The Effects of Varenicline on Neurocognitive Function in Non-Smokers with Schizophrenia

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Sciences
University of Toronto

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**Background:** Effective treatments for neurocognitive deficiencies among schizophrenia patients remain an unmet need. The nicotinic partial-agonist varenicline, approved for smoking cessation, has been shown to enhance neurocognition in schizophrenia, but its effects in non-smokers are unknown.

**Methods:** This study was a randomized, double-blind, placebo-controlled, cross-over design assessing effects of 3 different varenicline doses (0, 1, and 2mg/day) in non-smokers with schizophrenia (n=15) and non-psychiatric controls (n=15) on neurocognitive performance. Primary outcome assessed working memory, while secondary outcomes assessed other neurocognitive domains.

**Results:** Overall patients appeared to perform better on 1mg/day dose across visuospatial working-memory, verbal memory and executive function, while controls appeared to perform better on 2mg/day dose on these domains. Across time in the 2mg/day dose, patients demonstrated increased inattentiveness, whereas controls had greater executive function, attention, and decision-making performance. Varenicline was generally well-tolerated in both groups.

**Conclusions:** Varenicline may treat neurocognitive dysfunction in schizophrenia patients in a non-linear dose-related fashion.
Acknowledgements

This work is dedicated to my Babcia Ala and Dziadziu Władziu.

First I’d like to acknowledge all the participants without whom this research would not exist. They gave their time and genuine charisma to complete the study and I thank them greatly.

Secondly, I’d like to thank with the utmost sincerity, Dr. Tony P. George. He has provided me with this opportunity in the first place, along with so many others as his Master’s student. With his constant guidance, encouragement, and advice over these past two years, I have grown as an educated graduate. His continuous energy for me to develop as a scientist has driven my passion for research and mental health to a higher level. I hope I have made you proud.

I’d also like to thank Dr. Mera S. Barr for her continued support, expertise, as well as attention to detail. These have all translated into my own perfectionism for any presentation or exertion that I complete. You have always articulated your passion of research in a very intelligent and enthusiastic manner that has been very admirable to me. And of course, I will also miss our sporty conversations.

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Finally, I must express my gratitude to my sister, Kasia, for her continued support, cheer and love, to my brother, Krystian, who has always been a mentor for me in any science-related path I have endured, and to my mother and father, Mariola and Marek, for always believing in me.
Contributions

The study protocol from which this thesis was derived was developed by Dr. Tony George (PI), and Dr. Mera Barr, and was entitled “The Effects of Varenicline on Neurocognitive Function in Smokers with Schizophrenia”.

The candidate, Karolina Kozak, was responsible for recruitment, screening, and conduct of all study sessions, as well as conducting the Positive and Negative Syndrome Scale examinations on patients.

Emily Simpkin and Rachel Rabin were responsible for conducting the structured clinical interviews, and Emily Simpkin and Dr. George conducted physical examinations performed during the screening session.

Research Analysts Maryam Sharif-Razi and Marya Morozova trained the candidate on all neurocognitive measures, including working memory and pre-pulse inhibition.

The candidate, Karolina Kozak, conducted all data analyses, interpretation of research data, and drafting of the thesis. Dr. Mera Barr and Dr. Tony George assisted with analyses and interpretation of data.

The Program Advisory Committee (PAC) members, Dr. Rachel Tyndale and Dr. Martin Zack, assisted with the interpretation of the data.

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<td>Serotonin</td>
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<td>ACh</td>
<td>Acetylcholine</td>
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<td>AIMS</td>
<td>Abnormal Involuntary Movement Scale</td>
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<td>ANT</td>
<td>Attention Network Task</td>
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<td>ASR</td>
<td>Acoustic Startle Response</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BACDRL</td>
<td>Biobehavioural Addictions and Concurrent Disorders Research Laboratory</td>
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<tr>
<td>BARS</td>
<td>Barnes Akathisia Rating Scale</td>
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<td>BDI</td>
<td>Beck Depression Inventory</td>
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<tr>
<td>BID</td>
<td>Twice a Day</td>
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<tr>
<td>CAR</td>
<td>Continuous Abstinence Rates</td>
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<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CO</td>
<td>Carbon Monoxide</td>
</tr>
<tr>
<td>CPT</td>
<td>Continuous Performance Test</td>
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<tr>
<td>CPT-IP</td>
<td>CPT-Identical Pairs Version</td>
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<tr>
<td>CYPs</td>
<td>Cytochromes P450</td>
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<tr>
<td>CYP1A2</td>
<td>Cytochrome P450 1A2</td>
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<tr>
<td>D1</td>
<td>Dopamine Receptor 1</td>
</tr>
<tr>
<td>D2</td>
<td>Dopamine Receptor 2</td>
</tr>
<tr>
<td>dbA</td>
<td>A-Weighted Decibels</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>DHβE</td>
<td>Dihydro-Beta-Erythroidine</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DRD2</td>
<td>D2 Dopamine Receptor</td>
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<tr>
<td>DS</td>
<td>Digit Span</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders 4th edition-TR</td>
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<tr>
<td>DSM-V</td>
<td>Diagnostic and Statistical Manual of Mental Disorders 5th edition-TR</td>
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<tr>
<td>EDT</td>
<td>Experiential Delay Task</td>
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<tr>
<td>EKG</td>
<td>Electrocardiogram</td>
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<td>EOP</td>
<td>Endogenous Opioid Peptides</td>
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<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<td>hOCT</td>
<td>Human Organic Cation Transporter</td>
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<tr>
<td>HS</td>
<td>Healthy Subjects</td>
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<td>HVLT-R</td>
<td>Hopkins Verbal Memory Task-Revised</td>
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<tr>
<td>GABA</td>
<td>Gamma(\gamma)-aminobutyric Acid</td>
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<td>Glu</td>
<td>Glutamate</td>
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<tr>
<td>IGT</td>
<td>Iowa Gambling Task</td>
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<tr>
<td>IQ</td>
<td>Intelligent Quotient</td>
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<tr>
<td>KDDT</td>
<td>Kirby Delayed Discounting Task</td>
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<tr>
<td>MEC</td>
<td>Mecamylamine</td>
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<tr>
<td>MG</td>
<td>Milligram</td>
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<tr>
<td>MS</td>
<td>Millisecond</td>
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<tr>
<td>nAChR</td>
<td>Nicotinic Acetylcholine Receptor</td>
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NE  Norepinephrine
NRT  Nicotine Replacement Therapy
PANSS  Positive and Negative Syndrome Scale
PFC  Prefrontal Cortex
PI  Principal Investigator
PPI  Prepulse Inhibition
ppm  Parts Per Million
REB  Research Ethics Board
RM-ANOVA  Repeated Measures ANOVA
SAE  Serious Adverse Events
SCID  Structured Clinical Interview
SDR  Spatial Delay Recall
SEC  Second(s)
SNP  Single Nucleotide Polymorphism
SPSS  Statistical Package for the Social Sciences
TMT  Trail Making Test
TD  Tardive Dyskinesia
VSWM  Visuospatial Working Memory
VTA  Ventral Tegmental Area
WTAR  Wechsler Test of Adult Reading
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Chapter 1: Literature Review

1.1 Schizophrenia

1.1.1 Epidemiology, Etiology, and Pathophysiology

Schizophrenia is a severe neurodevelopmental disorder with annual incidence rates estimated to be 1% worldwide (Sutterland, Dieleman et al. 2013) and affecting all racial, ethnic, demographic and cultural lines (Correll, Penzner et al. 2007). However, with considerable heterogeneity in several of its core features, great variability exists in clinical presentation, disease course, psychological, and pharmacological treatments. In either sex, due to its particular age of onset in adulthood, this disease is devastating for individuals by interfering with their function in society, when achieving financial independence and launching their careers (Li, Hou et al. 2016). Traditionally, it has been accepted that schizophrenia is equally prevalent among males and females, however, the age of onset and first-episode psychosis appears to be a factor in presentation of symptoms (Hafner, Riecher-Rossler et al. 1993, Ochoa, Usall et al. 2012). Typical age of onset in men ranges between 15 and 25 years of age, while for women age of onset ranges between 25 to 35 years, commonly with two major peaks - first after menarche, and second once over 40 (Sutterland, Dieleman et al. 2013). The later onset could be explained by the estrogen hypothesis stating that female hormones in the developing brain protect its integrity and delay the expression of psychosis (Kulkarni, Riedel et al. 2001). Further, it has also been universally accepted that schizophrenia results from an interaction between environmental factors and genetic susceptibility (Sullivan, Kendler et al. 2003, Sutterland, Dieleman et al. 2013).
Many epidemiological studies have been conducted using classification schemes with a common consensus that multiple genes and environmental risk factors are involved that predisposition one to schizophrenia (Faraone, Kremen et al. 1995, Dealberto 2013). For example, direct observations of genetic factors in schizophrenia have demonstrated an association of fetal hypoxia, an environmental insult, as well as, decreased grey matter and increased cerebrospinal fluid (Fatemi and Folsom 2009). Further, gene expression alterations caused by epigenetic methods such as increased methylation on the D2 dopamine receptor gene, DRD2, may account for differences in schizophrenia as well (Petronis, Gottesman et al. 2003). However, the most significant evidence for a genetic component in this disorder results from twin studies with estimates of heritability as high as 80% (Tienari, Wynne et al. 2004). Moreover, genetic studies support the notion that this disorder begins before the onset of psychosis, expressing itself biologically in characteristic ways (Tsuang, Stone et al. 2000). This includes the dimensional view of “schizotaxia” which describes unexpressed genetic predispositions to schizophrenia (Meehl 1962), as well as, biological relatedness to a family member with schizophrenia, biological irregularities, and selected neuropsychological deficits (Helzer and Hudziak 2008). In addition, environmental factors including stressors such as drug use and social adversity in early adult life appear to modulate neurotransmitter function, and as such, progress to neurodevelopmentally impaired individuals (Howes, McDonald et al. 2004, van Os, Rutten et al. 2008). Nevertheless, despite these studies showing an association to schizophrenia, most of the heritability of this disorder remains unexplained given the complexity of their genetic and phenotypic relationships (Lee, DeCandia et al. 2012, Arnedo, Svrakic et al. 2015).
Additionally several studies have examined the role of neurotransmitters to explain the etiology and pathophysiology of schizophrenia. For example, the dopamine model suggests hypoactive dopamine transmission in the prefrontal cortex and hyperactive transmission in the mesolimbic areas (da Silva Alves, Figee et al. 2008). Dopamine belongs to the catecholamine family and is produced in the ventral tegmental regions and substantia nigra regions of the brain (Bogerts, Hantsch et al. 1983). Prominent in the central nervous system (CNS), dopamine receptors are a class of G protein-coupled receptors implicated in several neurological processes such as learning, memory and motor control, as well as neuroendocrine signaling (Girault and Greengard 2004). At least five subtypes of the dopamine receptors exist, however the most commonly studied in this disorder are D1 and D2 receptors of the D1-like and D2-like family, respectively (Vallone, Picetti et al. 2000). Coupling with their respective G proteins, D1 receptors, found exclusively postsynaptically on dopamine receptive cells, lead to the activation of adenylyl cyclase, a regulatory enzyme, resulting in increased levels of intracellular cyclic adenosine monophosphate (cAMP), a secondary messenger (Beaulieu and Gainetdinov 2011). Whereas D2 receptors, which are expressed both presynaptically on dopaminergic neurons and postsynaptically on dopamine target cells, prevent adenylyl cyclase enzyme activity, directly inhibiting the formation of cAMP (De Mei, Ramos et al. 2009). Originally it was proposed that hyperactivation of dopaminergic neurotransmission contribute to the prognosis schizophrenia (Yeragani, Tancer et al. 2010); however, hypoactivity of the prefrontal cortex and subcortical hyperactivity has gained the most recent support (Pogarell, Koch et al. 2012).
Recent research, however, has indicated that there are other anatomical anomalies and neurotransmitter systems that contribute to the pathophysiology of schizophrenia such as acetylcholine (ACh), glutamate (Glu), γ-aminobutyric acid (GABA) and serotonin (5-HT) (Brisch, Saniotis et al. 2014). Anatomical contributors include cortical grey matter volume reduction, specifically with right hemisphere atrophies, and decreased frontal and posterior volume on both sides, that may indicate abnormal synaptic plasticity occurring in this disorder (Harvey, Ron et al. 1993). As the brain matures, synaptic pruning results in the brain shedding weak or redundant connection between neurons. Thus researchers suggest that this activity primarily takes place in the prefrontal cortex (PFC) during adolescence and early adulthood and this process is accelerated in people who are at higher risk of developing schizophrenia (Sekar, Bialas et al. 2016).

Taken together, there is no single risk factor responsible for the existence of schizophrenia. Rather an integration of genetic, environmental, and neurotransmitter systems, contribute as fundamental factors in the epidemiology, etiology, and pathophysiology of this disorder. What is a distinct hallmark of schizophrenia, are the symptoms used in aiding diagnosis and taking preventative measures. These symptoms consist of positive, negative and neurocognitive deficiencies.

1.1.2 Symptoms

According to the Diagnostic and Statistical Manual of Mental Disorders fourth edition-TR (DSM-IV-TR), for an individual to meet criteria for a diagnosis of schizophrenia, one must have symptoms present for six months, including one month of active symptoms. These include delusions, hallucinations, disorganized speech, catatonic
behaviour and symptoms causing social dysfunction (Association 2000). As the disorder develops, progressive symptoms such as flattening or inappropriate affect may appear. The newest edition of the DSM, fifth edition, (DSM-V) requires an individual to exhibit at least two characteristic symptoms of schizophrenia raising the symptom threshold (Association 2013). Furthermore, subtypes of schizophrenia are no longer identified as too often patients present overlapping symptom subtypes, such that distinction among the five subtypes is blurred.

Typically patients with schizophrenia demonstrate three relatively independent categories of symptoms: positive, negative, and neurocognitive deficits. Several neuropsychological studies have demonstrated that this 3-axes model bears etiological, pharmacological and prognostic importance. Positive symptoms include delusions, hallucinations, conceptual disorganization, grandiosity, excitement, suspiciousness, and hostility (Kay, Fiszbein et al. 1987). Disturbed cortical pathways in the nucleus accumbens of mesolimbic pathway may attribute to the positive symptoms consequently from increased dopamine release via D2 receptor activation (Shen, Liao et al. 2012). Negative symptoms include blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, avolition, anhedonia, difficulty in abstract thinking, as well as lack of spontaneity and social drive. Dopamine function abnormalities in the mesocortical pathway may contribute to the negative symptoms resulting from reduced D1 receptor activation (Perez-Costas, Melendez-Ferro et al. 2010). Significant contributors to both positive and negative symptoms are known to be a result of serotonergic and dopaminergic deviations. Finally, compared to indices of positive and negative symptoms, neurocognitive deficits are the strongest predictors of functional
outcome in schizophrenia (Green, Nuechterlein et al. 2008). These deficits typically appear before the age of onset and present themselves in addition to disorganized symptoms, bizarre behaviour, inappropriate affect, and poor thinking and planning skills (Buchanan and Carpenter 1997). Contributing factors to neurocognitive deficiencies have strongly been associated with the dysregulation of the nicotinic acetylcholinergic system. Importantly, neurocognitive deficiencies are a much stronger predictor of functional outcome than psychotic symptoms. Thus understanding the functional and neuropharmacological role of nicotinic acetylcholine receptor system and key molecules such as acetylcholine (ACh) and nicotine may provide a better understanding of this co-morbid mental disorder and the substantial roles it has on neurocognitive performance and neurotransmission.

1.2 Nicotinic Acetylcholine Receptor System

1.2.1 Neuropharmacology of Nicotine, Tobacco and Nicotinic Acetylcholine Receptors

The function of several neurotransmitter systems including dopamine, norepinephrine (NE), 5-HT, Glu, GABA, endogenous opioid peptides (EOPs) and cannabinoids are altered by nicotine, the primary reinforcing component of tobacco (McGehee, Heath et al. 1995, Renda, Fecarotta et al. 2000, D'Souza and Markou 2011, Filbey, McQueeny et al. 2015). Playing critical neuromodulatory roles in the CNS, nicotinic acetylcholine receptors (nAChRs) are diverse members of the neurotransmitter-gated ion channel super-family (Picciotto 2003). nAChRs are located in thalamus, cerebral cortex, basal ganglia, hippocampus and cerebellum (Zoli, Pistillo et al. 2015).
There are 17 known nicotinic receptor subtypes consisting mainly of alpha (α2–α7), and beta (β2–β4) subunits, and to a lesser extent with gamma (γ), delta (δ), and epsilon (ε) subunits (Zouridakis, Zisimopoulou et al. 2009). Of the nAChRs, α3β4, α7, and α4β2 are the primary ones located in the brain. Responding to either its endogenous ligand acetylcholine, ACh, or its exogenous agonist, nicotine, activity of presynaptic nAChRs initiates both direct and indirect intracellular calcium signaling leading to increased neurotransmitter release (i.e., dopamine) (Hogg, Raggenbass et al. 2003). There are two general families of central nAChRs: 1) high-affinity–β2 subunit-containing nAChRs, existing in a heteropentameric combination of α3/4 and β2 subunits; and 2) low-affinity–α7 subunit-containing nAChRs, existing as homopentameric complexes (Picciotto, Caldarone et al. 2000, Dani and De Biasi 2001, Improgo, Scofield et al. 2010). High affinity receptors are sensitive to nicotinic antagonists such as mecamylamine (MEC), and are enriched on GABA interneurons controlling mesolimbic dopamine release and mesocorticolimbic dopamine neurons projecting to the nucleus accumbens and prefrontal cortex (Wooltorton, Pidoplichko et al. 2003). Low affinity receptors are sensitive to snake venom toxin α-bungarotoxin and the selective antagonist methyllycaconitine (MLA). They are found on hippocampal GABAergic neurons, which facilitate information processing and sensory integration, as well as on ventral tegmental area (VTA) Glu neurons that facilitate mesolimbic dopamine neuron firing (Leonard and Bertrand 2001, Dani and Bertrand 2007). Studies have found that central high-affinity nAChRs play an important role in nicotine reinforcement, clinical symptoms and some aspects of neurocognitive function (Dani and De Biasi 2001, George, Vescicchio et al.
2002), while there is a complementary role of low affinity nAChRs in information processing responses (Leonard and Bertrand 2001).

Of particular importance are the mesolimbic dopamine neurons that are profoundly involved with nAChRs and are involved with the reward pathways mediating reinforcing effects of nicotine. Nicotine, the primary addictive and reinforcing effect from cigarettes, has high affinity for β2-nAChRs. As evidenced by several preclinical, clinical and postmortem studies, chronic nicotine exposure from tobacco use and smoking, leads to significant upregulation of this receptor availability (Cosgrove, Batis et al. 2009). Briefly, chronic nicotine administration leads to significant desensitization such that the receptors remain in a stable closed state resulting in inactivation of nAChRs (Besson, Granon et al. 2007, Govind, Vezina et al. 2009, Grady, Wageman et al. 2012). This level of desensitization depends on the concentration of the agonist (i.e., nicotine) and the length of exposure, with deeper states of desensitization resulting from longer exposures and slower recovery (Grady, Wageman et al. 2012). The recovery period includes the paradoxical upregulation of the receptor sites resulting in functional nAChR antagonism (Knable and Weinberger 1997). These nAChRs are then resensitized after overnight abstinence, and become fully responsive to nicotine with the first cigarette of the day (Wing, Wass et al. 2012). Thus the first cigarette of the day produces immediate effects with acute tolerance and ultimately is a suitable indicator of nicotine sensitivity in smokers (Transdisciplinary Tobacco Use Research Center Tobacco, Baker et al. 2007). Thus it is not surprising that there is a higher prevalence of cigarette smoking in patients with mental health and addiction disorders such as schizophrenia. Interestingly, this
process of upregulation may be to a lesser extent in the schizophrenia population unlike otherwise healthy individuals. It is therefore clear that we need to examine the involvement nAChR systems vis-a-vis neurocognition that may help to understand the pathophysiology of schizophrenia with co-morbid tobacco dependence

1.2.2 Schizophrenia and Nicotinic Acetylcholine Receptor Function

Cholinergic neurotransmission involved in mediation of dopamine release via the mesolimbic pathway is dysregulated in schizophrenia (D'Souza and Markou 2012). Several studies have found reduced receptor regional availability of $\beta_2$–nAChR among post-mortem brains of smokers with schizophrenia in comparison to non-psychiatric smokers and nonsmokers, while there is similar upregulation among nonsmokers with and without schizophrenia (Breese, Lee et al. 2000, D'Souza, Esterlis et al. 2012). Thus the same extent of upregulation may not be present in smokers with schizophrenia as compared to the general population that may explain the higher prevalence and intensity of smoking in this disorder. The most commonly studied nAChRs in relation to schizophrenia and tobacco addiction are the $\alpha_4\beta_2$ and $\alpha_7$ nAChRs. Recently, one study found that smokers with schizophrenia showed an upregulation of the $\alpha_4\beta_2$ receptors in frontal and parietal cortices, and striatum regions of the brain compared to nonsmoking patients. This was also related with better executive functioning and reduced negative symptoms implicating the possible role of the upregulation nAChRs on symptoms of schizophrenia (Esterlis, Ranganathan et al. 2014). Furthermore, genetic studies have found that smoking severity in schizophrenia pertains to certain nAChR abnormalities.
For example, patients with schizophrenia have reduced levels of expression of α7 subunit gene (CHRNA7) polymorphisms in the prefrontal cortex (Mathew, Law et al. 2007).

