, out of thousands of people tested. This has never happened with the form of TMS used in this study. 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 this study is very low.

The peripheral nerve stimulation causes a twitch of the hand muscles. The effect of the stimulation is similar to a light pinch and should be annoying, but not painful. There are no known complications from stimulating peripheral nerves in this fashion.

1. Risks associated with having blood drawn include bruising, swelling, or infection at the site where the needle is inserted, and lightheadedness or feeling faint. If you feel faint, notify study staff. If you must stand up, please do so slowly. Precautions will be taken to avoid these difficulties.

The study physician will be on site at all times during the TMS/EEG procedure and will promptly assess and treat any side effects you may experience.

What can I not do when I am in the study?
Don’t wear metals or electrical devices during testing
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.*

You cannot have any metals in your body
Exposure to magnetic stimulation (as with TMS) or any strong magnetic field (as with the MRI) 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.*

Don’t participate in the study if you are pregnant or become pregnant during the study.
The risks of exposure to magnetic fields and varenicline during pregnancy are unknown. Therefore, until more is known we will limit the study to women who are not pregnant and are using effective means of birth control. These include hormonal methods (pill, injection, vaginal ring), male or female condoms, injectable contraceptives, intrauterine devices or abstinence. You will also be asked to give a urine pregnancy test that has to be negative in order for you to enter the study. *Please inform the investigator if you are pregnant or if you become pregnant during the study.*

What are possible benefits from taking part in this study?
There are no direct benefits to you from participation in this study. This research study is designed to help us in understanding brain function and the effects of smoking in schizophrenia.

What treatments or procedures are available if I decide not to take part in this research study?
If you decide not to take part in this research study, you may obtain cognitive (thinking and memory) testing outside of this study, if your current treating physician feels it is indicated.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?
You will be promptly notified if, during the course of this research study, any new information develops which may cause you to change your mind about continuing to participate.

Who will pay if I am injured as a result of taking part in this study?
Centre for Addiction and Mental Health researchers and their associates who provide services recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator, Dr. George, at (416) 535-8501 extension 4544, or Dr. Daskalakis at (416) 535-
Will personal information about me be kept confidential?
The research records will be kept confidential to the extent permitted by law. In accordance with Health Canada requirements, CAMH will maintain archived study records for 25 years. However, those documents that contain personal identifiers (i.e. consent forms) will be stored separately from data files.

The information from this study may be published in scientific journals or presented at scientific meetings but the patient’s identity will be kept strictly confidential, and no personal information will be made public.

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.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Will this research study involve the use or disclosure of my identifiable medical information?
This research study will involve the recording of current and future identifiable medical information from your hospital or other (e.g., physician, psychologist or pharmacy) records. The information that will be recorded will be limited to information concerning your demographics (such as your age, date of birth, education, occupation, religion, race, and marital status).

We will also record information about your mental health that we are unable to obtain during your interviews. In addition, we will record information about your physical health. This is done in order to assure the study quality and to make sure that you are safe to partake in the study. Your research record will include a copy of your signed consent form, cognitive testing results, progress notes and results from your laboratory tests.

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this consent form and their research staff, the following individuals will or may have access to identifiable information for the purpose of monitoring the appropriate conduct of this research study: Authorized representatives of CAMH and the Ethics Review Board Office may review your identifiable research information for the purpose of monitoring the appropriate conduct of this research study.
As part of the Research Services Quality Assurance role, studies may be audited by the Manager of Quality Assurance. Your research records and CAMH records may be reviewed, during which confidentiality will be maintained as per CAMH policies and to the extent permitted by law.

**Is my participation voluntary? What happens if I no longer wish to take part in the study?**
Your participation in this study is entirely voluntary. You may decide not to participate or you may withdraw at any time without penalty or loss of benefits to which you may otherwise be entitled. This will have no effect on your current or future relationship with the Centre/hospital or affiliated care provider. If you agree too, any research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

**Can I be excluded from the study?**
You understand that you may be removed from the study at any time if in the judgment of the investigators you are unable to follow the study requirements, such as attending study clinic appointments, or have a medical complication that would make it unsafe or difficult for you to continue participating in the study.

**Costs**
There will be no costs to you as a result of your participation in this study. Reimbursement will be provided for costs of participation (parking fees, travel, TTC tickets, etc.) and for your time, prorated to $15/hour.

**Conclusion**
If you agree to take part in the study, please sign this Informed Consent Form. Thank you in advance for considering this study. The study staff will be more than happy to answer any questions about this research. Their contact details are given below:

Please contact Dr. Mera Barr (Co-investigator) at (416) 535-8501, extension 33095 at any time if you have questions or concerns about this study, or if you decide to withdraw from this research.

For questions about your rights as a research participant, please contact Dr. Padraig Darby Chair, Research Ethics Board, at the Centre for Addiction and Mental Health at (416) 535-8501, extension 36876.

You may be invited to participate in more than one research study in the schizophrenia program. Very often the researchers use the same assessments as the ones in the study you are considering. To avoid repeating the same assessments and to reduce your time commitment, the researchers may share the results of common assessments completed within the past 3 months. Sharing results will be limited to research studies in the Schizophrenia Program which have research ethics review and approval. Results will only be shared if you
consent to participate in another study. You may indicate your decision to agree or decline sharing the results of the assessments by checking your choice below.

If you decline sharing information, you can still consent to study participation.

I agree  I decline

Signature Section:
Signing does not release investigators from liability for negligence.

