Neuroplasticity in the Dorsolateral Prefrontal Cortex of Older People with Schizophrenia Measured by Paired Associative Stimulation

by

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

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2014

Abstract

Cognitive impairments are a common symptom of schizophrenia, and may be caused in part by disrupted neuroplasticity. Aging is associated with further decline in both neuroplasticity and cognition. Cognitive ability is one of the strongest predictors of functional outcome for people with schizophrenia, thus understanding the nature of these impairments is an important step for improving quality of life for this population.

Paired Associative Stimulation (PAS) is a transcranial magnetic stimulation protocol that assesses neuroplasticity. PAS was used to evaluate increases in cortical excitability and theta-gamma coupling – a measure that describes the relationship between neural oscillations - in older people with schizophrenia and healthy controls.

Both forms of neuroplasticity were impaired in schizophrenia. Furthermore, plasticity of theta-gamma coupling decreased with increasing age. These results contribute to understanding aging in the dorsolateral prefrontal cortex of people with schizophrenia, however further research is needed to connect these deficits to cognition.
Contributions and Acknowledgments

The following contributors were vital for the completion of this thesis project:

Reza Moghaddam, for EEG analysis of the younger subject group.

Lisa Tran, for performing neuropsychiatric assessments on participants.

Daphne Voineskos, for performing clinical assessments on schizophrenia participants.

Angela Ziluk, for performing PAS testing on the younger participant group and 10 of the older participants.

Sincere thanks to Dr. Tarek Rajji, Dr. Jeff Daskalakis, Dr. Ariel Graff, Dr. Benoit Mulsant, Dr. Willy Wong, Dr. Faranak Farzan, Dr. Sanjeev Kumar, Bahar Salavati, Natasha Radhu, Jennifer Bennie and the members of the Temerty Centre for Therapeutic Brain Intervention for their guidance and support.
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List of Abbreviations

**AMPA:** alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

**CEA:** Cortical Evoked Activity

**CEP:** Cortical Evoked Potential

**fMRI:** Functional Magnetic Resonance Imaging

**LTD:** Long-Term Depression

**LTP:** Long-Term Potentiation

**MEP:** Motor Evoked Potential

**MI:** Modulation Index

**NMDA:** N-Methyl-D-Aspartate

**PAS:** Paired Associative Stimulation

**PET:** Positron Emission Tomography

**SEP:** Sensory Evoked Potential

**TMS:** Transcranial Magnetic Stimulation
1 Literature Review

1.1 Introduction

Schizophrenia is a severe mental illness with a prevalence of approximately 1% of the population (Jablensky, 1997; Torrey, 1987). The condition has a high disease burden due to healthcare costs, strain on caregivers and difficulties for affected individuals in securing employment (Rossler, Joachim Salize, van Os, & Riecher-Rossler, 2005). People with schizophrenia contend with increased risk of suicide, comorbid depression and drug addiction, unemployment and decreased life expectancy (Buckley, Miller, Lehrer, & Castle, 2009; Hor & Taylor, 2010; Van Os & Kapur, 2009). The illness is highly heterogeneous, and is characterized by positive and negative symptoms. The positive, or psychotic symptoms include hallucinations, delusions and disordered thought. The negative, or deficit symptoms include low motivation, apathy and anhedonia (Andreasen & Olsen, 1982). Cognitive deficits are also present, in particular in executive function, information processing, and memory (Friston, 2002; Heinrichs & Zakzanis, 1998; Weickert et al., 2000). The causes of schizophrenia are not well understood however certain genes have been identified to increasing susceptibility (Owen, Williams, & O’Donovan, 2004). Environmental factors such as childhood trauma, fetal infection, urban environment have all been found to increase susceptibility as well (Van Os & Kapur, 2009).

1.2 Aging and Cognition in Schizophrenia

1.2.1 Cognition in Schizophrenia

People with schizophrenia have been found to be impaired in a number of cognitive domains, namely attention, memory, problem solving, processing speed, social cognition, verbal
comprehension, verbal learning and working memory (Nuechterlein et al., 2004). The importance of cognitive ability in quality of life for people with schizophrenia has been demonstrated in a series of reviews (J. F. Green & King, 1996; Michael F Green, Kern, & Heaton, 2004; Michael Foster Green, Kern, Braff, & Mintz, 2000), which found that cognitive function – in particular verbal memory and fluency, immediate memory and executive function - was an important predictor of functional outcome for people with schizophrenia. These outcomes include social problem solving, greater facility during daily independent living and community involvement. A review of longitudinal studies further found that cognitive assessments could predict functional outcome up to 20 years afterwards (Michael F Green, Kern, & Heaton, 2004). Thus, understanding and minimizing the factors which contribute to cognitive impairments in this population is essential for improving their quality of life.