Taken together, patients with schizophrenia show reduced upregulation of nAChRs, thus contributing to dysregulation of dopamine levels as well as other neurotransmitter systems (Glu and GABA) expressed in the brain (Wing, Wass et al. 2012). Disrupted cortical regions of nAChR availability in the brain may mediate relationship to neurocognitive deficits, negative symptoms, and high rates of tobacco use. This observation is further supported by the demonstration that neurocognitive deficits in schizophrenia improve by administration of nicotine, nicotinic agonists and cigarette smoking. It has been demonstrated from multiple studies that certain neurocognitive impairments associated with schizophrenia are less severe in smokers with the disorder than when compared to non-smokers (Wing, Bacher et al. 2011, Wing, Moss et al. 2012). Furthermore, neurocognitive enhancement appears to be mediated by nAChR stimulation (Sacco, Termine et al. 2005, George, Termine et al. 2006, Hong, Thaker et al. 2011), with nicotine administration from cigarette smoking having positive effects on neurocognition (Sacco, Bannon et al. 2004, Wing, Wass et al. 2012). Thus nAChR dysregulation may ultimately have important implications relating the higher smoking prevalence rates and neurocognitive deficits in this disorder.

1.3 Tobacco Use

1.3.1 Smoking and Schizophrenia

Smoking is the leading avoidable cause of mortality and causes a significant burden on disease outcome. Presently, schizophrenia is associated with a fivefold higher
rate of tobacco addiction (70-90%) relative to the general population (20%) (Li, Hou et al. 2016). A meta-analysis of 42 studies across 20 nations found an odds ratio of 5.9 for cigarette smoking occurrence in schizophrenia (de Leon and Diaz 2005). Studies have found that smokers with schizophrenia extract more nicotine from each cigarette, smoke more heavily (e.g., more puffs per cigarette), and have shorter interpuff intervals (Tidey, Rohsenow et al. 2005, Williams, Ziedonis et al. 2005). Correspondingly this heavy tobacco usage is associated with greater difficulty of quitting, leading to higher rates of smoking-related cardiovascular and pulmonary disease (Kelly, McMahon et al. 2011). As a result, persons with schizophrenia have a reduced life expectancy, with smoking being the leading cause of mortality (Dickerson, Origoni et al. 2016).

Many reasons are proposed accounting for the increased susceptibility of the schizophrenia population to nicotine dependence. One of the most universally studied hypotheses suggests that patient vulnerability to co-morbidity is centered on the putative dysregulation of nAChR systems (D'Souza, Esterlis et al. 2012). It suggests that patients with schizophrenia are less sensitive to the effects of nicotine due to reduced expression of high-affinity nAChR in the brain and compensate for this by increasing nicotine intake (Breese, Lee et al. 2000). An additional premise suggests that patients smoke to alleviate negative symptoms, neuroleptic side effects, and neurocognitive deficits (D'Souza and Markou 2012). With increasing evidence supporting the notion that stimulation of the nAChR has beneficial effects on neurocognitive function as well as auditory sensory motor gating, a better understanding of the individual memory domains is of great importance (George, Termine et al. 2006). Thus taking into consideration the high prevalence of smoking, dysregulated nAChR in the dorsolateral prefrontal cortex
(DLPFC), and associated neurocognitive deficits, a solid foundation for pharmacotherapeutic strategies connecting all three factors is promising.

1.4 Learning, Memory and Schizophrenia

1.4.1 Neurocognitive Dysfunctions in Schizophrenia

With neurocognitive dysfunctions having a substantial impact on functional outcomes in schizophrenia, an accumulation of research into understanding the framework of neurocognition in this disorder has occurred in the last three decades (Harvey, Howanitz et al. 1998, Green, Nuechterlein et al. 2004, Barch and Ceaser 2012). Impairments in prefrontal regions of the brain characterize dopamine dysregulation, which underlie spatial working memory deficits that are heavily studied in schizophrenia patients (Goldman-Rakic 1994, Goldman-Rakic, Castner et al. 2004). Further, neurocognitive deficits in schizophrenia not only shows high prevalence, but is relatively stable over time, and independent of psychotic symptoms (Gold 2004). Neurocognitive deficits are also suggested to be a biomarker of schizophrenia given their presence in healthy relatives of schizophrenia relatives (Barrantes-Vidal, Aguilera et al. 2007). Moreover, neurocognitive discrepancies affect several domains including: working memory, executive function, verbal memory, attention, inhibition, processing speed, episodic memory, and sensory processing (Barch and Ceaser 2012). As a consequence, disturbances in critical neurocognitive domains are regarded as a fundamental feature of schizophrenia and as such are now viewed as potential treatment targets for psychopharmacology (Hyman and Fenton 2003).
There is ample evidence associating neurocognitive deficits as a core feature of schizophrenia (Bora and Pantelis 2013). A core feature means that neurocognitive impairments are not simply the result of current treatments or other symptoms present in schizophrenia. Instead, these deficits are a fundamental aspect of the illness itself, as otherwise it would make little sense to target neurocognition separately. Various aspects of schizophrenia support this premise. First, many patients present clear neurocognitive impairments before the onset of illness or any other clinical features of schizophrenia, (Jahshan, Heaton et al. 2010). Second, a pattern of similar neurocognitive deficits is demonstrated in a subgroup of the first-degree relatives of schizophrenia patients, proposing that certain neurocognitive impairments are a result of genetic vulnerability to this disease (Asarnow, Nuechterlein et al. 2002). Single nucleotide polymorphisms (SNPs) in the most common gene associated as a heritability site for schizophrenia (CHRNA7 gene) have also been linked to schizophrenia and deficits in sensory gating (Freedman, Coon et al. 1997). Third, although variable from patient to patient, the types of neurocognitive dysfunctions present in schizophrenia, whether in recent-onset or chronic, tends to fit a typical profile that differs from patterns of neurocognitive deficits present in bipolar disorder (Kahn and Keefe 2013), dementia and depression (Ting, Rajji et al. 2010). Finally, the discrepancy of both first- and second-generation antipsychotic treatments having small neurocognitive effects (with the latter showing advantages) and marked clinical effects on psychotic symptoms, suggests these treatments act on different neural systems than those underlying neurocognitive deficits (Wang, Hu et al. 2013).

A second premise centers on the neurobiology of schizophrenia and the critical role for impaired connectivity between brain regions that pronounce deficiencies in the
DLPFC (Fornito, Yoon et al. 2011), and alterations in GABAergic and glutamatergic neurotransmission leading to disturbed neural oscillations (Spencer, Nestor et al. 2003, Lewis, Hashimoto et al. 2005). Failure to integrate activity of local and disturbed neural circuits has been suggested to relate to the neurocognitive impairments associated with schizophrenia (Lewis and Gonzalez-Burgos 2000, Uhlhaas and Singer 2013). Several studies have correlated a decline in dopamine levels primarily at the level of D₁ receptors suggesting that an inverted U-shaped relation exists between activation of the prefrontal cortex and working memory in patients with schizophrenia (Callicott, Mattay et al. 2003, Goto and Grace 2006, Goto, Otani et al. 2007). An imbalance of D1 and D2 receptors in the prefrontal cortex further implicates the pathophysiology of this disorder (Laruelle 2014). As reviewed by Brisch et al. positive effects of dopamine enhancement on neurocognitive domains in schizophrenia patients have already been noticed (Brisch, Saniotis et al. 2014).

Thus all these related lines of evidence provide context of logical targets for treating this disorder. To date much research has focused on the effects of nicotine on neurocognitive domains impaired among schizophrenia patients. Pharmacological treatment of neurocognitive dysfunction in schizophrenia is in its infancy, thus identifying promising compounds is critical in advancing translational treatment strategies. The results may show the selectively of drug mechanisms but the specificity of disease outcome responsiveness to drug treatments. In accordance with standardized sets of measures of neurocognitive deficits by the Food and Drug Administration (FDA) for potential drug treatments this includes, working memory, attention/vigilance, verbal memory, executive function, speed of processing, and reasoning/problem solving.
Other commonly studied domains showing correlations to the former components include and are not limited to, sensorimotor gating, delay discounting, and impulsivity.

1.4.2 Division of Working Memory

The longest notion of the division of memory holds that there are separate memory systems for information over the short-term and long-term (James 1890). In the traditional view, while short-term memory serves to store information for a short time, long-term memory stores the meaning of words and ultimately encompasses memories that range from a few days to decades (Rose and Craik 2012). Today, one of the most influential and widely used models is the working-memory model (Baddeley 1992).

Working memory is most commonly defined as the ability of manipulating and maintaining information over short periods of time (Barch and Smith 2008, Sternberg 2015). Ultimately the concept of working memory serves not only to store information, but also to process it, thereby bridging the gap between the two previous components; manipulating short-term information, while holding the most recently activated portion of long-term memory, and moving it to temporary memory storage. (Unsworth 2010). There are five components in working memory: the visuospatial sketchpad (briefly stores some visual images), the phonological loop (briefly holds mainly verbal information), central executive (allocates attention within working memory), episodic buffer (how we integrate information) and other "subsidiary slave systems" (perform other neurocognitive or perceptual tasks) (Baddeley 1990, Baddeley 2007, Baddeley 2009). Together, these components composite the working-memory model that has been commonly shown to have several important implications in real functional outcomes in individuals. In
particular, those with mental health disorders, such as schizophrenia, have been shown to have deficiencies in working memory, among several other neurocognitive domains. All of these neurocognitive domains are considered important markers of real functional outcomes, thus are a key area of research to be studied.

1.4.4 Neurocognitive Domains in Schizophrenia

1.4.4.1 Delay Discounting, Impulsivity & Decision-Making in Schizophrenia

Delay discounting is an essential factor of behavioural economics involving cost in the form of time (Odum 2011). This involves choosing between alternative actions that could entail short-term sacrifice for long-term gain. The inability to initiate and maintain such goal-directed behaviour in schizophrenia contributes to the long-term disability of this illness (Weller, Avsar et al. 2014). Several studies have shown that compared to non-psychiatric controls, schizophrenia patients demonstrate higher delay discounting that is associated with greater impulsivity and worsened neurocognition (Heerey, Robinson et al. 2007, Heerey, Matveeva et al. 2011). However, mixed results have been yielded when considering smoking status of patients (Ahn, Rass et al. 2011, MacKillop and Tidey 2011, Wing, Moss et al. 2012, Weller, Avsar et al. 2014). Further, high levels of impulsivity found in patients with schizophrenia may be associated with risky decision-making (Wing, Rabin et al. 2013). Impaired decision-making abilities have been continuously found in schizophrenia patients and may have difficulty weighing risks and benefits with different choices, such as smoking (Yip, Sacco et al. 2009, Fond, Bayard et al. 2013). Additional investigation of the role of impulsive behaviour and neurocognitive
dysfunction without confounding effects of nicotine may permit a greater understanding of schizophrenia and improvement of future outcome.

1.4.4.2 Sensorimotor Gating (Prepulse Inhibition) in Schizophrenia

Prepulse inhibition (PPI) is a well established tool of sensorimotor gating measuring suppression of the acoustic startle eyeblink response by a prepulse (Graham 1975). Compared to non-psychiatric individuals, both preclinical and clinical studies have consistently shown reduced sensory gating in schizophrenia patients, which may be associated with neurocognitive domains such as attentional processes (Scholes and Martin-Iverson 2009, Witten, Oranje et al. 2014, Wu, Song et al. 2015). Of the few studies examining this deficit while controlling for smoking status, it has been found that smokers may partially improve sensory gating in schizophrenia patients (Chen, Li et al. 2011, Song, Chen et al. 2014). Moreover, PPI is impaired by antagonism of nAChRs, thus meriting potential pharmacotherapeutic treatment strategies on central receptors α4β2 and α7 (George, Termine et al. 2006).

1.4.4.3 Attention/Vigilance and Schizophrenia

One of the most replicated neurocognitive dysfunctions in schizophrenia patients is evident through the impairment of sustained attention (Hahn, Robinson et al. 2012). Attentional disturbances and impaired vigilance also appear to be a stable trait predating the onset of schizophrenia, thus may serve as a manifestation of the illness (Birkett, Sigmundsson et al. 2007). A long held notion holds that working memory depends on the ability to control attention, implicating a possible correlation between performance on tasks measuring both of these domains (Unsworth and Engle 2007, Giakoumaki, Roussos et al. 2011). A meta-analysis supporting this idea revealed that brain imaging trials
involving attentional tasks found least activity in the DLPFC of schizophrenia patients when compared to non-psychiatric controls (Langner and Eickhoff 2013). Thus the importance of such regions of the brain further implicates the need for pharmacotherapeutic treatments that target receptors specific to these regions.

1.4.4.4 Executive Function & Speed of Processing in Schizophrenia

Executive function is a large and complex domain comprised of a collection of processes critical for effective neurocognitive function, such as set-shifting, inhibition and goal maintenance (Orellana and Slachevsky 2013). Impaired performance on tasks evaluating executive function in patients with schizophrenia reflect inefficient sequencing of planning and acting (Knowles, Weiser et al. 2015). A meta-analysis of imaging studies conducted by Minzenberg et al. shows robust evidence that executive function is yet another neurocognitive impairment in schizophrenia characterized by reduced activity in DLPFC (Minzenberg, Laird et al. 2009). Incidentally, compared to other neurocognitive functions, executive function has a developmental course which is both later in life (<20 years of age) and develops for a longer period of time (Kalkut, Han et al. 2009). Yet many researchers suggest that since executive function is comprised of working memory as well, the deficiencies may be a result of a combination of neurocognitive processes (Logue and Gould 2014). As a result, longitudinal data are of great importance and acute clinical studies should assess a range of ability areas within neurocognition.

1.4.4.5 Verbal Memory and Schizophrenia

Verbal memory is another highly studied neurocognitive deficit found in patients with schizophrenia. As reviewed by Touloupooulos and Murray, verbal deficits are present both in the first episode and chronic schizophrenia, appearing throughout the
course of the illness; however, secondary effects of medication need to be considered (Toulopoulou and Murray 2004). Although the number of studies focusing solely on verbal memory are limited, it remains consistent that patients display poorer scores on verbal assessments when compared to non-psychiatric controls (Horan, Green et al. 2008). Similarly to the aforementioned neurocognitive processes, reduced activity of the DLPFC also implicates the role of nAChRs in better performance on verbal tasks and potential pharmacotherapeutic targets (Shad, Muddasani et al. 2004).

1.4.4.6 Working Memory Deficits and Schizophrenia

The greatest amount of literature and research on neurocognitive deficits in schizophrenia has focused on working memory. Defined as the ability to transiently hold and manipulate information leading to goal-directed behaviour, working memory has been found to correlate with intellectual aptitudes better than any other particular psychological process as it incorporates not only storage, but also processing of information (Cowan 2008). Primarily due to hypofrontality in the DLPFC, neurocognitive deficits in the prefrontal regions, affect several measures of working memory (i.e., visual, verbal, attentional, central executive, and episodic memory) (Park and Gooding 2014). Neuropsychological measures that test these components result typically with schizophrenia patients performing one to two standard deviations below non-psychiatric controls, with the greatest impairments appearing in the visuospatial domain (Lee and Park 2005, Smieskova, Marmy et al. 2013). Several meta-analyses over the last couple of decades have consistently revealed robust effect sizes ranging from 0.45 to 1.29 for the presence of working memory deficits in first-episode, drug-naïve, and chronic schizophrenia patients (Lee and Park 2005, Forbes, Carrick et al. 2009,
Mesholam-Gately, Giuliano et al. 2009, Fatouros-Bergman, Cervenka et al. 2014). As such, several studies have investigated reasons for these deficits.

For example, working memory in schizophrenia may have a genetic basis that involves a single nucleotide polymorphism at the catechol-O-methyltransferase (COMT), the enzyme responsible for metabolizing dopamine, with a major route in the PFC (Egan, Goldberg et al. 2001). This altered genotype (Val108/158Met) confers an unstable methionine-allelemorph leading to lower COMT enzyme activity thus increased PFC dopamine levels (Ceaser, Csernansky et al. 2013). Interestingly, researchers have shown that in the presence of the Met allele (either as Val/Met or Met/Met) in patients with schizophrenia, neurocognitive function improves in both visuospatial working memory and executive function (Egan, Goldberg et al. 2001, Wing, Tang et al. 2013). It has also been shown that neurocognitive deficits in schizophrenia improve by administration of nicotine (Smith, Singh et al. 2002), nicotinic agonists (Olincy, Harris et al. 2006) or cigarette smoking (George, Vessicchio et al. 2002, Sacco, Termine et al. 2005). This demonstrates the ability to recruit and study effects of potential neurocognitive enhancers on neurocognition in schizophrenia, and as a function of smoking status.

1.4.5 Smoking and Nicotine Effects on Memory
1.4.5.1 Smoking and Nicotine Effects on Neurocognition in Non-Psychiatric Controls

With such a high prevalence of smoking, it has been long appreciated that there must be some gains in memory from nicotine. Smoking benefits on concentration and memory are robustly reported, while abstinence is associated with poorer working memory efficiency (McCleron, Froeliger et al. 2015), impaired sustained attention
(Hendricks, Ditre et al. 2006), and difficulty concentrating (Harrison, Coppola et al. 2009) in smokers. For example, Kleykamp et al. found that following administration of 21-milligram (mg) nicotine versus placebo patch, the placebo condition resulted in worse performance on a working memory N-back task in smokers (Kleykamp, Jennings et al. 2011). Nonetheless results have been mixed, as other studies report none or selective changes in neurocognitive performance such as disruption on verbal and working memory but no changes on tasks such as the Continuous Performance Test (CPT) (measuring vigilance/attention) following 24-hour or overnight abstinence (Jacobsen, Krystal et al. 2005, Ashare and Hawk 2012). Another factor to consider is the varying effects of acute versus long-term nicotine abstinence. For example, one study found that prolonged abstinence (8 weeks) improved working memory performance in non-psychiatric smokers (George, Vessicchio et al. 2002), while Park et al. found that following overnight abstinence, acute smoking actually impaired spatial working memory performance (Park, Knopick et al. 2000). Thus direct pro-neurocognitive effects of smoking have been difficult to separate from the reversal of nicotine withdrawal effects. Accordingly, to avoid any confounding effects of withdrawal, clinical studies have examined the effects of nicotine administration in satiated smokers and non-smokers.

Literature on nicotine and neurocognitive function in non-smoking individuals is smaller and equivocal. For example, Barr et al. examined the effects of 14 mg transdermal nicotine on attention and found improved attentional performance on the CPT-identical pairs version, (CPT-IP) (Barr, Culhane et al. 2008). In contrast, Wignall et al. found that administration of 7 mg nicotine patch impaired neurocognitive and attentional performance, on the Attention Network Test (Wignall and de Wit 2011).
Another study found that nicotine improved PPI and attention in nonsmokers, but the level of effectiveness also depended on the baseline discrepancies between individuals (Baschnagel and Hawk 2008). Of most recent, Grundey et al. found that nicotine administration reversed abstinence induced working memory deficits in smokers, while decreasing accuracy and increasing number of errors in non-smokers (Grundey, Amu et al. 2015). Taken together, the mixed results of the aforementioned studies may be explained by the variances in methodologies used to assess the neurocognitive domains; hence further research in this area is warranted.

1.4.5.2 Smoking and Nicotine Effects on Neurocognition in Patients with Schizophrenia

The vast majority of patients with schizophrenia are smokers (70-80%) (Winterer 2010). Such high rates implicate the role of the cholinergic system in the pathophysiology of schizophrenia (de Leon 1996). Further, patients with this disorder suffer from pervasive neurocognitive deficits that are associated with dysregulation of central nAChRs. As such, smoking cessation treatment trials have demonstrated effects of nicotine remediating these neurocognitive deficits in smokers with schizophrenia. Research has primarily focused on the association of working memory with smoking as it has consistently been shown to be the most sensitive to trials such as overnight-abstinence paradigms. For instance, George and colleagues examined both acute (less than 1 week) and prolonged (8-10 weeks) smoking abstinence in patients with schizophrenia (George, Vessicchio et al. 2002). Interestingly, they found that visuospatial working memory was worsened at both time points in the patient group, while controls in contrast had improvements in performance. Likewise, Sacco and colleagues replicated a
similar finding by showing that smoking abstinence impaired visuospatial working memory, while smoking reinstatement reversed these effects in smokers with schizophrenia (Sacco, Termine et al. 2005). Furthermore, nonselective nAChR antagonist MEC was found to block the reversing effects of reinstatement. Consistently, Wing et al. found that overnight abstinence decreased visuospatial working memory in schizophrenia smokers at the 30-second (sec) delay of a working memory task (Wing, Wass et al. 2013). Thus these results not only demonstrate the selective benefits of nicotine in the schizophrenia population compared to non-psychiatric controls, but it also suggests targeting nAChRs as a possible pharmacotherapeutic treatment for neurocognitive deficits.