CONSENT: My signature below indicates:

- I have read the information provided above.
- The study procedures, purpose, risks and benefits have been explained to me.
- I have been assured that confidentiality will be maintained.
- I have a right to withdraw from the study.
- I understand that unexpected abnormal findings arising from my participation in the study will be communicated to my treating physician.
- I have been given the opportunity to ask questions and all of my questions have been answered to my satisfaction.
- I have been given a copy of this form.
- By signing this form, I consent to participate in the research as described.

Name of Subject  Signature  Date & Time Signed

I personally explained the research to the subject and answered all of his/her questions. I believe that s/he understands the information described in this document and freely consents to participate.

Name of Person  Signature  Date & Time Signed

Obtaining Informed Consent (Print)
Appendix B: Study Recruitment Flyer

Research Participants Needed
Varenicline, Brain activity and Cognition in Patients with Schizophrenia Study

We are seeking participants that are

- Between the ages of 18 to 55
- Having a diagnosis of schizophrenia
- Are not smoking tobacco
- Having no substance use problems
- Are willing to take varenicline (Champix®) for three days

The study is evaluating the effects of the quit-smoking drug varenicline (Champix®) on brain activity and cognition in people with schizophrenia. Participants will receive varenicline (Champix®) or placebo (sugar pill) and cognition will be measured using transcranial magnetic stimulation, electroencephalography (EEG) and a computerized cognitive test. Research assessments will be conducted prior to and during treatment.

Reimbursement will be provided for participation in the study.

To learn more about this study, please contact:
416-535-8501 x30462 or alanna.bridgman@camh.ca

The Centre provides other treatment options For mental illness and addiction. For more information about programs and services at CAMH, please visit www.camh.ca or call 416-535-8501 (or 1-800-463-6273).
Appendix C: Telephone Screen

<table>
<thead>
<tr>
<th>Screen ID:</th>
<th>Screen date:</th>
<th>Interviewer’s Initials</th>
<th>Eligible</th>
<th>Y</th>
<th>N</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name (first/last):</th>
<th>Phone # H/W/C:</th>
<th>Referral Source:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Age: _ (18 – 55)</th>
<th>DOB (yyyy/mm/dd):</th>
<th>Gender: ☐M ☐F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handedness: ☐L ☐R (exclude left handed)</td>
<td>VM ok?</td>
<td>☐No ☐Yes</td>
</tr>
</tbody>
</table>

Do you have health insurance? ☐ No ☐ Yes

**SMOKING:**
Do you smoke tobacco? ☐ No ☐ Yes (exclude)

Have you ever smoked tobacco? ☐ No ☐ Yes, When: ________________________________

(Exclude if a regular smoker less than a year ago)

**STUDY SPECIFIC:**
Have you ever used any medication for smoking cessation? ☐ No ☐ Yes,

Which one? ________________________________

Side Effects? ________________________________

Have you ever participated in a research study that involved taking medication? ☐ No ☐ Yes,

How long ago? ________________________________

(Exclude if < 3 months)

Have you ever participated in a study that involves TMS? ☐ No ☐ Yes (exclude if < 1 m)

Are you pregnant or think you might be? ☐ No ☐ Yes (exclude if yes)

If NO: Are you willing to use contraception for the study duration? ☐ No ☐ Yes (exclude if yes)

Are you hypersensitive to Varenicline? ☐ No ☐ Yes (exclude if yes)

Are you currently breastfeeding? ☐ No ☐ Yes (exclude if yes)

Have you recently had a heart attack? ☐ No ☐ Yes (exclude if yes)

Do you have a history of hepatic or renal failure? ☐ No ☐ Yes (exclude if yes)

Do you have/have had a serious cardiovascular disease? ☐ No ☐ Yes (exclude if yes)

**PSYCHIATRIC HISTORY:** (Any current Axis I diagnosis is exclusionary)

Have you ever seen a psychiatrist/social worker/psychologist for emotional or mental health problems? ☐ N ☐ Y

Details: ________________________________

If a dx of SCZ, are you an inpatient? ☐ No ☐ Yes,

Details: ________________________________

Who is your psychiatrist? ________________________________

What medications are you currently taking? ☐ No ☐ Yes,

Details: ________________________________
Have you ever had/currently have any problems with depression or anxiety? ☐ No   ☐ Yes, Details: ____________________________________________________________

Are you experiencing medical problems at this time? ☐ No   ☐ Yes, Details: ____________________________________________________________

CURRENT/PAST SUBSTANCE USE:

Alcohol: …………… Current: ☐ Y ☐ N    #/week: _____  Past: ☒ Y ☐ N  Last time: ______
Marijuana: …………. Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______
Cocaine: …………… Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______
Opiates: …………… Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______
Methadone: …………. Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______
Pills: ………………… Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______
Other: ………………… Current: ☐ Y (exclude) ☐ N  Past: ☒ Y ☐ N  Last time: ______

NEUROLOGICAL HISTORY:

Have you ever had a traumatic brain injury/concussion? ☒ No   ☐ Yes, Details: ____________________________________________________________

Have you ever had a seizure? ☐ No   ☐ Yes, Details: ____________________________________________________________

Consent to contact for participation in future studies or add to CAMH research registry: ☐ No   ☐ Yes

Eligibility: ☐ No   ☐ Yes   ☐ Yes, possibly (inform of urine drug screen and CO test upon arrival)

Availability: ____________________________________________________________

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

If Yes:

Date: __________________________

Time: __________________________

RA: ____________________________

☐ Fill out Participant Screening Log