1.2.2 Aging in Schizophrenia

In people with schizophrenia age affects both psychotic symptoms and cognition. Positive symptoms have been found to be reduced in older people, and antipsychotic doses are lower. Age is also associated with less severe depression symptoms in schizophrenia, as opposed to healthy people where depressive symptoms tend to increase with increasing age (Jeste et al., 2003). Over all, aging is associated with more stable, rather than worsening symptoms.

Due to the limitations of cognitive batteries, certain studies have failed to identify declining cognition in older people with schizophrenia (Bowie et al., 2002). Common neuropsychological assessments used to assess cognition, such as the Mini Mental State Examination (MMSE), are
not adequate for measuring differences when task performance is very low. On the other hand, assessments designed to measure cognitive ability in low-performing populations have been found to effectively show differences in cognition with age in schizophrenia. In one study that examined this phenomenon, the use of a scale for measuring cognitive impairment in Alzheimer's (Alzheimer's Disease Assessment Scale), can detect cognitive impairments in older people with schizophrenia where the MMSE cannot. (Bowie et al., 2002). Further evidence of cognitive decline in schizophrenia comes from a 6-year follow-up study that assessed patients using the Clinical Dementia Rating and MMSE. Results showed that people with schizophrenia had a higher risk of cognitive decline than unaffected people (Friedman et al., 2001). Similar to younger people with schizophrenia, older patients have been found to demonstrate impairments that differ in severity across cognitive domains. In a review of 23 studies of cognition in late-life schizophrenia, the majority of studies reported declines in executive function, verbal fluency and visuospatial ability (Tarek K Rajji & Mulsant, 2008). Given the link between cognition and functional outcome, this worsening of cognitive skills could lead to poor quality of life in older people with schizophrenia.

One research group has noted similarities between cognitive deficits in older healthy people and younger people with schizophrenia (Kirkpatrick, Messias, Harvey, Fernandez-Egea, & Bowie, 2008). They notes that the areas that are most impaired in schizophrenia – episodic verbal memory, processing speed and high-load information processing – are the same that are most vulnerable to deterioration in normal aging. (Bowie, Reichenberg, Patterson, Heaton, & Harvey, 2006). Similarly, cognitive skills like long-term verbal memory and recognition memory are
spared in both controls and schizophrenia (Kirkpatrick, Messias, Harvey, Fernandez-Egea, & Bowie, 2008). On the Weschler Adult Intelligence Scale and Rey Auditory Verbal Memory Tests, test scores of 50-60 year old people with schizophrenia are similar to those of 70-80 year old healthy older adults, whereas healthy 50-60 year-olds perform better. While these similarities might not necessarily indicate that aging and schizophrenia cause cognitive deficits through the same mechanism, it could indicate a particular circuit which is vulnerable in both cases.

1.3 Neuroplasticity

1.3.1 Long-Term Potentiation

Neuroplasticity refers to the ability of the brain to strengthen or weaken neural connections to adapt to the stimuli it receives. Long-Term Potentiation (LTP) is one form of plasticity which is considered essential for learning and memory. LTP acts at synapses to strengthen transmission between two or more neurons, and can last for over 24 hours (T. V. P. Bliss & Lømo, 1973; Malenka & Bear, 2004). The most common form of LTP is NMDA-receptor dependent, and results from coordinated neural activity that triggers calcium signaling through the NMDA receptor (Collingridge & Bliss, 1995; Morris, 1989). In order to allow calcium signaling, glutamate released from presynaptic terminals must bind to the NMDA receptor. At resting membrane potential, magnesium ions in NMDA receptor pores block calcium from entering through the receptor into the cell. When the post-synaptic neuron is depolarized, the magnesium ions are forced out of the NMDA channel. Both glutamate binding and magnesium expulsion must occur simultaneously in order for calcium to enter through NMDA channels, meaning that the pre- and post- synaptic neurons must fire simultaneously in order for signaling to be initiated.
Through this mechanism, the NMDA receptor acts as a coincidence detector (Ascher & Nowak, 1988; Collingridge & Bliss, 1995).