Nicotine also affects sensory gating which is mediated by the same mechanism of augmenting dopamine release. George and colleagues found that while at baseline patient and control groups had comparable PPI performance, overnight-abstinence significantly reduced PPI in patients and this effect was then reversed during reinstatement (George, Termine et al. 2006). Again, the blockade of nAChRs with MEC dose-dependently blocked PPI enhancement during reinstatement, contributing to understanding of nAChR in schizophrenia vulnerability to smoking.

However, some may argue that the proneurocognitive effects of nicotine are a result of abstinence induced deficits and consequential withdrawal. AhnAllen et al. attempted to avoid such confounds by examining the effects of nicotine in patient and non-psychiatric smokers while performing the Attention Network Task (ANT) (which evaluates alertness, orienting, and executive function) in three conditions: baseline, 8-hour overnight withdrawal, and 3-hour 21 mg nicotine patch (AhnAllen, Nestor et al.
The investigators found that the groups did not differ across the conditions on three ANT measures. In comparison to controls, schizophrenia patients also showed greater impairment in the withdrawal condition, and greater accuracy in the patch condition, thus supporting evidence of greater nicotine effects in schizophrenia, as seen in several other studies (Smith, Singh et al. 2002, Smith, Warner-Cohen et al. 2006, Beck 2015).

Thus the aforementioned studies demonstrate the possible beneficial effects of nicotine on neurocognitive function in schizophrenia. However, nicotine has several limitations as a therapeutic agent. This includes tachyphylaxis of nicotinic receptors and nicotine-induced desensitization, long-term health risks of chronic nicotine, and addictive properties that can lead to symptoms of withdrawal. Therefore, alternative nAChR agonists, such as varenicline, may be helpful as pharmacotherapy treatments in schizophrenia are of great economic and scientific interest.

### 1.5 Varenicline

Different treatments incorporate different mechanisms of smoking cessation. Recent work has shown α7’s effect on neurocognitive tasks and P50 gating improvements in schizophrenia. However, these findings are inconsistent (Olincy, Harris et al. 2006, Buchanan, Conley et al. 2008, Dyer, Freudenreich et al. 2008, Shiina, Shirayama et al. 2010). Thus using a pharmacotherapy which targets the other central brain receptor, α4β2, may provide more solid evidence of downstream proneurocognitive effects.
1.5.1 Pharmacological Properties

Varenicline (as the tartrate salt; Chantix® in the US; Champix® in Europe and Asia) is an approved drug worldwide, including US and Europe (2006), Canada (2007) and Japan and China (2008) (Faessel, Obach et al. 2010). Of the three first-line smoking cessation aids, (nicotine replacement therapy, (NRT), bupropion sustained-release (SR), varenicline), the efficacy of varenicline is most successful with a nearly 2-fold increased odds of quitting smoking compared with bupropion-SR or NRT, and 4-fold efficacy rate compared with placebo (Cahill, Stevens et al. 2014). Varenicline is generally prescribed at doses of 1-2 mg/day using twice daily dosing (BID), and has established dosing regimens for 12 weeks in large clinical trials (Gonzales, Rennard et al. 2006, Jorenby, Hays et al. 2006, Oncken. C. 2006, Tonstad, Tonnesen et al. 2006, Williams, Reeves et al. 2007).

1.5.1.1 Mechanism of Action

Based on the nAChR partial agonist (-)-cystisine, the development of varenicline was hypothesized to be an effective agent through its selective partial agonism for the α4β2 nAChR (Coe 2005). As a partial agonist, varenicline elicits a moderate and sustained increased in dopamine levels, such that full stimulation of the mesolimbic dopamine system is prevented and dopamine-induced behavioral reinforcement is blocked (Zhu 1996). Due to its higher affinity for α4β2 nAChR compared to nicotine (>20-fold), varenicline prevents nicotine from full activation of nAChRs (Rollema, Chambers et al. 2007). At high nicotine levels varenicline blocks the nAChR resulting in decreased pleasure when a smoker smokes a cigarette (Tonstad, Tonnesen et al. 2006).
Conversely, at low nicotine levels, varenicline stimulates downstream effects of α4β2 leading to the release of dopamine and decreased craving and withdrawal symptoms (Gonzales, Rennard et al. 2006). Varenicline is also a full agonist of α7 nAChR which of most recent has been a growing topic of interest for its implications in schizophrenia, and of α3β4; however, the relationship of this binding profile to its clinical actions is not fully understood (Mihalak, Carroll et al. 2006, Raybuck, Portugal et al. 2008, Shim, Jung et al. 2012).

1.5.1.2 Pharmacokinetics

After oral administration of varenicline, maximum plasma concentration (Cmax) is reached within 3-4 hours (Faessel, Obach et al. 2010). With a half-life of approximately 17-24 hours, repeated dosing results in steady state levels within 4 days (Obach, Reed-Hagen et al. 2006, Faessel, Obach et al. 2010). Dosing may be either single- or multiple- of up 3 mg/day, and results in linear pharmacokinetics relating renal and total clearance. Primarily through glomerular filtration via the renal human Organic Cation Transporter (hOCT), approximately 92% of varenicline unchanged is excreted unchanged in urine (Feng, Obach et al. 2008). Such metabolite and excretion patterns are the same in both smokers and nonsmokers while not undergoing significant hepatic metabolism (Faessel, Smith et al. 2006, Obach, Reed-Hagen et al. 2006). Varenicline has no drug interaction with compounds metabolized by the cytochrome P450 (CYPs) enzyme system, an important factor to consider as antipsychotics like olanzapine and clozapine are metabolized through cytochrome P450 1A2 (CYP1A2) (Prior and Baker 2003, Faessel, Obach et al. 2010).
1.5.2 Preclinical and Phase I Studies

Various preclinical and Phase I trials studied the aforementioned pharmacokinetics of varenicline (Coe, Brooks et al. 2005, Obach, Reed-Hagen et al. 2006). Ultimately this resulted in much discussion as to whether the downstream effects of varenicline owe to activation of α4β2 or α7. Researchers have demonstrated that with pretreatment, varenicline was able to dose-dependently attenuate nicotine-enhanced brain-stimulation reward (Spiller, Xi et al. 2009). Blockade of these effects with MEC and Dihydro-Beta-Erythroidine (DhβE) (a selective α4-containing nAChR antagonist), and not by methyllycaconitine (a selective α7 nAChR antagonist) suggested that the α4β2 nAChR subtype underlies the action of varenicline (Spiller, Xi et al. 2009).

*In vitro* functional patch clamp studies have demonstrated that varenicline has 45% of nicotine's maximal efficacy of α4β2 nAChRs, with a 500-20,000-fold selectivity for α4β2 than any other nAChR subtype (Coe, Brooks et al. 2005, Rollema, Coe et al. 2007, Ivy Carroll, Yokota et al. 2008). In vivo studies of neurochemical models have found varenicline to increase dopamine release from rat nucleus accumbens, and when combined with nicotine, effectively attenuate nicotine-induced dopamine release (Rollema, Chambers et al. 2007). Further, animal models of addiction have shown varenicline to exhibit multiple trajectories, such as inhibiting nicotine self-administration and reinstatement, and nicotine-enhanced brain stimulation reward (Spiller, Xi et al. 2009, O'Connor, Parker et al. 2010). With such promising preclinical results, a strong theoretical foundation relevant to human trials was developed.
1.5.3 Therapeutic Efficacy

1.5.3.1 Phase II Trials

There have been a number of phase II clinical trials of varenicline to further evaluate its safety and efficacy. Oncken et al. conducted a multicenter trial that examined the tolerability and efficacy of four different dose regimens of varenicline (Oncken, Gonzales et al. 2006). They found that 0.5 and 1 mg BID were most efficacious for smoking cessation, and most tolerable when titrated up to full dose rather than fixed dosing (nontitrated). In another placebo-controlled phase II trial, Nides and colleagues investigated the optimal doses of varenicline (0.3 mg/day, 1 mg/day and 1 mg/BID) in non-psychiatric smokers while measuring continuous abstinence rates (CARs) (for any 4-week period) over a 52-week period (Nides, Oncken et al. 2006). The authors found no dose-related increases in relation to adverse events and varenicline to be effective both in the short-term (4 weeks) and long-term (52 weeks), with either the 1 mg/day or 1 mg/BID dosing.

Nakamura et al. similarly examined the efficacy of slightly different dosing of varenicline (0.25 mg/day, 0.5 mg/day and 1 mg/BID) for 12-week and 40-week timepoints. Varenicline was found to dose-dependently maintain smoking abstinence, with highest efficacy in the 1 mg/BID group compared to placebo (Nakamura, Oshima et al. 2007). Likewise, three additional studies found that varenicline dosed at 1 mg/BID was both tolerable and efficacious as a pharmacotherapy for smoking cessation (Tsai, Cho et al. 2007, Niaura, Hays et al. 2008, Wang, Xiao et al. 2009). Taken together, the most commonly found efficacious dosing was 0.5 mg/BID and 1 mg/BID, both of which were found tolerable for smokers and efficacious for smoking abstinence.
1.5.3.2 Phase III Trials

A vast majority of studies completed with varenicline have been larger-scale Phase III clinical trials that assess the efficacy of varenicline and monitor side effects, while in comparison to the two other commonly used smoking cessation treatments, bupropion-SR and NRT. Jorenby et al. and Gonzales et al. both conducted double-blind, placebo-controlled studies to determine the efficacy and safety of varenicline (titrated up to 1 mg BID) in comparison to bupropion-SR and placebo (Gonzales, Rennard et al. 2006, Jorenby, Hays et al. 2006). Similarly both studies consisted of a 12-week treatment period and 52-week follow-up periods and pooled data analysis found that varenicline had greater CARs compared to bupropion and placebo (44.0%, 29.7%, and 17.7%, respectively; both comparisons P < 0.001) (Nides, Glover et al. 2008). Another phase III study compared the differences in efficacy of varenicline to NRT. Aubin et al. found that after 12 weeks of open-label treatment varenicline resulted in significantly more smokers with CAR compared to NRT (Aubin, Bobak et al. 2008).

Elucidating optimal duration of maintenance pharmacotherapies such as varenicline, may ultimately lead towards successful cessation rates beyond end of treatment in schizophrenia. Thus Tonstad et al. conducted a study such that smokers who maintained abstinence after first being treated for 12 weeks of open-label varenicline (1 mg/BID), (62.8%) were then randomized to receive an additional for an additional 12 weeks (Tonstad, Tonnesen et al. 2006). It was found that the percentage of CARs in the varenicline group was significantly greater than placebo, and no difference in reporting of adverse events between the two groups was found. Given that adverse event reporting up to this point have been mostly avoided, a recent study examined as to whether
varenicline increased dosing (up to 5 mg/day) would cause a response in smokers with no/low response to the standard dosage. After a 12-week treatment, it was found that increasing varenicline dose had no significant on smoking cessation thus physicians should warrant that such an approach may not optimize cessation outcomes.

1.5.3.3 Phase IV Trials

Phase IV trials of varenicline gather information on its efficacy as a smoking cessation aid in various populations while also associating any side effects with long-term usage. As such, one study conducted across 10 countries with 61 centers examined varenicline treatment (1 mg/BID) for a 24-week period and 28-week follow-up for increasing CAR among smokers not willing or able to quit within the next month (Ebbert, Hughes et al. 2015). The authors found that the varenicline group had significantly increased CAR at end of treatment compared to placebo thus offering a treatment option for smoking reduction. Another study conducted across 8 different countries evaluated the efficacy of retreatment with varenicline in smokers attempting to quit (Gonzales, Hajek et al. 2014). It was found that CAR for 12-week treatment with varenicline was comparable for varenicline-naive smokers and significantly greater compared to placebo. Thus the efficacy of varenicline across several populations and long-term usage seems to remain tolerable as well.

1.5.4 Side Effects and Tolerability

Reports concerning increased suicide ideation and other dangerous behaviours when taking varenicline emerged after its regulatory approval as a smoking cessation aid. One study examined post-marketing case reports from the FDA AE Reporting System and found varenicline users showing increased risk of reporting depression and suicidal
behaviour compared to bupropion or NRT users (Moore, Furberg et al. 2011). In response, the FDA required information regarding risk of neuropsychiatric event and black box section of varenicline labeling. However, despite early reports of varenicline’s exacerbation of psychiatric symptoms varenicline has continuously been found well tolerated and safe in both non-psychiatric and psychiatric smokers. Smoking has been shown to increase metabolism of antipsychotic drugs via increased activity of the enzyme CYP1A2 leading to differences in psychiatric symptoms (Prior and Baker 2003). Thus reduction of smoking may result in increased plasma levels of these medications, potentially explaining previous study findings that questioned varenicline's safety in the schizophrenia population (Fatemi, Yousefi et al. 2013). Hence therapeutic drug monitoring and dose reduction over the first few days of tobacco abstinence may further avoid toxicity (Faber and Fuhr 2004). Notably, George et al. found that atypical agents may be superior to typical agents in combination with NRTs for smoking cessation in schizophrenia (George, Ziedonis et al. 2000). In line with this, future studies may consider to investigate whether effects of varenicline are similar and safe across these two agent groups, in both short-term and long-term smoking cessation trials, which could also account for why some trials are found to result in improved negative psychiatric outcomes.

Finally, numerous trials have been demonstrating that varenicline does not differ form placebo for depression, suicidal ideation, attempted suicide, or suicide (Thomas, Martin et al. 2015). In fact, a major meta-analysis along (Thomas, Martin et al. 2015) with two large observation studies found no significant association between varenicline and suicidal ideation (Kotz, Viechtbauer et al. 2015, Molero, Lichtenstein et al. 2015). A
recent large multinational trial with 8144 participants (4116 psychiatric cohort and 4028 non-psychiatric smokers) examined the efficacy and safety of varenicline and bupropion with nicotine patch and placebo in smokers with and without psychiatric disorders (Anthenelli, Benowitz et al. 2016). Overall it was found that varenicline was more effective than bupropion, nicotine patch and placebo. Further, there was no significant increase in rates of psychiatric adverse events with either varenicline or bupropion treatments compared to placebo or nicotine patch in either smoking group. Thus the safety and tolerability of varenicline has been strongly supported over several clinical trials. Nonetheless, individuals experience some adverse events (primarily nausea) and non-significant serious adverse events (SAEs) (O'Malley 2011), therefore clinical vigilance is warranted.

1.5.5 Effects of Varenicline on Neurocognitive Function

To date, several studies have examined cigarette smoking on neurocognitive function and clinical outcome measures. Additionally, there is indirect evidence of nicotinic receptor stimulation mediating such effects as neurocognitive enhancement by smoking, using pre-treatment with MEC at doses of 10 mg/day (speculated to have some selectivity for α4β2 nAChR) (Young, Shytle et al. 2001). Utilization of investigation drugs (e.g., ABT-089, ABT-594, SIB-1663, RJR-2402) for potential studies that are nicotinic report agonists are currently under development but still several years away (Decker, Meyer et al. 2001, Beinat, Banister et al. 2015). Thus since varenicline is a partial α4β2 agonist and full agonist of α7, and the purported beneficial effects of cigarette smoking on neurocognitive function, a pharmacotherapy based on the nAChR
system for treatment of neurocognitive dysfunction is of great scientific interest (Decker, Brioni et al. 1994, Decker, Meyer et al. 2001).

1.5.5.1 Effects of Varenicline on Neurocognitive Function in Non-Psychiatric Individuals

It has long been suggested that as a partial nAChR agonist, varenicline may subsequently have neurocognitive-enhancing effects; however, research into this hypothesis in non-psychiatric individuals is limited but optimistic. In smokers, a neuroimaging study conducted by Loughead et al. found that following 3 days of nicotine abstinence, varenicline (titrated up to 1 mg/BID over 13 days) increased working memory-related brain activity, and in highly dependent smokers was also associated with improvements in neurocognitive performance (Loughead, Ray et al. 2010). However, reports of varenicline's potential to treat nicotine addiction and improve neurocognition may have been a result of varenicline or ceasing smoking itself, thus Mocking et al. conducted a study in non-smoking individuals (Mocking, Patrick Pflanz et al. 2013). This double-blind study found higher working memory (n-back task) and declarative memory (verbal task) scores in the varenicline group (0.5 mg/day first 3 days, then 1 mg/day for 4 days) compared to placebo thus supporting proneurocognitive effects. In contrast, a study by Roh et al. showed no significant improvements on either attention (CPT task) or working memory (visuospatial working memory task) performance in non-smokers who received a single dose of varenicline (1 mg) (Roh, Hoeppner et al. 2014). However some of these discrepancies of results may owe to the fact that these studies used different tasks to assess the same domains, along with different dosing strategies. Working memory may perhaps only be enhanced if steady state levels of varenicline are reached, and single
dosing may not accomplish this (Faessel, Smith et al. 2006). Taken together, the majority of studies examining the effects of varenicline in non-psychiatric individuals have focused on its effects as a smoking cessation aid rather than its effects on neurocognition. However among these relatively few studies, varenicline's neurocognitive-enhancing properties led to the suggestion of being therapeutically useful in patients with neurocognitive deficits, such as schizophrenia.

1.5.5.2 Effects of Varenicline on Neurocognitive Function in Schizophrenia Patients

Presently approved pharmaceutical treatments for schizophrenia are typically effective for positive symptoms but have little or no effect on neurocognitive dysfunction (Keefe, Haig et al. 2016). Patients with schizophrenia show a high rate of cigarette smoking, and accordingly neurocognitive deficits may be related to dysregulation in their nAChRs. A pharmacotherapeutic treatment such as varenicline, which is shown to be efficacious as an anti-smoking aid while also acting a partial \( \alpha 4\beta 2 \) agonist and full \( \alpha 7 \) agonist, may ultimately have potential as a repurposed drug for disorders with neurocognitive deficiencies (Lett, Voineskos et al. 2014).

1.5.5.2.1 Effects of Varenicline on Neurocognitive Function in Schizophrenia Smokers

The effects of varenicline targeting nAChRs for neurocognitive deficits in smokers with schizophrenia have been mixed. Two studies found varenicline improved executive function in smokers with schizophrenia (Hong, Thaker et al. 2011, Shim, Jung et al. 2012), while other studies have found significant improvements primarily in verbal memory and not visuospatial or attentional indices (Smith, Lindenmayer et al. 2009), or
no changes in neurocognitive outcomes at all (Smith, Amiaz et al. 2016). Notably, Hong et al. used a varenicline treatment of 1 mg/day over 8 weeks while Shim et al. used a 2 mg/day (titrated up over 8 weeks). This suggested that although dosing half of the recommended 2 mg dosage for smoking cessation results in over 50% reduction of the primary side effect of nausea (Oncken, Gonzales et al. 2006), it still has similar effects on executive functional outcome. However, another study found selective dosing effects, where repeated 1 mg/day treatment over a three day period resulted in attenuated abstinence-induced deficits in visuospatial working memory at the 30 sec delay but limited effects were found in 2 mg/day treatment (Wing, Wass et al. 2013). This highlighted the importance of 1-week titration to reduce potential α4β2-nAChR antagonistic effects of higher doses of varenicline. Furthermore, the study that found no neurocognitive effects using varenicline was an 8-week double-blind trial that had no "quit date" as part of the study design varenicline (titrated up to 1 mg/BID). This suggests that in smokers, some positive neurocognitive effects of varenicline are stronger only after acute cigarette abstinence. Perhaps this lack of efficacy is related to its receptor pharmacology. Varenicline’s much higher affinity to α4β2 is associated with dopamine release that may compensate for nicotine’s actions, may also mean it has a 24 times less functional potency for α7 due to its lower affinity (Mihalak, Carroll et al. 2006). Thus these differing factors of functional potency and affinity may explain the lack of clear neurocognitive enhancing effects. Moreover, varenicline may have selective effects on specific neurocognitive domains that may vary due to dosing strategies as well.

Perhaps an ideal method to efficiently be able to distinguish the effects of varenicline in this neurocognitive dysfunctional disease would be in non-smoking
populations, thereby avoiding any confounding effects of nicotine or withdrawal. To date, no study has examined the effects of varenicline in nonsmokers with and without schizophrenia as a pharmacotherapeutic neurocognitive enhancer. Thus given these mixed proneurocognitive effects of varenicline which may or not be a result of reversing abstinence induced deficits, further research is warranted in non-smoking populations.

1.5.5.2.2 Effects of Varenicline on Neurocognitive Function in Schizophrenia Non-Smokers

The subset group of non-smokers with schizophrenia represents a minority of this mental illness population (approximately <30%). As such, little is known about the functional effects of nAChR modulation independent of cigarette smoking effects in people with schizophrenia. The importance of conducting studies using non-smoking populations allows one to examine and determine possible proneurocognitive effects from targeted nAChRs without the confounding effects of nicotine dependence or withdrawal from smoking. Presently, there are a limited amount of studies in non-smokers, though of the few, modest effects of acute nicotine administration are demonstrated, warranting further research in this subgroup (Avila, Sherr et al. 2003, Harris, Kongs et al. 2004, Barr, Culhane et al. 2008). Only one study has compared the effects of varenicline on neurocognitive performance in non-smokers with and without schizophrenia. Roh and colleagues reported no differences in working memory between the varenicline and placebo group (Roh, Hoeppner et al. 2014). However, the authors also found a worsening in performance after treatment with MEC, suggesting selective nAChRs may in fact exert neurocognitive benefits.
Two other studies that had non-smoking groups matched to smokers have reported mixed results of the effects of varenicline. One of the studies found treatment differences of varenicline where in comparison to smokers with schizophrenia, non-smokers showed no improvement in attention (Shim, Jung et al. 2012). In contrast, Hong et al. found varenicline reduce P50 sensory gating deficits in non-smokers but not in smokers, and improved executive function by reducing antisaccade error and reduced startle reactivity irrespective of smoking status (Hong, Thaker et al. 2011).

At this time, there are no standardized treatment options for working memory deficits in schizophrenia. Thus the rationale for varenicline as a neurocognitive augmentation strategy in non-smokers with schizophrenia may shed light into its effect as pharmacotherapeutic for neurocognitive impairment. However, to date no study has examined the effects of varenicline solely on neurocognitive function in non-smokers with and without schizophrenia thus further research is warranted.

1.6 Overall Summary

Neurocognitive dysfunction is a core feature of schizophrenia and is one of the strongest predictors of poorer functional outcomes that may relate to the high smoking prevalence in this disorder. Neuronal nAChR that are widely distributed throughout the central nervous system, play an important role in working memory as well as other neurocognitive domains including verbal memory, executive function, attention, impulsivity, and decision-making. Thus the importance of pharmacotherapeutic approaches targeting nAChR is fundamental.
The administration of nicotine has been shown to transiently improve neurocognitive function in schizophrenia patients. At therapeutic levels, the nicotinic partial agonist varenicline, which is approved for smoking cessation and is highly selective for α4β2, has been shown to enhance neurocognition in schizophrenia. However, the effects of varenicline in non-smokers are still not clearly known. Therefore, this study assessed the effects of varenicline on neurocognitive function in non-smoking schizophrenia patients compared to non-psychiatric controls. Improving treatments for neurocognitive deficiencies in patients is important, as it has been shown by several studies how these deficits affect their functional abilities. For instance, limitations on ability to acquire, retain, and relearn skills such as forming relationships and undertaking employment have been shown to be a result of impairments in working memory and executive functioning (Lasser, Nasrallah et al. 2007).

1.7 Thesis Objectives and Hypotheses

The primary objective of this thesis was to determine the effect of varenicline on neurocognitive performance including the primary outcome visuospatial working memory (VSWM). We hypothesized varenicline would improve VSWM performance in both patients with schizophrenia and non-psychiatric controls, with a greater enhancement among patients.

The secondary objectives were: 1) to evaluate neurocognition in non-smoking patients with schizophrenia compared to non-psychiatric controls at baseline; 2) to determine the effects of varenicline on other neurocognitive domains in non-smoking patients with schizophrenia to non-psychiatric controls; and 3) to determine if varenicline
would improve neurocognition in a dose-dependent manner (0, 1, 2 mg/day).

Accordingly, we hypothesized that: 1) at baseline patients with schizophrenia would
demonstrate deficits in neurocognition compared to non-psychiatric controls; 2)
varenicline would improve neurocognitive performance in both patients with
schizophrenia and controls, with a greater enhancement among patients; and 3)
neurocognitive performance would improve in a dose-dependent manner of varenicline
(0, 1 and 2 mg/day).
2. Methods

2.1 Study Design

This study was a randomized, placebo-controlled cross-over design, approved by the Centre for Addiction and Mental Health's (CAMH) Research Ethics Board (REB #067/2009) and registered on www.clinicaltrials.gov. Study duration took an approximate total of four weeks to complete. All participants had undergone an initial phone screen (Appendix A) followed by an in-person screen. Signed study informed consents were obtained during this visit (Appendix B). The optional genotyping portion of the study was also completed during this screening visit; all participants were provided with a separate consent form (Appendix C). If participants meet eligibility criteria, then baseline testing was completed. Those found eligible were then randomized to receive three different doses of varenicline across three separate weeks for three consecutive days each week (Day 1, Day 2, and Day 3). Neurocognitive testing would occur on baseline, Day 2, Day 3AM and Day 3PM (Figure 2.1). Further study visit details are provided in section 2.5 (Study Procedure).

2.2 Participants

All participants were required to obtain an estimated Intelligent Quotient (IQ) ≥80 using the Shipley scale (Shipley and Burlington 1941) and WTAR (Wechsler 2001). Participants were also required not to be taking any form of NRT. The Structured Clinical Interview (SCID) for DSM-IV-TR confirmed a diagnosis of schizophrenia/schizoaffective disorder for patients and no current diagnosis of Axis I
disorders for controls (except past history of major depression). Also a stable remission from positive symptoms of psychosis as judged by Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein et al. 1987) total PANSS score <70 and psychiatric evaluation was required by patients. All patients with schizophrenia were on stable doses of antipsychotic medication(s) for the past 3 months. Chlorpromazine (CPZ) equivalents of antipsychotics were calculated for all patients (Woods 2003).

Exclusion criteria for all participants included any substance abuse including cigarette smoking (urine drug screen, and carbon monoxide (CO) level measured on every visit), as well as any history of alcohol/drug abuse in the 3 months before study enrollment and use of opioids (meperidine, oxycodone, methadone, etc.,). Non-smoking

**Figure 2.1**: Study Design. After screening and baseline sessions, testing each week lasted for 3 consecutive days and included 4 laboratory sessions (day 1 morning, Day 2 morning, day 3 morning, day 3 afternoon). There were 3 separate testing weeks in which participants were administered varenicline (0, 0.5 or 1 mg BID for 3 days).

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participants (defined as never smoking, or being a former smoker for at least 1 year) were screened for study eligibility and provided written informed consent. Participants were also excluded if currently taking psychotropic medications, or have a history of renal insufficiency, a hypersensitivity to varenicline, dementia, or any other neurological illness like epilepsy or medical condition known to significantly influence neurocognitive function at the discretion of the Principal Investigator (PI). Inability to learn the neuropsychological tasks during the training session, or failure to demonstrate a deficit of at least 0.5 standard deviations below average levels of non-psychiatric control performance on the VSWM task (greater than 1.5 cm distance from target at baseline performance) were also excluded from the study.

Both groups were age- and sex-matched. Non-psychiatric controls were recruited from flyers, Craigslist ads and cross-referral meetings. Schizophrenia patients were recruited through CAMH flyers (Appendix D), recruitment booths at outpatient clinics, cross-referral meetings, and psychiatrist referrals.

2.3 Measures

2.3.1 Psychiatric Measures

Structured Clinical Interview (DSM-IV-TR)

The Structured Clinical Interview (SCID) DSM-IV-TR is a semi-structured interview assessing current and lifetime Axis I disorders (First, Spitzer et al. 2002) to confirm diagnosis of schizophrenia or schizoaffective disorder in patients, and to rule-out psychiatric diagnoses in controls at the screening session.

Positive and Negative Syndrome Scale
The Positive and Negative Syndrome Scale (PANSS) is a 30-item used to judge stable remission in patients with schizophrenia from positive and negative symptoms, along with their general and total severity of illness. Constructed using a 7-point rating scale starting with 1 (absent) to 7 (extreme), the PANSS provides a validated criterion with both high inter-rater and internal reliability (Kay, Fiszbein et al. 1987, Kay, Opler et al. 1988). The PANSS was administered to patients at screening, as well as at every neurocognitive assessment day prior to completing neurocognitive tasks.

*Beck Depressive Inventory*

The Beck Depressive Inventory (BDI) scale is a self-report 21-item rating inventory used to measure symptoms and characteristic attitudes of depression. Answers are rated on a scale from 0 (absent) to 3 (severe), with higher total scores indicating more severe depression symptoms (Beck, Ward et al. 1961). The BDI was completed by all participants at screening, as well as at every neurocognitive assessment day prior to completing tasks. At one extreme, a score between 0-10 indicated ups and downs considered normal, while at the other extreme over 40 indicated extreme depression; 11–13 (minimal), 14–19 (mild), 20–28 (moderate), and 29–63 (severe). Non-psychiatric participants tend to report an average item score between 0-3, while schizophrenia patients mean scores are around 11 (Chemerinski, Bowie et al. 2008).

2.3.2 Extrapyramidal Side Effects

*Abnormal Involuntary Movements Scale*

The Abnormal Involuntary Movements Scale (AIMS) is a 12-item scale used in schizophrenia populations on neuroleptic regimes to assess dyskinetic symptoms allowing for early detection and on-going surveillance of tardive dyskinesia (TD) (NIMH
1974). With exception of items 11 and 12 that assess dental care, and are answered with either a "yes" or "no", all other answers are rated using a 5-point scale starting with 0 (none) to 4 (severe). Evidence of TD occurs when ratings are or higher. The AIMS was administered to patients at screening, as well as at every neurocognitive assessment day prior to completing neurocognitive tasks.

*Barnes Akathisia Rating Scale*

The Barnes Akathisia Rating Scale (BARS) is a 4-point scale used in patients with schizophrenia to rate both subjectively and objectively for drug-induced akathisia (Van Putten 1986, Van Putten and Marder 1987, Barnes 1989). Subjective awareness of restlessness, and distress to restlessness are also assessed. A 5-point scale is used to rate a global clinical assessment. The BARS was administered to patients at screening, as well as at every neurocognitive assessment day prior to completing neurocognitive tasks.

*Simpson Angus Rating Scale for Extrapyramidal Symptoms*

The Simpson Angus Rating Scale (SARS) is a 4-point scale used to rate for extrapyramidal, also commonly referred to as Parkinsonian symptoms. There are 10 items that are measured including measure gait, arm dropping, rigidity, glabella tap, salvation and tremor. The SARS was administered to patients at screening, as well as at every neurocognitive assessment day prior to completing neurocognitive tasks.

2.3.2 Physical Examination Measures

The physical examination was consisted of medical history, vitals, height and weight, current and past substance use, and a physical examination of the head, ears, neck, and abdomen for medical clearance of the study medication. The assessments were conducted during the screening visit either by the Research Nurse or PI (Physician).
Other measures also included an electrocardiogram (EKG), to check for any medical issues with the electrical activity of the heart, a pregnancy test (if applicable), and urine toxicology with the Medtox™ urine toxicology screening to test for any recent drug use. There was also biochemical verification of non-smoking status (CO<10ppm).

2.3.3 Blood

Blood samples were collected at screen to assess medical eligibility. At screening, if consented for, participants also had extra blood taken for genetic and biomarker analysis to examine genes that may be related to nicotine receptor function, metabolism and schizophrenia (Appendix B). Sampling for varenicline plasma levels (on Day 3PM) per study week (3 times in total) was also collected.

2.4 Study Medication

Three different doses (0, 1, 2 mg/day) of varenicline tartrate (Pfizer Pharmaceuticals) tablets were administered over consecutive 3 days per week. Generic purple capsules composed only of lactose monohydrate were re-compounded by the CAMH Pharmacy and matching placebo were produced. Pre-treatment with varenicline or placebo started on the morning of Day 1 of each test session, followed by the second dose at 6pm. Participants had taken the second and fourth dose at home, while the other doses were administered under staff supervision. Most participants completed testing in 3 consecutive weeks allowing testing days to be separated by at least 1-week to rule out medication carry-over effects. Ordering of varenicline doses across the test weeks were counter-balanced to control for medication sequencing effects. Thus, participants received study medications on a twice-daily basis for a final total of 6 doses beginning 24 hours prior to
the first test day (either 0.0 mg/day, 2) 0.5 mg twice daily (BID) or 3) 1 mg BID per week).

2.5 Systematic Assessment of Treatment Emergent Events

The Systematic Assessment of Treatment Emergent Events (SAFTEE) is a 5-point scale log that was used to assess any potential varenicline-related adverse events (Levine and Schooler 1992). This 33-item log included primarily gastrointestinal, and include nausea, vomiting, diarrhea, anorexia, weight loss, and insomnia (Appendix E). The SAFTEE was administered to patients at baseline, as well as at every neurocognitive assessment day prior to completing neurocognitive tasks, with the exception of Day 3PM, where the SAFTEE was conducted at the end of the session. The PI was also notified via email of any adverse events reported, and if SAEs occurred, the REB would be notified as well.

2.5 Neuropsychological Testing

An extensive neurocognitive battery was administered at baseline that was then repeated 3 times on each dose. Training on the battery ensured understanding of the tasks and minimized practice effects. This method has been previously shown to result in asymptotic performance with repeated testing (Sacco, Termine et al. 2006).

2.5.1 Spatial Delayed Response Task

The Spatial Delay Response (SDR) task involves participants being instructed to fixate on a fixation cross during which a stimulus appears and disappears in the peripheral area. This is followed by a delay period of 5, 15 or 30-sec to assess shorter- vs. longer-term visuospatial working memory. After participants are instructed to point on the screen where they saw the stimulus to be; during the task participants must press the
spacebar when the diamond shape appears. The difference from where they pointed and the actual stimulus was calculated and reported as the averaged “distance from target” (mm) after 10 trials per delay condition; the greater the number, the worse the performance. Based on a previous MEC study using the same task, the authors showed overnight abstinence was found to induce a schizophrenia-specific deficit in VSWM, but was reversed by smoking reinstatement (Sacco, Termine et al. 2005).

2.5.2 Hopkins Verbal Learning Task-Revised

The Hopkins Verbal Learning Task-Revised (HVLT-R) is a 12-item verbal list-learning and memory test that includes three semantic categories of four words (Shapiro, Benedict et al. 1999). There are three learning trials in the immediate recall phase, followed by a 20-25 minute delay period during which other tasks are administered. There is a fourth recall trial and a recognition and discrimination trial during the delayed phase. There are six forms that are highly inter-correlated, which is ideal for repeated assessment.

2.5.3 Continuous Performance Test-X

Continuous Performance Test-X (CPT-X) is a computer task designed to measure sustained attention, reaction time, and motor response inhibition (Connors 1995). Participants were required to monitor a continuous presentation of stimuli of target letters on the screen and respond by pressing the space bar while withholding their response when the letter specific target “X” appeared. Trials were divided into six blocks, with 1, 2, or 4 sec interstimulus intervals, with three 20-sec subblocks. Measures reported from this task include % Hits, % Omissions, % Commissions, Reaction Time (millisecond
(ms), Reaction Time Variability, and Attentional Index (d’) (a measure of overall attentiveness).

2.5.4 Trail Making Test, A and B

The Trail Making Test (TMT) is a paper-and-pencil test consisting of two parts. Part A involves drawing lines to connect consecutively numbered circles, assessing visuo-constructional processing speed. Part B involves alternating between a number and lettered sequence requiring the participant to shift sets, and measures executive functioning (Lezak 2004).

2.5.5 Digit Span, Forward and Backward

The Digit Span (DS) is a subtest of the Wechsler intelligence battery divided into two parts, DS-Forward and Backwards. DS Forward involves the examiner reading a series of numbers and the participant is to repeat the digit sequence back to the examiner exactly which is thought to be indicative of efficiency of attention. In DS Backward, the participant repeats the sequence in reverse order, a process which requires storage of information for mental manipulation of the digits thus considered a measure of working memory (Lezak 2004).

2.5.6 Iowa Gambling Task

The Iowa Gambling Task (IGT) is a highly sensitive computerized task measuring impaired decision-making (Bechara, Damasio et al. 1994). Four decks of cards (A, B, C, and D) are displayed on a screen of which participants are asked to pick a card from any deck one at a time. The goal of the game is to win as much money as possible on a $2000 loan that they receive before the beginning of the task. They are informed prior to starting the task, that they receive a monetary reward or penalty for every card they choose and
are permitted to choose from any deck at any time. Of the four decks, C and D offer smaller monetary rewards but smaller penalties, compared to decks A and B which offer larger monetary rewards but also larger penalties.

2.5.7 Kirby Delayed Discounting Task

The Kirby Delayed Discounting Task (KDDT) is a 27-item task that is a measure of impulsivity by assessing whether individuals discount hypothetical monetary amounts (Kirby, Petry et al. 1999). The participants are offered a smaller reward now, or a larger reward in a specified number of days; the three different delayed-reward magnitudes are: small ($25-35), medium ($50-60) and large ($75-85). K-values can be thought as an impulsiveness parameter, with higher values corresponding to higher levels of impulsiveness. Adequate test-retest reliability (r=0.71) and internal consistency between the three different reward magnitudes has been shown (Kirby 2009).

2.5.8 Experiential Delayed Task

The Experiential Delayed Task (EDT) is a 4-block task of at least 16 choices using a real-time adjusting-amount paradigm to assess delay discounting (Reynolds and Schiffbauer 2004). Each block has a different delay period (0, 15, 30, or 60 sec). All participants are asked to choose between an adjusted immediate amount of money to be deposited into their bank, or a probabilistic monetary certain amount ($0.30) with a delay period. Next to the computer is a coin dispenser, which dispenses money anytime a reward is won. The probabilistic option has a 35% certainty to be received, while the immediate option will always immediately be received, though decrease in amount (begins at $0.17) every time it is chosen.
2.5.9 Prepulse Inhibition

Prepulse Inhibition (PPI) of the acoustic startle response (ASR) is a 36-trial measure of sensorimotor gating (Braff, Geyer et al. 2001). First participants were screened only during the baseline session, for normal hearing using an audiometer (>45dB at 500, 1000 and 6000 Hz). If passed, participants then completed the 30-minute PPI session at the end of each neurocognitive testing day. A weak sound (prepulse) between 30 and 120 ms is presented before a louder startling sound resulting in attenuation of the startle response that is measured by eyeblink responses. A commercially available startle system (SR-lab; San Diego Instruments) recorded the start response in a sound attenuated testing room. In an upright position, participants were seated in a comfortable chair, and orbicularis oculi electromyographic (EMG) activity was recorded with 2 disc electrodes (Ag-AgCl) placed 1 cm below and 1 cm lateral to the external canthus of the right eye. The ground electrode was placed on the mastoid bone behind the ear. A 70 A-weighted decibels (dbA) background noise was superimposed on all acoustic stimuli that were delivered through headphones. Impedance was kept below 10 kohms and EMG activity was filtered (1-1500 Hz), digitalized for 250 milliseconds from the onset of the acoustic stimuli, rectified and stored for off-line analysis. Stimuli were delivered in 4 trials (0, 30, 60, and 120 ms pre-pulses) in 9 blocks for a total of 36 trials per session.

The four conditions examined were: 1) Pulse alone, in which participants heard an acoustic startle pulse of 116 dbA and 40 ms duration; 2) 30 ms pre-pulse to pulse interval, with a pulse to pre-pulse interval of 30 ms; 3) 60 ms pre-pulse to pulse interval, with a pulse to pre-pulse interval of 60 ms; 4) 120 ms pre-pulse to pulse interval, with a
pulse to pre-pulse interval of 120 ms; the latter 3 conditions had the above startle pulses preceded by a pre-pulse of 85 dbA and 20 ms duration and were presented in a randomized order after the initial pulse alone.

2.6 Study Procedure

2.6.1 Screening Session

There was a total of eleven study sessions done by all study completers. After initial phone screen eligibility, participants were invited for the one-day screening visit, which would take approximately 3 hours to complete for controls and 4 hours for patients. All study visits were conducted in the Biobehavioral Addictions and Concurrent Disorders Research Laboratory (BACDRL; Principal Investigator: Dr. Tony P. George, M.D., FRCPC) at CAMH. The candidate, Karolina Kozak (KK), with the exception of the SCID and physical examination, conducted all screening, and remaining session measures. Emily Simpkin (ES, Research Nurse) and Dr. George conducted the physical examinations. ES and Rachel Rabin (RR, PhD Candidate) conducted the SCIDs.

Participants upon arrival for screening assessment completed informed consents, followed by completion of a post-consent quiz (minimal score requirement of 80%) to determine understanding of study procedures. IQ testing was then administered using the Shipley and WTAR, followed by completion of demographic information, past smoking status, vitals, CO testing, urine testing, and medication forms. Clinical assessments were completed with the BDI, PANSS (schizophrenia patients only), SCID-IV, AIMS, BARS, and SARS. Finally physical examination and blood draws were taken.
2.6.2 Neurocognitive Sessions

Once deemed eligible by the PI, participants were assigned a neurocognitive battery randomization order and scheduled for their baseline session. Baseline assessment would take approximately 4 hours to complete. If ability to learn the neurocognitive tasks (mentioned in section 2.5) was successful and threshold was met for the SDR task (only for non-psychiatric participants), the pharmacy was notified and randomization of varenicline ordering was issued. Participants were scheduled for their medication weeks accordingly (for more detail see Table 2.1), and were contacted as a reminder prior to their Day 1 each week. All baseline and study medication assessment weeks were conducted by KK.

2.6.3 Varenicline Randomization and Administration

Dose order for participants was randomized within their respective groups using a block design. A secure location at the CAMH Queen Street research pharmacy kept the randomization code, with an on-call pharmacist having access to the codes should a treating physician require the blind to be broken due an adverse event occurrence after hours. After completion of the study and with approval by the PI, the pharmacists emailed broken blind ordering. Medication was administered twice daily under double-blind conditions for 3 consecutive days per week. The second and fourth dose were given to participants to take at home at 6PM, and were asked to return their empty blister pack and medication compliance log the following day. The other doses were administered under supervision of KK.
2.6.4 Compensation

All participants were compensated for their time, with an average hourly rate of $10, and possibility to earn up to $380 for completion of the entire study. An additional $20 of compensation was given to those who consented for the Genotyping portion of the study.
Table 2.1: Summary of Enrolled Study Assessments. After baseline session, participants were randomized for their respective weeks of varenicline dose (0, 1, or 2 mg/day). Morning and evening doses were repeated over three consecutive days, with neurocognitive testing on Day 2 and Day 3AM and PM, while monitoring any adverse events with the SAFTEE.

<table>
<thead>
<tr>
<th>Week</th>
<th>Begin Medication (Day 1)</th>
<th>1st Neurocognitive Testing Session (Day 2)</th>
<th>2nd Neurocognitive Testing Session (Day 3AM)</th>
<th>3rd Neurocognitive Testing Session (Day 3PM)</th>
</tr>
</thead>
</table>

2.7 Statistical Analysis

2.7.1 Demographics and Clinical Characteristics

All statistical calculations were completed using the Statistical Package for the Social Sciences (SPSS) for Windows version 23.0. Significance was set at $p<0.05$, two-tailed. Demographic and clinical characteristics were analyzed using Chi-Square ($\chi^2$) for categorical variables and independent t-tests for continuous variables. This included age, race, education, IQ scores and BDI for both groups, and CPZ equivalents and PANSS mean scores were calculated $\pm$ standard deviation in the patient group.
2.7.2 Neurocognitive Performance

2.7.2.1 Baseline Outcomes

Baseline neurocognitive differences were determined using independent samples t-tests comparing performance at the Day 2 placebo week. The assumption of homogeneity of variance was tested using Levene's Test of Equality of Variances. Boxplot analysis was used to determine and remove outliers greater than three standard deviations from the group mean. All data are expressed as mean ± standard deviation (SD).

2.7.2.2 Effect of Study Medication Day 3PM

To determine the effect of study medication on neurocognitive performance, a 2-way ANOVA with diagnosis (schizophrenia vs. non-psychiatric controls) and study medication (0, 1, 2 mg/day) as between subject factors and given neurocognitive outcomes (SDR, HVLT, CPT, TMT, DS, KDDT, IGT) as the within-subjects factor, was conducted for the Day 3PM session. This session was used, as it would have the highest potential plasma levels of varenicline in participants thus serving as the definitive time point of each week for comparing doses. This was followed by one-way ANOVAs for Day 3PM within each diagnosis to determine if there were any differences of neurocognitive performance between the low and high dose within each diagnosis. Post-hoc independent samples t-tests were performed if significance was found in the ANOVAs. The order of the varenicline dose in the counterbalanced sequence (e.g., 0, 0.5 or 1 mg BID on the first test week) was also analyzed among significant findings in neurocognitive performance.
2.7.2.3 Effect of Varenicline Across Time

To determine the effect of study session, a two-way repeated measures ANOVA (RM-ANOVA) with diagnosis as the between factor and study medication and session (Day 2, 3AM, 3PM) as the within factor, was conducted. 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 study medication.

2.7.3 Adverse Events and Clinical Symptoms

Pearson chi-square tests were used to determine if adverse events differed during varenicline treatment weeks compared to the baseline session in each of the groups separately. Other outcome measures including BDI, PANSS, AIMS, BARNES, and SARS were analyzed separately for each group (if applicable) using 2-way ANOVAs for dose and session.
3. Results

3.1 Study Sample

A total of 60 non-smoking (defined as never smoking, or being a former smoker for at least 1 year) participants (schizophrenia=32, non-psychiatric controls=28) were screened for study eligibility and provided written informed consent. All participants were able to consent on their own and did not require significant others to be involved. Of those screened, 15 patients and 8 non-psychiatric controls were excluded at screen (irregular EKG, n=1; lost interest, n=4, CO level >10 parts per million (ppm)/occasional smoker, n=2; IQ<80, n=3; did not pass medical exam, n=5; older than 55 yo, n=1; unstable psychiatric evaluation, n=5; other, n=2; Figure 3.1). This resulted in thirty-seven participants (schizophrenia=15, non-psychiatric controls=18) completing the baseline session. Of these four non-psychiatric controls dropped out after baseline (inability to complete neuropsychological task, n=1; other, n=3). During their first week of medication treatment, two patients and one non-psychiatric controls dropped out (noncompliance of regular antipsychotics, n=1, adverse event, n=2). This left thirty participants between the ages of 18 and 55 whom completed the protocol. Fifteen were non-psychiatric controls (males=10, females=5), and fifteen were patients diagnosed with either schizophrenia or schizoaffective disorder (males=10, females =5). All those who completed the study were contacted for a follow-up phone-call, 21 of which were confirmed.
3.1.1 Demographic and Clinical Characteristics

Patients and non-psychiatric controls were comparable on age, sex, race, years of education, WTAR IQ score and BDI, but differed on the Shipley IQ score (Table 3.1). That is, schizophrenia patients had lower IQ scores than non-psychiatric controls ($t_{18}=-1.925, p=0.07$). Five patients and three controls were past smokers. Shipley IQ scores for never smoker patients was 88.6 ± 12.7 which was higher than past smokers 80.2 ± 13.50; controls had similar mean scores between never vs. past smokers (98.83±13.12 vs. 98.33 ± 14.84, respectively). WTAR scores between never and past smokers within both diagnoses were similar (data not shown). Two non-psychiatric controls had a past history of depression, as did four patients.

With respect to patients, nearly all patients (n=12) were taking atypical antipsychotic medications alone, one was taking typical, and two were taking both. Of these, patients were prescribed aripiprazole (n=5), clozapine (n=2), loxapine (n=2), flupentixol (n=1), quetiapine (n=2), risperidone (n=3), and olanzapine (n=2). Mean CPZ equivalents and mean PANSS scores at baseline are also summarized in Table 3.1.
Figure 3.1: CONSORT Diagram. Details of recruitment, screening, drop-outs and completion rates of participants for this study.
Table 3.1 Demographics and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>SZ (n=15)</th>
<th>HS (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.1 ± 11.1</td>
<td>39.8 ± 10.6</td>
</tr>
<tr>
<td>Race (C/A/O)</td>
<td>6/7/2</td>
<td>11/2/2</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.8 ± 1.9</td>
<td>16.0 ± 3.5</td>
</tr>
<tr>
<td>Shipley IQ Score</td>
<td>85.8 ± 13.1</td>
<td>98.7 ± 12.9 *</td>
</tr>
<tr>
<td>WTAR IQ Score</td>
<td>98.7 ± 5.6</td>
<td>103.4 ± 5.4</td>
</tr>
<tr>
<td>BDI</td>
<td>5.1 ± 7.27</td>
<td>1.3 ± 2.7</td>
</tr>
<tr>
<td>CPZ Equivalents (14)</td>
<td>454.2 ± 384.0</td>
<td>--</td>
</tr>
<tr>
<td>PANSS (+)</td>
<td>14.5 ± 4.7</td>
<td>--</td>
</tr>
<tr>
<td>PANSS (-)</td>
<td>12.7 ± 2.8</td>
<td>--</td>
</tr>
<tr>
<td>PANSS General</td>
<td>24.3 ± 5.5</td>
<td>--</td>
</tr>
<tr>
<td>PANSS Total</td>
<td>51.5 ± 8.4</td>
<td>--</td>
</tr>
</tbody>
</table>

Values given in mean ± standard deviation; *, values are in numbers; *p<0.05
SZ, Schizophrenia Patients; HS, Healthy Subjects; C, Caucasian; A, African; WTAR, Wechsler Test of Adult Reading; BDI, Beck Depression Inventory; CPZ, Chlorpromazine; PANSS, Positive and Negative Symptom Scale

3.2 Neuropsychological Performance

3.2.1 Baseline Neurocognitive Performance

Baseline neurocognitive performance (*i.e.*, in the Day 2 session of the placebo week) is summarized in Table 3.2. Outliers for the respective tasks were also removed.

Overall it was observed, that across SDR, HVLT, TMTB, and KDDT patients performed worse than controls (Figure 3.2). In other words, patients had poorer VSWM, verbal memory, executive function, and were more impulsive than controls. There was also a trending difference between the two groups in the DS, backwards component representing worse working memory performance in patients compared to controls. No difference was observed between patients and controls on the CPT, IGT, DS forwards, and TMTA (Table 3.2).
Figure 3.2: Baseline Neurocognitive Performance Between Groups. Performance is significantly worse in the SZ group across all delay conditions in the SDR, HVLT, TMTB and KDDT tasks; p<0.05. Asterisks (*) represent significant findings. Bars represent standard deviations.
Table 3.2 Baseline (Placebo Day 2) Neurocognitive Performance

<table>
<thead>
<tr>
<th>Cognitive Measure (n=SZ:n=HS)</th>
<th>Subset</th>
<th>Between Group Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDR (14:13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-second delay</td>
<td></td>
<td>(t(25)=2.738, p=0.01)</td>
</tr>
<tr>
<td>15-second delay</td>
<td></td>
<td>(t(25)=2.056, p=0.05)</td>
</tr>
<tr>
<td>30-second delay</td>
<td></td>
<td>(t(25)=2.647, p=0.01)</td>
</tr>
<tr>
<td>HVLT (15:14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td></td>
<td>(t(27)=-2.126, p=0.04)</td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td>(t(27)=-3.075, p&lt;0.01)</td>
</tr>
<tr>
<td>Trial 3</td>
<td></td>
<td>(t(27)=-4.293, p&lt;0.001)</td>
</tr>
<tr>
<td>Sum of Trial 1-3</td>
<td></td>
<td>(t(27)=-3.265, p&lt;0.01)</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td></td>
<td>(t(27)=-3.276, p&lt;0.01)</td>
</tr>
<tr>
<td>Repetitions</td>
<td></td>
<td>(t(27)=0.389, p=0.70)</td>
</tr>
<tr>
<td>Intrusions</td>
<td></td>
<td>(t(20)=1.164 p=0.26)</td>
</tr>
<tr>
<td>True Positive</td>
<td></td>
<td>(t(27)=-3.239, p&lt;0.01)</td>
</tr>
<tr>
<td>False Positives</td>
<td></td>
<td>(t(27)=1.560, p=0.13)</td>
</tr>
<tr>
<td>False Positives Unrelated</td>
<td></td>
<td>(t(13)=-1.000, p=0.34)</td>
</tr>
<tr>
<td>% Retention</td>
<td></td>
<td>(t(27)=-1.601, p=0.12)</td>
</tr>
<tr>
<td>Discrimination Index</td>
<td></td>
<td>(t(27)=-2.785, p=0.01)</td>
</tr>
<tr>
<td>CPT (15:13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Hits</td>
<td></td>
<td>(t(21)=-1.648, p=0.11)</td>
</tr>
<tr>
<td>% Commission</td>
<td></td>
<td>(t(21)=1.648, p=0.11)</td>
</tr>
<tr>
<td>% Omission</td>
<td></td>
<td>(t(23)=0.454, p=0.65)</td>
</tr>
<tr>
<td>Hit Rate</td>
<td></td>
<td>(t(26)=0.441, p=0.66)</td>
</tr>
<tr>
<td>Variability</td>
<td></td>
<td>(t(15)=-0.183, p=0.86)</td>
</tr>
<tr>
<td>Attentiveness</td>
<td></td>
<td>(t(26)=0.422, p=0.677)</td>
</tr>
<tr>
<td>TMT (15:15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (sec)</td>
<td></td>
<td>(t(26)=1.457, p=0.156)</td>
</tr>
<tr>
<td>B (sec)</td>
<td></td>
<td>(t(28)=2.376, p=0.03)</td>
</tr>
<tr>
<td>B minus A (sec)</td>
<td></td>
<td>(t(28)=2.492, p=0.02)</td>
</tr>
<tr>
<td>DS (15:15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forwards</td>
<td></td>
<td>(t(28)=-1.402, p=0.17)</td>
</tr>
<tr>
<td>Backwards</td>
<td></td>
<td>(t(28)=-1.835, p=0.08^*)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>(t(28)=-1.762, p=0.09^*)</td>
</tr>
<tr>
<td>IGT (14:15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net Total</td>
<td></td>
<td>(t(27)=-0.284, p=0.78)</td>
</tr>
<tr>
<td>Total Money</td>
<td></td>
<td>(t(27)=-0.202, p=0.84)</td>
</tr>
<tr>
<td>KDDT (13:11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k for small reward magnitude</td>
<td></td>
<td>(t(22)=1.748, p=0.09^*)</td>
</tr>
<tr>
<td>k for medium reward magnitude</td>
<td></td>
<td>(t(22)=2.246, p=0.04)</td>
</tr>
<tr>
<td>k for large reward magnitude</td>
<td></td>
<td>(t(22)=1.528, p=0.14)</td>
</tr>
<tr>
<td>Geomean</td>
<td></td>
<td>(t(22)=2.291, p=0.03)</td>
</tr>
</tbody>
</table>

* trending to significance; SDR, Spatial Delay Recall; HVLT, Hopkins Verbal Learning Test; CPT, Continuous Performance Test; TMT, Trail Making Test; IGT, Iowa Gambling Test; KDDT, Kirby Delay Discounting Test 3.2.2 Effect of Varenicline Dose Between Diagnoses Day 3PM
3.2.2 Were there Differential Effects of Dose on Neurocognition?

3.2.2.1 Spatial Delay Response Task: Effect of Varenicline on Visuospatial Working Memory

After removing 2 outliers (one schizophrenia and one non-psychiatric control), a two-way ANOVA for data collected at Day 3PM sessions indicated a significant effect of diagnosis at each delay period (5 second delay; 15 second delay; 30 second delay; $F_{(1,78)}=19.750, p<0.001$) on VSWM, but no significant dose or diagnosis x dose interaction were found (Table 3.3). This was followed up by one-way ANOVAs within each diagnostic group. There was no significant main effect of varenicline on any of the three delay conditions in neither the schizophrenia group (30 sec: $F_{(2,39)}=0.761, p=0.474$; 15 sec: $F_{(2,39)}=1.184, p=0.317$; 5 sec: $F_{(2,39)}=0.441, p=0.647$) nor the control group (30 sec: $F_{(2,39)}=0.021, p=0.979$; 15 sec: $F_{(2,39)}=0.460, p=0.634$; 5 sec: $F_{(2,39)}=0.289, p=0.750$) (Figure 3.3). The order of the varenicline dose in the counterbalanced sequence (e.g., 0, 0.5 or 1 mg BID on the first test week) did not significantly alter neurocognitive results in either group (data not shown), suggesting no significant carryover effects between weeks.

Based on visual inspection, an independent t-test was run comparing the high (SZ: $t_{(26)}=-0.259, p=0.797$; HS: $t_{(26)}=-0.606, p=0.550$) and low dose (SZ: $t_{(26)}=1.389, p=0.177$; HS: $t_{(26)}=-0.979, p=0.337$) of varenicline to placebo in the SZ and HS group separately in the 15 sec delay condition. Notably, the patient group seems to improve in the low dose (Mean=18.79, SD=5.10) compared to placebo (Mean=21.71, SD=6.02), while no such effect is seen in HS. Further, it is visually apparent that in the 30 sec condition, patients seem to perform worse in the high dose compared placebo.
Table 3.3 *Dose by Diagnosis Differences on Day 3PM Session Neurocognitive*

<table>
<thead>
<tr>
<th>Cognitive Measure</th>
<th>Subset</th>
<th>Difference Between Doses (regardless of diagnosis)</th>
<th>Difference Between Diagnosis (regardless of dose)</th>
<th>Diagnosis by Dose Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDR (14:14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-second delay</td>
<td>F(2.78)=0.324, p=0.724</td>
<td>F(1.78)=5.094, p=0.027</td>
<td>F(2.78)=0.420, p=0.658</td>
<td></td>
</tr>
<tr>
<td>15-second delay</td>
<td>F(2.78)=0.445, p=0.642</td>
<td>F(1.78)=13.766, p&lt;0.001</td>
<td>F(2.78)=1.344, p=0.267</td>
<td></td>
</tr>
<tr>
<td>30-second delay</td>
<td>F(2.78)=0.450, p=0.639</td>
<td>F(1.78)=19.750, p&lt;0.001</td>
<td>F(2.78)=0.423, p=0.657</td>
<td></td>
</tr>
<tr>
<td><strong>HVLT (15:15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td>F(2.84)=1.579, p=0.212</td>
<td>F(1.84)=20.947, p&lt;0.001</td>
<td>F(2.84)=2.863, p=0.063*</td>
<td></td>
</tr>
<tr>
<td>Trial 2</td>
<td>F(2.84)=0.483, p=0.619</td>
<td>F(1.84)=38.850, p&lt;0.001</td>
<td>F(2.84)=0.963, p=0.386</td>
<td></td>
</tr>
<tr>
<td>Trial 3</td>
<td>F(2.84)=0.603, p=0.550</td>
<td>F(1.84)=44.763, p&lt;0.001</td>
<td>F(2.84)=0.035, p=0.966</td>
<td></td>
</tr>
<tr>
<td>Sum of Trial 1-3</td>
<td>F(2.84)=1.084, p=0.343</td>
<td>F(1.84)=43.800, p&lt;0.001</td>
<td>F(2.84)=1.098, p=0.338</td>
<td></td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>F(2.84)=1.253, p=0.291</td>
<td>F(1.84)=38.858, p&lt;0.001</td>
<td>F(2.84)=1.046, p=0.356</td>
<td></td>
</tr>
<tr>
<td>% Retention</td>
<td>F(2.84)=1.493, p=0.231</td>
<td>F(1.84)=22.068, p&lt;0.001</td>
<td>F(2.84)=1.301, p=0.278</td>
<td></td>
</tr>
<tr>
<td>Discrimination Index</td>
<td>F(2.84)=0.014, p=0.986</td>
<td>F(1.84)=35.000, p&lt;0.001</td>
<td>F(2.84)=0.434, p=0.649</td>
<td></td>
</tr>
<tr>
<td><strong>CPT (15:14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Hits</td>
<td>F(2.81)=1.835, p=0.166</td>
<td>F(1.81)=6.462, p=0.01</td>
<td>F(2.81)=0.156, p=0.856</td>
<td></td>
</tr>
<tr>
<td>% Omission</td>
<td>F(2.81)=1.835, p=0.166</td>
<td>F(1.81)=6.462, p=0.01</td>
<td>F(2.81)=0.156, p=0.856</td>
<td></td>
</tr>
<tr>
<td>% Commission</td>
<td>F(2.81)=0.344, p=0.710</td>
<td>F(1.81)=3.121, p=0.08*</td>
<td>F(2.81)=0.190, p=0.827</td>
<td></td>
</tr>
<tr>
<td>Hit Rate</td>
<td>F(2.81)=0.330, p=0.720</td>
<td>F(1.81)=0.596, p=0.442</td>
<td>F(2.81)=0.081, p=0.922</td>
<td></td>
</tr>
<tr>
<td><strong>TMT (14:14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (sec)</td>
<td>F(2.78)=0.344, p=0.710</td>
<td>F(1.78)=11.576, p&lt;0.001</td>
<td>F(2.78)=0.228, p=0.797</td>
<td></td>
</tr>
<tr>
<td>B (sec)</td>
<td>F(2.78)=0.203, p=0.817</td>
<td>F(1.78)=19.088, p&lt;0.001</td>
<td>F(2.78)=0.736, p=0.482</td>
<td></td>
</tr>
<tr>
<td>B minus A (sec)</td>
<td>F(2.78)=0.117, p=0.890</td>
<td>F(1.78)=13.936, p&lt;0.001</td>
<td>F(2.78)=0.863, p=0.426</td>
<td></td>
</tr>
<tr>
<td><strong>DS (15:15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forwards</td>
<td>F(2.84)=0.816, p=0.446</td>
<td>F(1.84)=6.682, p=0.011</td>
<td>F(2.84)=0.221, p=0.802</td>
<td></td>
</tr>
<tr>
<td>Backwards</td>
<td>F(2.84)=0.062, p=0.940</td>
<td>F(1.84)=4.514, p=0.037</td>
<td>F(2.84)=0.071, p=0.932</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>F(2.84)=0.292, p=0.748</td>
<td>F(1.84)=6.394, p=0.013</td>
<td>F(2.84)=0.093, p=0.911</td>
<td></td>
</tr>
<tr>
<td><strong>IGT (15:14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net Total</td>
<td>F(2.80)=0.187, p=0.830</td>
<td>F(1.80)=0.012, p=0.913</td>
<td>F(2.80)=0.256, p=0.775</td>
<td></td>
</tr>
<tr>
<td>Total Money</td>
<td>F(2.80)=0.137, p=0.872</td>
<td>F(1.80)=0.773, p=0.382</td>
<td>F(2.80)=0.041, p=0.959</td>
<td></td>
</tr>
<tr>
<td><strong>KDDT (15:13)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k for small reward</td>
<td>F(2.78)=0.397, p=0.674</td>
<td>F(1.78)=13.050, p&lt;0.001</td>
<td>F(2.78)=0.041, p=0.959</td>
<td></td>
</tr>
<tr>
<td>k for medium reward</td>
<td>F(2.78)=0.110, p=0.896</td>
<td>F(1.78)=14.486, p&lt;0.001</td>
<td>F(2.78)=0.048, p=0.953</td>
<td></td>
</tr>
<tr>
<td>k for large reward</td>
<td>F(2.78)=0.217, p=0.805</td>
<td>F(1.78)=10.989, p&lt;0.001</td>
<td>F(2.78)=0.298, p=0.743</td>
<td></td>
</tr>
<tr>
<td>Geomean</td>
<td>F(2.78)=0.181, p=0.835</td>
<td>F(1.78)=15.907, p&lt;0.001</td>
<td>F(2.78)=0.042, p=0.959</td>
<td></td>
</tr>
</tbody>
</table>

* *trending to significance*
Figure 3.3 SDR Performance at Day 3PM Between Diagnoses Across Study Medication. A significant effect of diagnosis at each delay period (5 second delay; 15 second delay; 30 second delay; p<0.001) on VSWM, but no significant dose or diagnosis x dose interaction was found. There was no significant main effect of varenicline on any of the three delay conditions in neither SZ (n=14) nor HS (n=14) group; p>0.05. Asterisks (*) represent significant findings. Bars represent standard deviations.

3.2.2.2 Effect of Dose on Other Neurocognitive Outcome Measures

Further assessment of the effects of study medication between schizophrenia patients and non-psychiatric controls across other neurocognitive measures with two-way ANOVAs revealed significant effects of diagnosis across all subsets of HVLT, TMTA, TMTB, DS forwards, DS backwards, and KDDT, but no dose or diagnosis x dose
interaction (Table 3.3). Notably, there was a trending diagnosis x dose interaction in the HVLT Trail 1 subset ($F_{(2,84)}=2.863, p=0.063$). All significant and non-significant relationships are presented in Table 3.3, with the task respective outliers removed.

To determine the effect of the varenicline within each group one-way ANOVAs were conducted between the three doses. There was no main effect of varenicline in the SZ and HS group across all subsets of outcomes in HVLT, CPT, TMTA, TMTB, DS forwards, DS backwards, IGT and KDDT. Among non-psychiatric controls, trending significance was observed in HVLT Trial 1 ($F_{(2,42)}=3.122, p=0.054$) with greater recall on the high varenicline dose (Mean=8.67, SD=2.22), and poorer performance on the lower dose (Mean=6.73, SD=1.79) when compared to placebo (Mean=7.33, SD=2.44) (Figure 3.4). On the other hand, patients had greater recall on the low dose (Mean=6.07, SD=1.75) compared to placebo (Mean=5.53, SD=1.51) and high dose (Mean=5.67, SD=1.40), but this difference was not significant. Interestingly, the same pattern was observed for TMTB, where control non-smokers appeared to have greater executive function on the high dose (Mean=37.53 ± 11.95) compared to both placebo (Mean=42.18±22.10) and low dose (Mean=48.01 ± 31.16). Whereas patient non-smokers appeared to have greater executive function on the low dose (Mean=61.85 ± 19.94) compared to placebo (Mean=68.24 ± 25.65) and high dose (Mean=65.61 ± 27.05); though these differences were not significant ($p>0.05$) (Figure 3.4). Data from the EDT task were not included in this M.Sc. thesis. Due to continuing technical difficulties, PPI analysis is also not included in this M.Sc. thesis.
3.2.3 Was there an Effect of Time within Doses on Neurocognition within Diagnoses?

3.2.3.1 Spatial Delay Response Task: Effect of Time on Visuospatial Working Memory

A one-way RM-ANOVA using 9 time-points (Week1Day2 (W1D2), W1D3AM, W1D3PM, W2D2, W2D3AM, W2D3PM, W3D2, W3D3AM, W3D3PM) demonstrated no significant changes in working memory performance in either group, across time in the 5 sec delay condition in the low dose (SZ: $F_{(2,24)}=1.923, p=0.168$; HS: $F_{(2,22)}=1.970, p=0.163$) and high dose (SZ: $F_{(2,24)}=0.866, p=0.434$; HS: $F_{(1,3,13.8)}=0.343, p=0.617$). Two patients and three controls were deemed outliers as based by boxplot analysis (data not shown), resulting with a sample of SZ=13 and HS=12. No significant change was
observed in both groups for the 15 sec delay condition in the low dose (SZ: $F(2,24)=1.763$, $p=0.193$; HS: $F(2,22)=0.630$, $p=0.542$) and high dose (SZ: $F(2,24)=0.087$, $p=0.917$; HS: $F(2,22)=0.006$, $p=0.994$). There was no significant change in SDR performance over time in the 30 sec delay condition in both diagnoses with either the low dose (SZ: $F(2,24)=0.112$, $p=0.895$; HS: $F(1.4,15.1)=0.247$, $p=0.701$) or high dose of varenicline (SZ: $F(2,24)=0.925$, $p=0.410$; HS: $F(1.4,15.0)=0.398$, $p=0.601$). Graphically, compared to baseline, SZ patients showed improvement in the low dose and some variability in the high dose, whereas the HS showed no changes compared to placebo (Figure 3.5).

### 3.2.3.2 Effect of Time on Other Neurocognitive Outcome Measures

In both diagnoses, one-way RM-ANOVA demonstrated no significant changes on verbal memory performance in HVLT subset outcomes over time in either high or low dose ($p>0.05$; data not shown). However, in the schizophrenia group, a significant effect of time was observed in the placebo week in the HVLT % Retention ($F(2,28)=3.713$, $p=0.037$). Paired t-tests revealed that there was no significant difference between Day 2 and Day 3AM ($t_{(14)}=2.024$, $p=0.062$) but a significant difference between Day 3AM and Day 3PM ($t_{(14)}=2.619$, $p=0.020$). In the CPT, three patients and 4 controls were removed since were found to be outliers. We observed a significant effect of time on % Commission in the high dose in the SZ group ($F(2,22)=4.730$, $p=0.020$) (Figure 3.6). Post-hoc analysis revealed that performance significantly differed between Day 2 and Day 3PM ($t_{(11)}=-2.569$, $p=0.026$). Similarly, a significant effect of time was found in the HS group in the high dose ($F(2,20)=5.358$, $p=0.014$), with significant different performance between both Day 2 and Day 3PM ($t_{(10)}=-2.318$, $p=0.043$) and Day 3AM and Day 3PM.
(t_{10}= -3.396, p=0.007) and also in the low dose (F_{(2,20)} = 4.073, p=0.033) with significant increase in % Commissions between both Day 2 and Day 3PM (t_{10}= -2.780, p=0.019). There was also a significant effect of time on the Attentiveness subset outcome in HS in the high dose (F_{(2,20)} = 4.679, p=0.022), where paired t-test analysis revealed a significant difference in performance between Day 3AM and Day 3PM (t_{10}=3.435, p=0.006).

Regarding the TMT task, no significant effects of time on TMTA and TMTB were seen in the patient group (n=14) in either dose (p>0.05). Among controls (n=13), no significant change was in TMTA scores, however a significant change total scores for TMTB across time emerged in the high dose (F_{(2,24)} = 3.450, p=0.048) and for TMTB-A (F_{(2,24)} = 4.594, p=0.020). Post hoc analyses revealed that the performance differed in the Day 2 and Day 3PM sessions (t_{12}=3.347, p=0.006; t_{12}=4.616, p=0.001) respectively (Figure 3.6).

In DS, no significant effects of time on DS forwards and backwards were seen in the patient group (n=15) in either dose (p>0.05). While in controls (n=15) showed a significant difference in DS forwards performance across time in the high dose (F_{(1.4,19.6)} = 4.997, p=0.027). Post-hoc analysis revealed a difference in the Day 2 and Day 3PM (t_{14} = -4.432, p=0.001) (Figure 3.6). No difference was seen in the backwards subset in either dose in HS, as well as no main effect of time on DS total in either diagnoses in either dose (data not shown).

Among patients (n=13) there was no significant main effect of time on IGT net total and total money (data not shown). Controls (n=13), showed a significant main effect of time in IGT total money in the low dose (F_{(2,24)} = 5.118, p=0.014). Paired t-test analysis revealed a significant difference in performance between Day 2 and Day 3PM (t_{12}= -
The high dose showed a trend towards significance for the main effect of time ($F_{(2,24)}=3.305, p=0.054$).

Patients demonstrated no significant main effect of time on KDDT k for medium reward magnitude for low dose, but a trend towards significance in the high dose ($F_{(1,0,12.5)}=3.637, p=0.079$). Similarly, among controls, RM-ANOVAs showed a trend towards significance for the main effect of time k for medium reward magnitude in the high dose ($F_{(2,18)}=2.935, p=0.079$) and no difference was seen in low dose (data not shown). There was also a trend towards significance for the main effect of time in HS in KDDT geomean for low dose ($F_{(1,6,9.6)}=3.980, p=0.073$).

### 3.2.4 Was there a Different Effect of Time Between Diagnoses?

#### 3.2.4.1 Effect of Time Between Diagnoses on Visuospatial Working Memory

A two-way RM-ANOVA using 9 time-points demonstrated an effect of session ($F_{(2,46)}=5.196, p=0.009$) and session x diagnosis interaction ($F_{(2,46)}=3.460, p=0.040$) in the 5 second delay, but no three-way interaction (dose x session x diagnosis) was observed ($F_{(3,0,68.8)}=0.500, p=0.683$); Mauchly’s test of sphericity was violated ($p=0.046$) thus the latter reports Greenhouse-Geiser statistics. No session (15 sec: $F_{(1,6,36,2)}=0.101, p=0.859$; 30 sec: $F_{(2,46)}=0.680, p=0.511$), session by diagnosis (15 sec: $F_{(1,6,36,2)}=0.054, p=0.912$; 30 sec: $F_{(2,46)}=0.956, p=0.392$) or three-way interaction (15 sec: $F_{(3,0,68,4)}=0.789, p=0.503$; 30 sec: $F_{(4,92)}=0.478, p=0.752$) was observed in the 15-second delay nor the 30 sec delay respectively. However, evidenced from Figure 3.4 are the selective effects of
the study medication in the schizophrenia group across all three delay periods when compared to controls.

3.2.4.2 Effect of Time Between Diagnoses on Other Neurocognitive Outcome Measures

Regarding verbal memory, two-way RM-ANOVAs demonstrated significant dose x diagnosis interaction ($F_{(2,50)}=4.342, p=0.018$) and dose x session interaction ($F_{(4,100)}=2.559, p=0.043$) in HVLT Trial 1 (SZ: $n=15$; HS: $n=15$). A significant effect of session was found in delayed recall HVLT ($F_{(2,50)}=3.887, p=0.027$) and in % retention ($F_{(2,50)}=4.154, p=0.021$). There was also a trend in a dose x session interaction in the sum of trials 1-3 ($F_{(4,100)}=2.407, p=0.054$). The CPT % commissions (12:11) revealed a significant session effect regardless of dose and diagnosis ($F_{(2,42)}=4.742, p=0.014$), as well as a trend towards a dose x diagnosis interaction in CPT Attentiveness ($F_{(2,42)}=2.978, p=0.062$). A trend was found in the DS forward (15:15) performance in dose ($F_{(2,56)}=2.613, p=0.082$) and dose x session interaction ($F_{(4,112)}=2.019, p=0.096$). There was a significant effect of session in IGT net total ($F_{(2,48)}=7.216, p=0.002$) and IGT total money ($F_{(2,48)}=5.738, p=0.006$), though no interaction effects were found (data not shown). Finally, KDDT k for medium reward magnitude showed a significant dose x session x diagnosis interaction ($F_{(2.4,51.2)}=3.263, p=0.037$), as well as a trending session x diagnosis interaction in both k for medium reward magnitude ($F_{(2,42)}=2.614, p=0.085$) and k for small reward magnitude ($F_{(2,42)}=2.493, p=0.095$).
Figure 3.5. SDR Performance in Low and High Dose of Varenicline Across Time within SZ (n=13) and HS (n=12) Compared to Placebo. There were no significant changes in working memory performance in either group, across time in all delay condition in the low dose and high dose. An effect of session (p=0.009) and session x diagnosis.
interaction \((p=0.040)\) in the 5 sec delay, but no three-way interaction \((dose \times session \times diagnosis)\) was observed. No interactions were observed in the 15 and 30 sec delay. Bars represent standard deviations.

### 3.3 Clinical Symptoms and Adverse Events

#### 3.3.1 Depression, Extra-Pyramidal, and Clinical Symptoms

Two-way ANOVAs demonstrated no changes in BDI scores in either group as a result of dose (SZ: \(F_{(2,126)}=0.293, p=0.747\); HS: \(F_{(2,119)}=0.598, p=0.552\)) or session (SZ: \(F_{(2,126)}=0.005, p=0.995\); HS: \(F_{(2,119)}=1.576, p=0.221\)). In patients \((n=15)\), there were no significant changes as a result of varenicline dose (high or low or placebo) in PANSS positive \((F_{(2,126)}=0.496, p=0.979\), PANSS negative \((F_{(2,126)}=0.174, p=0.841\), PANSS general \((F_{(2,126)}=0.083, p=0.921\), PANSS total \((F_{(2,126)}=0.059, p=0.942\), AIMS \((F_{(2,126)}=0.023, p=0.978)\), BARNES total \((F_{(2,126)}=0.299, p=0.742)\), and SARS \((F_{(2,126)}=0.016, p=0.984)\) scores. There were also no changes as a result of session in PANSS positive \((F_{(2,126)}=2.452, p=0.902)\), PANSS negative \((F_{(2,126)}=0.000, p=1.000)\), PANSS general \((F_{(2,126)}=0.055, p=0.947)\), PANSS total \((F_{(2,126)}=0.057, p=0.944)\), AIMS \((F_{(2,126)}=0.196, p=0.822)\), BARNES total \((F_{(2,126)}=0.019, p=0.982)\), and SARS scores \((F_{(2,126)}=0.038, p=0.963)\).

#### 3.3.2 Adverse Events

Among patients, chi-square tests showed significant associations between medication weeks and headache occurrence \((X^2(6)=15.229, p=0.019)\) with patients reporting less headaches across all three weeks when compared to baseline \((\text{count}=5(33\%))\). The remaining side effects showed no significant changes over time in either of the two doses in patients. No changes were found in the control group across all adverse events. All reported side effects reported are summarized in Table 3.4.
Figure 3.6. Neurocognitive Performance in Low and High Dose Varenicline Across Time in Respective Tasks and Diagnoses. SZ had greater % Commissions on high dose
(p=0.020), while HS had greater % Commissions on high (p=0.014) and low dose (p=0.033) but greater attentiveness with time (p=0.022). HS also had better executive function (p=0.048), DS attention (p=0.027) on high dose, and decision-making (p=0.014) on low dose across time. Bars represent standard deviations.
Table 3.4 Adverse Events Reported for Placebo and Varenicline Low and High Dose

<table>
<thead>
<tr>
<th>Symptom Type</th>
<th>Placebo</th>
<th>Low Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SZ (n=15)</td>
<td>HS (n=15)</td>
<td>SZ (n=15)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Indigestion</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Increased Appetite</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Decreased Appetite</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mood/Behaviour/Neurocognition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>1 (6.7%)</td>
<td>3 (20%)</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Poor Memory</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Troubles Concentrating</td>
<td>2 (13.3%)</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nightmares</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Waking too Early in the Morning</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Waking up During the Night</td>
<td>2 (13.3%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Trouble Falling Asleep</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nervous/Musculoskeletal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td>2 (13.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Backache</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Stiffness in Muscles</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Tremors/Shakiness</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Irregular/Pounding Heartbeat</td>
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<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chest Pain</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
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<td>0 (0%)</td>
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<tr>
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<td>0 (0%)</td>
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</tr>
<tr>
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<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
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<td>0 (0%)</td>
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<tr>
<td>Faintness/Lightheadedness</td>
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<td>0 (0%)</td>
<td>1 (6.7%)</td>
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<tr>
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<tr>
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<td>1 (6.7%)</td>
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<tr>
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<tr>
<td>Dry Mouth</td>
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<tr>
<td>Difficulty Sitting Still</td>
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<td>0 (0%)</td>
<td>1 (6.7%)</td>
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<tr>
<td>Blurred Vision</td>
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4. General Discussion

Neurocognitive deficits remain one of the most central features of schizophrenia that have been strongly linked to the high concurrent smoking rates in this disorder. Hence dysregulation of nAChR system that is implicated in the pathophysiology of schizophrenia is a main target of drug developmental efforts for treatment of schizophrenia, neurocognitive deficits, and nicotine dependence. The goal of developing adjunctive medications for this debilitating disorder may be accomplished by moving closer to functional and clinical outcomes. Consequently, this study was undertaken to evaluate the effects of varenicline, a partial nAChR agonist, in two different doses on neurocognitive function in non-smokers with schizophrenia with a matched non-psychiatric control group. Studying nonsmokers further eliminates any confounding nicotine dependence and withdrawal effects, thus may provide a clearer representation of varenicline’s proneurocognitive effects. Consideration that the non-smoking population of schizophrenia patients is in minority should be warranted in interpreting our results.

Our primary hypothesis was that there would be a dose-dependent improvement in working memory, among a battery of secondary neurocognitive domains (verbal memory, attention, executive function, impulsivity, decision-making), where an apparent deficit would be observed in the schizophrenia group at baseline. Overall, as we hypothesized patients had deficits across neurocognitive tasks including our main outcome of visuospatial working memory compared to non-psychiatric controls. We also observed that varenicline mediated selective effects in different domains, and differed in
dose and time effect within the two diagnoses. Varenicline was also found to be a safe medication in both groups in both doses across time.

4.1 Effects of Varenicline on Neurocognition

4.1.1 Effect of Varenicline on Visuospatial Working Memory

In line with previous research, our findings supported a clear deficit in visuospatial working memory performance both at baseline and during the medication weeks in the schizophrenia group compared to controls. Although we found no main effect of varenicline within either diagnoses, by visual inspection, it is apparent that there is some selectivity of varenicline affecting working memory in the patient group, whereas no such effect is observed in controls. Patients seemed to perform better with the low dose and worse with the high dose. This may be explained by the partial agonistic properties of varenicline, such that low dose varenicline may have agonistic effects on visuospatial working memory, whereas high dose varenicline result in antagonist effects, worsening visuospatial working memory in patients.

There have been few other studies that evaluated the effect of varenicline on working memory indices in nonsmokers and results have been mixed. Roh et al. (2014) found that performance on verbal working memory tasks while taking varenicline was better than when taking the antagonist MEC, though not better than placebo. However, this study used a single dose (1 mg) of varenicline, suggesting that repeat dosing may lend to beneficial effects of varenicline on neurocognition. In another study, Smith et al. (2012) evaluated high dose varenicline 2 mg/day for 8 weeks in smokers and non-smokers with schizophrenia. The authors found no differences between placebo and varenicline groups between baseline and week 8 on working memory. Lastly and notably,
this study is part of a larger study, which used a similar dosing regimen (0, 1, or 2 mg/day over 3 days). Wing et al. (2013) evaluated the effects of varenicline on cognitive function in smokers with schizophrenia in comparison to non-psychiatric controls under three conditions: smoking ad libitum, overnight abstinence, and reinstatement conditions. Interestingly, similar to our finding, patients performed better on the low dose of varenicline, such that changes in visuospatial working memory induced by smoking abstinence and reinstatement in the schizophrenia group were attenuated, while the high dose worsened memory during the regular smoking on day 2. Interestingly, these detrimental effects were not observed in the final two sessions possibly implying that tolerance developed over repeated dosing. No such effects were observed in controls. A possible explanation for these findings is that there was a ceiling effect during the regular smoking sessions, which prevented varenicline from having a proneurocognitive effect. Or perhaps the higher 2 mg dose of varenicline had an antagonistic effect on nAChR function, which would align with the concept of patients with schizophrenia smoking to normalize nAChR deficits and consequential neurocognitive impairments. Nonetheless, our findings suggest potential dose dependent effects in a non-linear fashion in the patient group compared to controls, and are consistent with deficits in nAChR function in this disorder. Even though one might have expected a larger effect on non-smokers than smokers due to reduced pre-exposure to nicotinic agents, our findings suggest that perhaps compared to smokers, the extent of deficits in nAChR function may not be the same.

There are several explanations for the lack of significant proneurocognitive effects of varenicline on visuospatial working memory. A possible source of
heterogeneity is that patients in our schizophrenia group performed above average than normal on the visuospatial working memory task at all three doses. One of the first studies examining spatial working memory in patients with schizophrenia, found that performance in the 30 sec delay condition were on average 45-50 mm from the target in patients and approximately 18 mm for controls, which is much greater than the performance compared to our placebo week with patients averaged distances from target (22.42 mm ±5.79), while controls were similar to this study (17.5 mm± 3.30); however, this study did not mention smoking status of participants (Keefe, Roitman et al. 1995).

There are a limited number of studies which compare performance of non-smokers on the SDR task, given the greater amount of research has focused on smokers. Nonetheless, according to our previously published protocols, schizophrenia non-smokers average distance error at the 30 sec delay was approximately 60 mm and controls with 20 mm from the target (George, Vessicchio et al. 2002). Roh et al. (2014) found that patient non-smokers averaged 40 mm in both the varenicline and placebo condition, and controls averaged to 36 mm in either condition. Thus perhaps having exclusionary criteria that is greater than our standard of 15 mm from the target is necessary in order to detect possible proneurocognitive effects of varenicline in patients.

Furthermore, one may suggest associating mean IQ scores with above average performance scores. However, our patient sample had mean IQ scores slightly lower than the general consensus of 90-95, and slightly higher premorbid IQ scores of the norm being 93; controls had IQ scores in align with the general population of 100 (Woodberry, Giuliano et al. 2008, Khandaker, Barnett et al. 2011). Therefore perhaps patients with higher premorbid IQ scores, perform better on visuospatial working memory tasks.
regardless of IQ measured post-symptom onset in schizophrenia. Additionally, we also found that never smokers had higher mean IQ scores than past smokers, while mean scores were similar between never and past smokers in the control group. Unfortunately, these subsets of patients is a relatively small sample size, and as such, future studies could recruit even numbers of such subset populations to stabilize statistical parameter estimates while minimizing variability when investigating effects of varenicline on working memory.

Finally, the indices of working memory are not the same across these studies, along with varying tasks being used to assess working memory performance. This may explain the great variability that has been reported thus far with effects of varenicline on working memory. Nonetheless, contrary to our hypothesis, we did not find a dose dependent effect of varenicline improving working memory in either group, but rather a non-linear effect that warrants further investigation.

4.1.2 Effect of Varenicline Dose on Other Neurocognitive Outcomes

In line with previous research, we observed that compared non-psychiatric controls, schizophrenia patients had worse verbal memory, attention, visuo-constructional processing speed, executive function, working memory, and delay discounting/impulsivity regardless of medication week. Furthermore, we found no improvement with either dose of varenicline on several domains including sustained attention, visuo-constructional processing speed, working memory (DS backwards), and delay discounting/impulsivity. Although contrary to what we predicted, our findings are similar to several studies.
First, Roh et al. (2014) found that there were no differences in performance on varenicline (single 1 mg dose) compared to placebo, and there were no treatment by diagnosis interactions. Notably, the authors did however find that MEC (a noncompetitive nAChR antagonist) did worsen sustained attention as assessed by CPT-Identical Pairs (IP) with outcome measures including hit reaction time, when compared to placebo and varenicline. CPT-IP is a specialized neurocognitive measure designed where participants respond when two identical pairs of numbers are presented in sequence (Cornblatt, Lenzenweger et al. 1989). This result suggested that non-smokers with schizophrenia are particularly sensitive to nAChR blockade but require greater dosing for activation. A study conducted by Barr et al (2008) found that a single dose (7-14 mg) of transdermal nicotine resulted in improved attention and inhibitory control assessed by CPT-IP, thus perhaps longer treatment with the partial agonist varenicline (greater than a single or 3-day paradigm) are required to result in proneurocognitive effects. However, another study conducted by Hong et al. (2011) found similar findings to ours, in that moderate dose varenicline (1 mg/day) in non-smokers with schizophrenia did not improve sustained attention (measured by Connors CPT d’), processing speed (measured by Digit Symbol Substitution) or in Composite or Domain scores of the MATRICS battery, and yet this study used an 8-week paradigm (slow titration of 0.5 mg daily for 1 week and then 0.5 mg bid for 7 weeks). This study however reported composite scores, which may minimize the selective effects of varenicline on working memory. Further, this study used a smoking population, which may result in varying effects of varenicline on working memory than in non-smokers. Finally, Shim et al. (2012) also found no effect of varenicline improving working memory (DS backwards), attention (DS forward, CPT-
II and Stroop Color Word Test) in non-smokers, but did in smokers with schizophrenia (for the later two tasks). Taken together, there is great variability in which tasks are used across these studies to assess yet the same neurocognitive domains. Thus some of the inconsistencies with using different and increased dosing strategies may reflect different task sensitivity to improvements resulting from varenicline, rather than varenicline itself not mitigating any proneurocognitive effects. Future studies need to use similar measures of neurocognition and outcome in order to provide reliable and consistent results. This may ultimately provide a better understanding of the link between neurocognition and real functional outcome, which can be translated into clinical practice.

Notably, when comparing the dose weeks within each diagnosis, the control group appeared to have improved executive function and initial verbal recall on the high dose as evident from the graphs (Figure 3.3) whereas patients seem to improve both executive function and verbal recall in the low dose. Though speculative, the fact that a similar pattern was observed in the visuospatial working memory domain in regards to patients, supports the idea that low dose varenicline may have agonistic properties in patients. Interestingly, the opposite may be true for controls with the high dose varenicline having agonistic properties. A possible explanation for this observation may relate to the fact that non-psychiatric control non-smokers with normal nAChR function require higher plasma levels of varenicline in order to induce proneurocognitive effects by increased activity and expression of nAChR.
4.2 Effect of Treatment and Time on Neurocognition

4.2.1 Effect of Time on Effects of Varenicline on Visuospatial Working Memory

As this is the first cross-over study to examine repeated dosing of varenicline alongside repeated neurocognitive testing in non-smokers with schizophrenia, our interpretation of the following results are preliminary. A common phenomenon is that progressive improvement would be seen in neurocognitive functions following repeated battery administration (Kantrowitz, Revheim et al. 2009). Thus the importance of examining an effect of time in a cross-over design allows one to detect any learning effects in both diagnoses.

We found that regardless of diagnosis, there was no effect of time (i.e. session Day 2, Day 3AM, Day 3PM) on visuospatial working memory performance in the three different delay periods and in three different dose groups (placebo, low, high dose). In contrast, the smoker portion of our study found there was a significant time effect in the placebo condition, no change in visuospatial working memory across time in the low dose, and impaired performance in the Day 2 session in the high dose. Accordingly, the placebo condition showed abstinence induced visuospatial working memory deficit effects, which were then attenuated with the low dose. Taken together, the ability of varenicline to attenuate negative changes in the visuospatial working memory deficit following abstinence contributes to its efficacy as a smoking cessation aid, which would make sense as to why we were not able to find any difference in visuospatial working memory in non-smokers across such a short-term. Other previous studies conducted in those without psychiatric illnesses have demonstrated that repeat dosing designs result in
beneficial effects of varenicline on neurocognition over time (Patterson, Jepson et al. 2009), however these studies are generally conducted in smokers. Since no stress was induced on the non-smokers, as is done in smokers when abstaining overnight from smoking (i.e., withdrawal and craving), one may expect time may not have as such an immediate effect. Additionally, since there was no improvement in the placebo week across time, we were able to rule out any practice effects and validate the use of our 3-day cross-over paradigm using the SDR task in non-smokers.

4.2.2 Effect of Time on Other Neurocognitive Outcomes

The effect of time across our secondary outcomes had much different results. We found that within the patient group, high dose varenicline led to a significant increase in % Commissions from Day 2 to Day 3PM. Generally, increase in commission rates indicates inattentiveness (Conners 2000), and in line with our previous observational finding of better performance in the low dose (verbal recall and executive function), it seems that the antagonistic properties of high dose varenicline are also being seen over time within the schizophrenia group, however, within the attention domain. In the control group, an increase in % Commissions was found in both the low and high dose, along with a decrease in Attentiveness over time in the high dose. Thus in the control group, varenicline may have selective effects over time and serve antagonistically at both doses in controls.

Better performance was observed in controls over time in a few neurocognitive outcomes. There was a significant effect of time in improving executive function (TMTB) and DS forwards (attention) in the high dose for controls. This finding is consistent with our earlier observation that controls perform better in the high dose
(verbal recall and executive function) (Figure 3.4). DS forwards is a measure of attention, whereas CPT-X measures sustained attention and as such the opposite time effects between the two may be owed to the subtle differences of subsets of attention being measured. Finally, there was also a significant increase in decision-making overtime in the low dose in the control group. Previous research has shown that less hypothetical monetary winnings by the end of the IGT task in schizophrenia patients compared to non-psychiatric controls, are due to significantly lower scores resulting from more disadvantageous picks (Kester, Sevy et al. 2006). However, we found that across time patients did not differ with decision-making choices, but rather that controls were making more advantageous picks in the low dose of varenicline.

Of the three previous studies in non-smokers with schizophrenia previous, Hong et al. (2011) only collected data for week 2 and week 8, Roh et al. (2014) conducted neurocognitive testing at a single session, while Shim and colleagues (2012) administered neuropsychological assessment at baseline, weeks 1, 2, 4, and 8. The later study found that CPT detectability, Digit Symbol Substitution Test, Digit Span Forward, Stroop Interference, and Wisconsin Card Sort Test categories were among the tests that showed improvement marked by repeated measures models estimating changes from baseline at each of the timepoints within the placebo group. We found across all secondary domains no practice effects. The only task which had a significant difference in performance during the placebo week was in the patient group in the HVLT % Retention outcome between Day 3AM and Day 3PM. However, contrary to what one might expect, patients retained less in the later session. This decrease in performance may reflect fatigue, which should be considered with repeated testing sessions. As an exploratory analysis, we also
found there were differential effects of time on any varenicline doses between the
diagnoses with a session by diagnosis interaction in the SDR 5 sec delay condition, as
well as dose by session interaction in the HVLT Trial 1 Recall Task. Taken together,
varenicline seems to not only have selective effects in term of cognitive domains, but also
across time with dose.

4.4 Safety and Tolerability of Varenicline Doses

*Psychiatric Symptoms (PANSS) and Depression (BDI)*

There have been concerns to the FDA (FDA 2009) and case reports (Freedman
2007) over the psychiatric safety profile of varenicline. Varenicline at both low and high
dose was not associated with the potential increase of depression, psychosis and suicide
in patients with schizophrenia. This is line with other randomized double-blind placebo-
controlled designs with varenicline used as either a smoking cessation therapy (Williams,
Anthenelli et al. 2012, Wing, Wass et al. 2013, Smith, Amiaz et al. 2016) or as a
neurocognitive enhancer (Hong, Thaker et al. 2011, Roh, Hoeppner et al. 2014). We
found there were no correlations of dose changing clinical symptoms including psychotic
negative and positive symptoms, as measured by the PANSS in the patient group, as well
as depressive symptoms in either diagnoses. Additionally, no changes were observed in
extra-pyramidal measurements between the treatment weeks, further supporting the
safety of varenicline within patients.

*Adverse Events*

In the current study we found no significant changes in side effects between the
three testing weeks. Unpredictably we also found that reports of headache occurrence
significantly decreased when compared to baseline across medication weeks. However,
these side effects are all based on self-reporting and should be taken into consideration. Nonetheless, individual participants experienced some adverse events (e.g., drowsiness, nausea, skin rash) therefore clinical vigilance is warranted. In line with this, future studies may consider to investigate whether effects of varenicline are similar and safe across these two dose groups, in both short-term and long-term trials in non-smokers. Notably, previous reporting of concerns was generally found in smoking cessation trials. One should be cautious with these medications, because while smoking cessation does not alter the metabolism of varenicline, cessation can lead to the de-induction of the liver enzyme, CYP1A2 (Prior and Baker 2003), leading to an elevation of plasma levels of certain psychotropic medications (e.g., clozapine, tricyclics, caffeine) (Desai, Seabolt et al. 2001, de Leon 2004, Haslemo, Eikeseth et al. 2006).

4.5 Limitations

The results of this study must be interpreted within the circumstance of several limitations. First, due to removing outliers respective to individual neurocognitive tasks, which also then varied when examining effect of time, our findings are limited by the relatively small sample size. This may have attributed to the lack of an effect of varenicline on visuospatial working memory, along with an effect on the secondary cognitive domains. Future replication studies assessing the effect of different doses of varenicline on visuospatial working memory may consider using subset groups of past smokers, never smokers, within both diagnoses to further examine this relationship in a non-smoking group. Nonetheless, the fact that some differences were observed across dose and time of the visuospatial working memory task, which was not observed in
controls, suggests that a greater sample size may capture significant results in the patient group.

Second, though this 3-day paradigm has been found effective in several acute trials, in a non-smoking group, titrating varenicline upward (i.e., 0.5 mg for days 1–3, 0.5 mg twice per day for days 4–7, then 1 mg twice daily for weeks 2–8) may generalize to more clinically relevant practice. Chronic dosing may also have significant pharmacodynamics effects, as well as effects on receptor and subunit expression. Further, there is a possibility that individuals may differ in their varenicline plasma levels and though collected, we did not analyze the blood plasma levels collected at day 3PM to evaluate these levels on cognitive performance. Nevertheless, 4 out of 6 doses were administered under staff supervision.

Third, although the majority of the neurocognitive tests have proven to be reliable, practice effects as well as ceiling/floor effects are still of risk on neurocognitive outcomes. Alternative options may include using different neurocognitive tests such as the California Verbal Learning Test (CVLT) (Delis 2000), which has 15 words instead of 12 as in HVLT. Additionally, although this study was a cross-over design and purposely similar to the smokers portion to allow for the future comparison to the smokers subset of data collected by our lab previously, carry-over along with practice effects are a major issue. Perhaps by spreading out testing sessions, (i.e. once per week at the end of the week), participants may minimize practice effects, and a two-week washout period would be more than sufficient to eliminate varenicline from the body (6 half-lives of varenicline, approximately 12-18 hours = 72-108 hours).
Fourth, although we set certain minimums on the visuospatial working memory task before continuing our study, patients seemed to perform above average throughout the entire study. Thus there may have not been a severe enough deficit in visuospatial working memory along with other neurocognitive domains to allow for apparent improvement during varenicline therapy.

Finally, though the tests employed in our study are robust to repeated administration, as any test, they are susceptible to changes in motivation/habituation, as well as repetition priming effects from the same stimuli (e.g., CPT). Extensive repeated testing on our data (with 9 repetitions of the key tasks) might ultimately result with effects of multiple exposures obscuring the effects of varenicline dose. This may especially be true when combined with group differences in overall proficiency, which add a great deal of systemic variance even before the varenicline dose is introduced.
5. Conclusions

To our knowledge, this is the first study using a randomized, placebo-controlled, cross-over design to evaluate two different doses of varenicline on neurocognitive in schizophrenia non-smokers. Unlike studies in smokers with schizophrenia and non-psychiatric individuals, the effects of varenicline on neurocognitive function independent of smoking cessation outcomes, is complex and nonlinear. Accordingly, we found that regardless of varenicline dose, patients performed worse than controls. Further, our data provide preliminary evidence that overall patients seem to perform better on the 1 mg/day dose across SDR, TMTB, and HVLT Trial 1 Recall, while controls perform better on the 2 mg/day dose in TMTB, HVLT Trail 1 Recall. We also demonstrated that there was no change in symptom severity with varenicline indexed by the PANSS supporting the safety and tolerability of varenicline at both high and low dose.

Further selective effects of time on varenicline effects also varied. Patients have greater % Commissions (increased inattentiveness) over time in the high dose, whereas controls were more inattentive in both doses, but had greater executive function, performed better on DS forwards (attention), and had better decision-making performance over time in the high dose. Thus it follows that pre-existing neurocognitive impairments in schizophrenia may be improved by low dose varenicline, a dose at which this smoking cessation aid serves primarily as an agonist in non-smokers. Whereas controls, may require more time and higher dose of varenicline in order to mitigate any procognitive effects.
Notably, the results of this study are preliminary and may be best explained by a model which suggests that the differential effects of varenicline dose (high versus low) in the schizophrenia patient group compared to the control non-smokers, may relate to higher sensitivity in patients to the 1 mg/day dose. Accordingly, this is best explained by findings found by Breese et al. (2000), whom demonstrated that schizophrenia patients have significantly lower expression of high-affinity nAChRs in key regions of the brain (i.e., hippocampus, PFC) than controls. Increases in nicotinic receptor levels have been shown to result from modifications to the receptor protein as a result of nicotine binding (Vallejo, Buisson et al. 2005). Thus it follows that perhaps the conformational state of the nicotinic receptors and subsequent desensitization may be related to the increases in nicotinic receptor number and decreased rate of receptor turnover (Govind, Vezina et al. 2009). Furthermore, Fenster et al. (1999) found that a requirement for receptor upregulation, particularly of the α4β2 receptor subtype, is receptor desensitization. Therefore it is possible that nicotinic receptors are not desensitized in patients, and in turn, fail to upregulate to the same extent as controls. This would support our proposed mechanism of action of varenicline, in that lower doses give maximal neurocognitive effects in schizophrenia patients, and the modest response effects that are seen in controls may be due to normal nAChR expression (Figure 4.1).

An alternative way to conceive our visuospatial working memory data in schizophrenia may relate to cortical dopamine function and spatial working memory (e.g., inverted U dose responses). It has been shown that acute nicotine administration leads to desensitization of α4β2 nAChRs projecting on the VTA in the mesocortical system. This may result in increased DA firing ultimately remediating prefrontal cortex-
dependent neurocognitive deficits (George 2007). Further, it has also been shown that chronic nicotine administration as occurs in smokers, leads to a shift in which nAChRs are activated as a result of desensitization and inactivation properties of different nAChR subtypes (Picciotto 2003). Thus smoking may have proneurocognitive effects in schizophrenia patients (George, Vessicchio et al. 2002), however, impairing spatial working memory function in healthy smokers (Park, Knopick et al. 2000).

Regardless of the mechanism resulting in abnormal levels of high affinity nAChRs in schizophrenia patients, several secondary effects exist, including neurocognitive dysfunctions. Given that the nAChR system is so widespread with roles modulating numerous components, including presynaptic receptor activation and nicotine-stimulated release of multiple types of neurotransmitters such as GABA, it is interesting to speculate that the decreased levels of nAChRs in patients may reflect in aberrant modulation in important regions of the brain that are associated with memory. Thus the implications for pharmacotherapy treatments with effective doses to modulate cognitive impairments are warranted.
Figure 4.1 Proposed Mechanisms of Low and High Dose Varenicline and Therapeutic Implications
6. Future Directions

Future studies evaluating the potential proneurocognitive effects of varenicline among those with schizophrenia as well as non-psychiatric individuals are warranted. This includes the above mentioned suggestions, such as using consistent testing regimens for commonly studied domains, as well as a greater sample size that could further establish any relationships of proneurocognitive effects by varenicline as several of our findings were trending to significance.

Additionally, by using a cross-over design we were able to reduce any confounding covariates such as demographic variability between the two diagnoses, and also within the schizophrenia group regarding antipsychotic medications. Nonetheless, several participants found the three consecutive sessions to be somewhat tedious, and may have also effected motivation to perform well in the tasks. Perhaps future studies could use bi-weekly washout period in order to reduce carry-over effects, practice effects, as well maintaining motivation in completing the study.

In clinical practice, varenicline is titrated over a one-week period to establish consistent steady state varenicline levels. Here we specifically used a 3-day paradigm in order to eventually conduct four-way comparisons with the smoker portion of the study. Nonetheless, given that neurocognitive dysfunction in schizophrenia is well-established and chronic, future studies could evaluate a longer-term treatment (i.e., 8 to 12-weeks) with the high and low dose, as it may reveal significant improvements in neurocognitive domains. One may then examine if a longer trial predicts clinical outcome later as well. Further, previous studies have found that the increase of nicotinic receptor up-regulation
exists in key regions of the human brain (i.e., thalamus, caudate, hippocampus and PFC) is both dose-dependent and reversible in control smokers but abnormal regulation of the high affinity nAChR exists in schizophrenia patients (Breese, Marks et al. 1997, Breese, Lee et al. 2000). Correspondingly, longer treatment paradigms in non-smokers examining the effect of varenicline may then be divided into four subgroups, (i.e., schizophrenia never smokers, schizophrenia past-smokers, control never smokers and control past smokers) to further assess possible differential non-smoker neurocognitive responses to varenicline.

Finally, currently within the field of schizophrenia, the \(\alpha_7\) receptor has become a novel agonist strategy. Given that varenicline is a full agonist of \(\alpha_7\), one may also question as to how we might ascertain that the discussed neurocognitive findings are owed to the \(\alpha_4\beta_2\) receptor activity. For example, a recent study found that chronic varenicline elicited upregulation of \(\alpha_4\beta_2\)-nAChR sites similar to that of chronic nicotine, whereas varenicline significantly increased \(\alpha_7\)-nAChR sites to a greater extent than nicotine (Marks, O'Neill et al. 2015). More importantly, several fMRI studies have shown that nicotine administration has improved working memory performance and attentional performance in non-smokers (Sutherland, Ross et al. 2011). Thus perhaps the most important aim for future schizophrenia research would be to conduct studies using positron emission tomography (PET) sessions with a selective \(\alpha_4\beta_2^*\) radioligand, such as the widely used 2-[(18)F]fluoro-A-85380 (2-FA) (Lotfipour, Mandelkern et al. 2011) to better identify the pharmacological actions of varenicline whilst performing neurocognitive tasks.
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Appendix

Appendix A: Telephone Screen

<table>
<thead>
<tr>
<th>Screen Initials:</th>
<th>Phone Screen date (dd/mm/yy):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer’s</td>
<td>Screen date (dd/mm/yy):</td>
</tr>
<tr>
<td>Initials:</td>
<td>Eligible?</td>
</tr>
<tr>
<td></td>
<td>□ Y   N</td>
</tr>
</tbody>
</table>

**BACDRL Phone Screen**

**Demographics:**

<table>
<thead>
<tr>
<th>Name (first/last):</th>
<th>Gender:</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Phone #</th>
<th>Voice mail message ok?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home</td>
<td>Work</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age:</th>
<th>DOB</th>
<th>Height:</th>
<th>Weight:</th>
</tr>
</thead>
<tbody>
<tr>
<td>_____ (dd/mm/yyyy):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(AGE - Cannabis 16-55, Var Lapse: 18-55, rTMS: 18-55)
(Weight: 40 – 100 kg or 88 – 220 lbs)

**Referral Source:**

<table>
<thead>
<tr>
<th>News Ad</th>
<th>CAMH flyer</th>
<th>CAMH program</th>
<th>Other</th>
</tr>
</thead>
</table>

Are you currently working right now? No Yes, Part-time Full-time

Do you currently have health coverage? No Yes

Would you be able to commit to 3 consecutive days over 3 consecutive weeks? No (exclude varcog) Yes

**Smoking: Do you smoke cigarettes?**

Yes, currently
No, never
No, past smoker since: ________________________________

Have you ever used any medication for smoking cessation? No Yes
If yes: What was it? When was it the last time you took it?

Did you experience any side effects with the smoking cessation drugs?
No Yes (Exclude Var Lapse if Champix®)

Now I am going to ask you a few questions about your **PSYCHIATRIC HISTORY**:

Have you ever seen a psychiatrist, social worker, or psychologist due to psychiatric or mental health issues? Yes No

If NO: Have you ever visited a therapist or councilor for psychological or emotional issues?
Yes No

If YES: What kind of disorder(s) have you been diagnosed with?

- Schizophrenia;
- Other psychiatric or mental disorder:
- High risk drinking
- Cannabis dependence
- Other substance abuse disorder
When diagnosed? and where?

Have you ever been an inpatient?
Yes No

If Yes: ______

If currently inpatient exclude and get in touch 3 months after they leave inpatient facility.

**Now I’d like to ask about your CURRENT MEDICATIONS:**

Are you currently taking any medication on a regular basis? Yes No

*Probe: Any homeopathic medication? Any over the counter drugs?*
If YES: please list the medication(s), including duration and dosage:

Make sure no recent dose changes!

Exclude from rTMS if:
- Wellbutrin/bupropion
- higher than 2 mg of Lorazepam or 1 mg of Clonazepam

Exclude from Varenicline if:
- Wellbutrin/bupropion
- Naltrexone
- Insulin
- Steroids
- Theophylline
- Warfarin
- NRT
- Stimulant
- Anorectic
- Mood stabilizers

Must be on stable dose of antipsychotic for at least one month for all studies

Have you participated in a research study that involved taking medication?  No  Yes
If YES, how long ago was it that you took part in the study?  
(Exclude, if less than 3 months)

Have you participated in a study that involves rTMS or ECT (electroconvulsive therapy)?  No  Yes
If YES, how long ago was it that you took part in the study?  
(Exclude, if less than 3 months)

Now, I’d like to ask you about your CURRENT SUBSTANCE USE:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Ever used?</th>
<th>Date of last use</th>
<th>Amount of use</th>
<th>Amount / week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiates (Heroin, Percocet)</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>----</td>
<td>-----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marijuana</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pills (Valium, Xanax, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (XTC, acid, speed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Alcohol: (exclude: Men>14 SD/wk; Women>9 SD/wk)
Marijuana: (exclude if current of former use for rTMS and Lapse, refer to Rachel, if no other current substance abuse)
rTMS, Var Lapse: exclude if abuse or dependence in past 3 months, CAN: 6 months

**Now I’d like to ask you some study specific questions to see which study you qualify for:**

**Cognition Study:**

Do you have one of the following:

- History of renal insufficiency
  - No
  - Yes (exclude)

- History of dementia, other neurological illness like epilepsy
  - No
  - Yes (exclude)

- Irritable bowel syndrome or any other GI problems
  - No
  - Yes (exclude)

- Are you currently taking any NRT?
  - No
  - Yes (exclude)

**ELIGIBILITY Notes**

- No
- Possibly
- Cognition
- Cannabis
- rTMS
- VARPAS

If Eligible – Schedule Screen (inform subject of urine drug screen)
Date: Time:
If not eligible: would you be interested in other studies? I could put you on the cross-referral list: Yes No
If interested in smoking cessation programs,
Refer to: NDC (175 College): 416-535-8501 ext. 7400, NDC (QS): ext. 6374
Appendix B: Study Informed Consent Form

Title of Study: “Effect of varenicline on neurocognitive function in cigarette smokers with schizophrenia”
Principal Investigator: Tony P. George, M.D., FRCPC
Co-Investigator: Dr. Mera Barr, Ph.D.
Form A: Non-Smokers with Schizophrenia

Overview:
You are invited to participate in a research study. This study will examine the effects of the investigational drug varenicline (CHAMPIX®) on neurocognitive function (i.e. attention, concentration, memory, information processing) in non-smokers and smokers with or without schizophrenia.
This study is not part of any treatment. It is expected that forty male and female participants will be enrolled.
In order to decide whether or not you wish to be a part of this research study, you should know about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study that a member of the research team will discuss with you. This discussion will go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures and possible benefits. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Voluntary Participation:
You are free to choose to participate, and, if you do so, you are free to withdraw from this study at any time. If you withdraw, it will not adversely affect your ability to receive treatment or any benefits at CAMH.

Benefits:
This study is not designed to be of direct benefit to you. However, we may develop a better understanding of how cigarette smoking and abstinence influence neurocognitive function in smokers with schizophrenia.

Description of Procedures:
If you are found to be physically healthy, you may qualify for participation in this study. You will also be asked to give the research team permission to access your medical record if necessary for the purpose of confirming your medication and treatment status (REQUEST FOR DISCLOSURE OF PERSONAL HEALTH INFORMATION Form).
A urine drug screen and carbon monoxide indicator will be used to check for the presence of other substances than your standard medication in your system (e.g., alcohol, other drugs).
If you are a woman, you will have a pregnancy test performed. While you are in the study and through 30 days after the last dose of the study medication you will have to use an adequate contraception such as a male and female condom, oral contraceptives, patch or an intrauterine device (IUD).
You will participate in three, 3-day sessions. These sessions are planned to be one week apart, which means the duration of the study will last three weeks.
Day 1: At 9.30 AM you will start taking the study medication. There will be a medical evaluation and you will be asked to fill out questionnaires and have a brief training session on the neurocognitive tests.

Day 2: At 9.30 AM you will complete the first neurocognitive testing (Session 1).

Day 3: You will have testing sessions at 9.30 AM (Session 2) and 2 PM (Session 3). You will remain under the supervision of the research assistant, during the test day from 9.30 AM to 6 PM.

The study procedures (Day 1 – 3) will be repeated during first, second and third weeks of the study.

**Neurocognitive Testing:**
The neurocognitive testing sessions will involve “paper and pencil” and computerized tests that evaluate your attention, impulsivity, concentration, memory, and ability to solve problems.

You will also participate in a procedure called pre-pulse inhibition that tests your ability to process information. The testing involves listening to white noise and loud startle “pulses” through earphones, and measures your eye-blink response with probes attached around your eye.

**Testing schedule:**
Within one week of completing the third test session, you will receive a phone call from a member of the research staff to assess your impressions of the study and to make sure you didn’t experience any problems after you finished the study protocol.

**Blood Sampling:**
During the entire study approximately 15 ml of blood will be collected which is the equivalent of about 1 teaspoon. Blood sampling for varenicline plasma levels (on D3PM) per study week (3 times in total) will also be done. If you consent extra blood will be taken for genetic and biomarker analysis (see Genetic and Biomarker Consent Form). 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.

**Study Medication:**
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.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.30 AM: Start receiving study medication</td>
<td>9.30 AM: First cognitive testing session</td>
<td>9.30 AM: Second cognitive testing session</td>
</tr>
<tr>
<td>and training on the cognitive battery</td>
<td>(Session 1)</td>
<td>(Session 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 PM: Third cognitive testing session</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Session 3)</td>
</tr>
</tbody>
</table>

Placebo or Varenicline (0.5 or 1 mg) twice daily
On Days 1 to Day 3 of each test week you will receive placebo, 0.5 mg or 1.0 mg twice daily varenicline. You will be assigned randomly (i.e. by a process like a flip of a coin) to take one of the two doses of varenicline (0.5 mg and 1.0 mg twice daily) or placebo (sugar pill). You, the research assistants or the study physician will not know whether you are taking varenicline or placebo during the test weeks.

You will be given a dose of the placebo or varenicline to take during the mornings on Days 1 and 2 under research staff supervision, and one dose to be taken at home on these days. On Day 3 of the study, you will receive the study medication under supervision in the morning and afternoon.

Overview of the medication schedule: Please note: The week order varies due to which condition you were randomly assigned to. You and the study physician will not know in which week you will receive which study medication.

<table>
<thead>
<tr>
<th>Study Week</th>
<th>(Day 1 - Begin Study Medication)</th>
<th>(Day 2, 9.30AM)</th>
<th>(Day 3, 9.30AM)</th>
<th>(Day 3, 2PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Placebo twice daily</td>
<td>Placebo twice daily</td>
<td>Placebo twice daily</td>
<td>Placebo twice daily</td>
</tr>
<tr>
<td>2</td>
<td>Varenicline, 0.5mg twice daily</td>
<td>Varenicline, 0.5mg twice daily</td>
<td>Varenicline, 0.5mg twice daily</td>
<td>Varenicline, 0.5mg twice daily</td>
</tr>
<tr>
<td>3</td>
<td>Varenicline, 1.0mg twice daily</td>
<td>Varenicline, 1.0mg twice daily</td>
<td>Varenicline, 1.0mg twice daily</td>
<td>Varenicline, 1.0mg twice daily</td>
</tr>
</tbody>
</table>

**Risks:**
There are no risks associated with the completion of the questionnaires and tasks. However, you may feel slight fatigue during the testing sessions. You may take as many breaks as you wish during the testing sessions.

There are no risks associated the pre-pulse inhibition. However, the procedure involves listening to some loud sounds through headphones which may result in mild discomfort. The maximum loudness you will hear is 116 dB which is similar to a loud rock concert but you will only hear the sounds for 0.5 to 2 seconds at a time and this is therefore unlikely to cause any damage to your hearing.

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 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 illness (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.

**Exclusion from the Study:**
If you test positive on pregnancy, alcohol or street drugs you will not be able to participate in this study. Reasons for being removed from the study may include: a serious side effects (e.g. an effect of the study medication that requires hospitalization), or not following study requirements. Also the investigator may decide to have you withdrawn from the study for any other reason.

**Confidentiality:**
All information collected about you during this study will be kept confidential and your identity will not be revealed when the results of this study are reported in presentations and publications. Your confidentiality will be protected to the extent permitted by law and your name will not be disclosed outside the clinic and institution you have attended. According to the Canadian Regulations, your records will be kept for 25 years. As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law. This study is under the authority of Health Canada as it, for example, involves evaluating a new drug. Your records may therefore be assessed by the Health Canada Therapeutic Products Programme. 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.

You may be invited to participate in more than one research study in the schizophrenia program at CAMH. Very often the researchers use the same assessments as the ones in the study you are considering. To avoid repeating the same assessments and reduce your time commitment, the researchers may share the results of common assessments completed within the past 6 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

**Reimbursement:**
You will get reimbursed $10.00 per hour for time it takes to complete the two assessment interviews (approximately 3 hours, therefore $30) and $20.00 for your blood sample to participate in the genotyping part of this study. You would be reimbursed $30.00 for your training session. You would be reimbursed $10.00 for your visit on Day 1, $30.00 for baseline evaluations (Day 2), $60.00 for completing the Day 3 morning and afternoon assessments, for a total of $100.00 per test week. Thus, you can receive up to
approximately $380.00 if you complete the entire study protocol, which you will be given on your last study visit.
If you withdraw or are removed from the study, you will be reimbursed based upon the amount of the study completed.
New Information:
If new information becomes available that is relevant to your participation to continue in the trial, you will be informed in a timely manner.
Contacts:
If you have any further questions or desire further information about this study, you may contact Dr. Tony George at 416-535-8501 x4544. If you have any questions about your rights as a study participant, you may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre for Addiction & Mental Health, at 416-535-8501 x36876.
Consent:
I, ____________________________ (name of study participant) have read the information form for the study named “Effect of Varenicline on neurocognitive function in smokers with schizophrenia”. I understand that my role is that of a participant in this study. I have been given an opportunity to ask questions about this study. Any questions that I have had have been answered to my satisfaction, so that I now understand the study procedures, the potential risks of participating, and my right to the confidential treatment of the information that is collected about me. I also understand that my participation in this study is entirely voluntary, and that I may refuse to participate or withdraw from the study at any time, without any consequences for my continuing care. I understand that I may have to leave the study without my consent if I need other treatment, do not follow the study plan, have a study-related injury, or for any other reason. If I leave the study for any reason, the study doctor may ask me to have some end-of-study tests which I may decline if I wish.
If I have any questions about my rights as a study participant, I may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre for Addiction and Mental Health, at 416-535 8501, extension x36876.
I have received a copy of this consent form for my own record.
Participant’s Initials: __________
In regards to all aspects of this consent, by signing this consent form, I am not waiving any of my legal rights.

_________________________________________   ___________________   ___________________
Study Participant Name                      Signature                  Date and Time (Printed or Typed)

_________________________________________   ___________________   ___________________
Name of Person Obtaining Consent           Signature                  Date and Time (Printed or Typed)
Appendix C: Genotyping Informed Consent Form

Title of Study: “Effect of varenicline on neurocognitive function in cigarette smokers with schizophrenia” - Genotyping Part

Principal Investigator: Tony P. George, M.D., FRCPC
Co-Principal Investigator: Dr. Mera Barr, Ph.D.

Overview:
You are invited to participate in the genotyping part of the study entitled “Effect of varenicline on neurocognitive function in cigarette smokers with schizophrenia”. We examine genes that are known to be related to cigarette smoking and schizophrenia. Information relating to these genes will be collected and added to a database of similar data from other studies so that we can understand if these genes are related to cigarette smoking in schizophrenia.

This study is not part of any treatment.

It is expected that forty male and female participants will be enrolled.

In order to decide whether or not you wish to be a part of this research study, you should know about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study that a member of the research team will discuss with you. This discussion will go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures and possible benefits. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Voluntary Participation:
You are free to choose to participate, and, if you do so, you are free to withdraw from this study at any time. If you withdraw, it will not adversely affect your ability to receive treatment or any benefits at CAMH.

Benefits:
This study is not designed to be of direct benefit to you. However, we may develop a better understanding of how cigarette smoking and abstinence influence neurocognitive function in smokers with schizophrenia.

Blood sampling:
20 ml of blood will be taken from your arm for our genotyping analysis. (15ml = one teaspoon)

Risks:
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.

**Genotyping:**
There will also be a blood sample taken to examine genes that are known to be related to nicotine receptor function, metabolism and schizophrenia. Information relating these genes will be collected and added to a database of similar data from other studies so that we can understand if these genes are related to the function of these receptors. Your genetic information will only be used for these purposes and no other. At the end of the study, your blood sample will be destroyed and only the information related to nicotine receptors, metabolism and schizophrenia will be retained in the same confidential manner as other study information (see confidentiality section).

**Confidentiality:**
All information collected about you during this study will be kept confidential and your identity will not be revealed when the results of this study are reported in presentations and publications. Your confidentiality will be protected to the extent permitted by law and your name will not be disclosed outside the clinic and institution you have attended. According to the Canadian Regulations, your records will be kept for 25 years. As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you (if your contact information is available) to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law. This study is under the authority of Health Canada as it, for example, involves evaluating a new drug. Your records may therefore be assessed by the Health Canada Therapeutic Products Programme. 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.

**Reimbursement:**
You will get reimbursed $20.00 for your participation.

**New Information:**
If new information becomes available that is relevant to your participation to continue in the trial, you will be informed in a timely manner.

**Contacts:**
If you have any further questions or desire further information about this study, you may contact Dr. Tony George at 416-535-8501 x4544. If you have any questions about your right as a study participant, you may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre of Addiction & Mental Health, at 416-535-8501 x36876.

**Consent:**
I, __________________________ (name of study participant) have read the information form for the study named “Effect of Varenicline on neurocognitive function in cigarette smokers with schizophrenia”. I understand that my role is that of a participant in this study. I have been given an opportunity to ask questions about this study. Any questions that I have had have been answered to my satisfaction, so that I now understand the study procedures, the potential risks of participating, and my right to the confidential...
treatment of the information that is collected about me. I also understand that my participation in this study is entirely voluntary, and that I may refuse to participate or withdraw from the study at any time, without any consequences for my continuing care. I understand that I may have to leave the study without my consent if I need other treatment, do not follow the study plan, have a study-related injury, or for any other reason. If I leave the study for any reason, the study doctor may ask me to have some end-of-study tests **which I may decline if I wish.** If I have any questions about my rights as a study participant, I may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre for Addiction and Mental Health, at 416-535 8501, extension36876.

I have received a copy of this consent form for my own record. Participant’s Initials: ____

In regards to all aspects of this consent, by signing this consent form, I am not waiving any of my legal rights.

________________________  _______________  ____________

Study Participant Name  Signature  Date and Time (Printed or Typed)

________________________  _______________  ____________

Name of Person Obtaining Consent  Signature  Date and Time (Printed or Typed)
Appendix D: Study Recruitment Flyer

Have you been diagnosed with schizophrenia?

✓ 18-55 years old?
✓ diagnosed with schizophrenia?
✓ taking antipsychotic medication?
✓ currently not taking street drugs?

If your answers are yes, you could qualify for our research study on Varenicline and Cognitive function. (REB 069/2009).

Please call 416 535 8501 x30463 for more details.

You will be reimbursed for your time.

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

VARENICLINE & COGNITION STUDY: ADVERSE EVENTS (SAFTEE GI Short Form, SGI, ver. A)

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>EVENT</th>
<th>DATE OF ONSET (dd/mm/yy)</th>
<th>DURATION (days)</th>
<th>SEVERITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. GENERAL INQUIRY</td>
<td></td>
<td></td>
<td></td>
<td>MN</td>
</tr>
<tr>
<td>Have you had any physical or health problems during the past week?</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>YES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you noticed any changes in your physical appearance during the past week?</td>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>YES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you cut down on the things you usually do because of not feeling well physically during the past week?</td>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>YES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5.</td>
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<tr>
<td>6.</td>
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<tr>
<td>7.</td>
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</tbody>
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Staff Initials

MN = Minimal
MI = Mild
MO = Moderate
S = Severe
VS = Very Severe
<table>
<thead>
<tr>
<th>QUESTION</th>
<th>EVENT</th>
<th>DATE OF ONSET (dd/mm/yy)</th>
<th>DURATION (days)</th>
<th>SEVERITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1. STUDY SPECIFIC EVENTS Have you had any of the following problems in the past week?</td>
<td>YES NO</td>
<td></td>
<td></td>
<td>MN MI MO S VS</td>
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<tr>
<td>1. Headache</td>
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<tr>
<td>2. Constipation</td>
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<tr>
<td>3. Backache</td>
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<tr>
<td>4. Poor memory</td>
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<td>5. Indigestion</td>
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<td>6. Nausea or vomiting</td>
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<td>7. Feeling drowsy or sleepy</td>
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<td>8. Blurred vision when reading</td>
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<td>9. Increased appetite</td>
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<td>10. Difficulties starting urination</td>
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<tr>
<td>11. Troubles concentrating</td>
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<tr>
<td>12. Nightmares</td>
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<td>13. Difficulty sitting still</td>
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<td>14. Irreg./pounding heart beat</td>
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<td>15. Chest pain</td>
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<tr>
<td>16. Diarrhea</td>
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<tr>
<td>17. Frequent need to urinate</td>
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<td>YES NO</td>
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<td></td>
<td>MN MI MO S VS</td>
</tr>
<tr>
<td>Have you had any of the following problems in the past week?</td>
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<tr>
<td>18. Dry mouth</td>
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<td>19. Decreased appetite</td>
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<td>20. Tremors/shakiness</td>
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<td>21. Skin rash</td>
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<td>22. Ringing in the ears</td>
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<td>23. Sweating</td>
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<td>24. Faintness / Light-headedness</td>
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<td>25. Poor coordination</td>
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<td>26. Stiffness in muscles</td>
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<td>27. Trouble falling asleep</td>
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<td>28. Waking up during the night</td>
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<td>29. Waking too early in the a.m.</td>
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<tr>
<td>30. Anxiety</td>
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<td>31. Dizziness</td>
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<tr>
<td>32. Flu-like symptoms</td>
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<tr>
<td>33 Runny nose</td>
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