The entry of calcium triggers a signaling cascade that strengthens the synapse. Calcium activates calmodulin-dependent protein kinase II and protein kinase C (Ling et al., 2002; Malinow, Schulman, & Tsien, 1989). Another compounds triggered during this signaling cascade is adenylyl cyclase, which supports the formation of cyclic adenoside monophosphate and activates protein kinase A and transcription factors (Wong et al., 1999; Wu et al., 1995). The activation of protein kinases leads to phosphorylation of AMPA receptors, which enhance ion conduction at the synapse (Barria, Muller, Derkach, Griffith, & Soderling, 1997; Soderling & Derkach, 2000). The signaling pathway also causes additional AMPA receptors to be inserted in the membrane at the synapse, further strengthening synaptic strength (T. V Bliss & Collingridge, 1993; Lu et al., 2001; Malenka & Nicoll, 1999).

LTP is modulated by a multitude of neurotransmitters, one of which is dopamine. Dopamine signals through five receptor subtypes, classified into D1-like and D2-like groups. The D1-like receptors are D1 and D5, which are coupled to the Gs protein through which they activate adenylyl cyclase. D2-like receptors are D2, D3 and D4, and these are coupled to Gi and G0 proteins that inhibit adenylyl cyclase (Iversen, 1975; Kebabian & Calne, 1979). signaling though D2 receptors enhances LTP by inhibiting GABAergic interneurons, resulting in increased glutamatergic transmission (Cooper & Stanford, 2001; Hu & White, 1997; Seamans, Gorelova, Durstewitz, & Yang, 2001; Smialowski & Bijak, 1987). Evidence that dopamine is important for
LTP comes from studies reporting that dopamine facilitates LTP in the amygdala and hippocampus (Bissière, Humeau, & Lüthi, 2003; Roggenhofer et al., 2010).

The physiologic mechanism of LTP induction coupled with its long-lasting effect make it a likely candidate for a physiologic mechanism of learning and memory, and a number of experiments indicate that it does in fact play an important role in memory formation (Collingridge & Bliss, 1995). Early evidence comes from a group that performed spatial learning experiments in rats using a water maze task. The researchers demonstrated that the animals were impaired in spatial learning when given an NMDA antagonist (Morris, 1989). In addition, mice lacking NMDA receptors in the hippocampus demonstrate impaired spatial memory (Tsien, Huerta, & Tonegawa, 1996). Dysregulation of LTP might therefore be expected to result in cognitive changes such as those seen in schizophrenia.

1.3.2 Paired Associative Stimulation

Transcranial Magnetic Stimulation (TMS) is non-invasive technique for stimulating the cortex. By generating a rapidly fluctuating magnetic field in a hand-held coil, TMS allows researchers to induce a current in the intracortical fibers that run parallel to the scalp, over an area several square centimeters with the exact area depending on stimulation intensity (Thielscher & Kammer, 2004). Different stimulation parameters allow for TMS to be used to either stimulate or inhibit the cortex (Pascual-Leone, Walsh, & Rothwell, 2000). EEG recorded during TMS pulses shows a pattern of cortical evoked potentials that have been consistently replicated in a number of TMS-EEG studies in both the motor and prefrontal cortices (Bonato, Miniussi, & Rossini,
The TMS paradigm Paired Associative Stimulation (PAS) is used to assess neuroplasticity. The paradigm pairs electrical stimulation of peripheral nerves with TMS stimulation of the cortex, resulting in increased cortical excitability. Conventionally, PAS has been used to increase excitability of the connection between motor cortex and median nerve (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). An electrical stimulus is delivered to the median nerve, followed 25 ms later by a TMS pulse to the contralateral motor cortex. The 25 ms delay allows for the two stimuli to arrive simultaneously in the cortex, inducing spike-timing dependent plasticity. As PAS is typically performed by stimulating the motor cortex, plasticity can be quantified by measuring the magnitude of the motor evoked potential (MEP) measured from the abductor pollicis brevis muscle in the hand that is caused by single TMS pulses to the hand area of the motor cortex. The ratio of this measure after PAS to before PAS gives a value for potentiation. In a seminal study, 22 participants underwent PAS and demonstrated increases in motor evoked potential amplitude ranging from 5%-85%, with two thirds of participants showing at least a 30% increase. Potentiation of motor evoked potential was greatest when a 25 ms interval was used between peripheral and cortical stimulation (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000).

Regulation of inhibitory GABAergic transmission has been suggested to play an important role in the increase in cortical excitability brought on by PAS. In theory, excitation in a particular
region could be potentiated by removing GABAergic inhibition to pyramidal cells. In one study, participants treated with a GABAergic agonist displayed reduced potentiation in response to PAS (Ilić Nela, Ivana, Mirko, & Ilić Tihomir, 2012). Further evidence indicates that GABA$_A$ receptors in particular are important for this effect (Elahi, Gunraj, & Chen, 2012). Another group used a protocol that combines GABA$_A$-mediated short intracortical inhibition with PAS. They found that by enhancing SICI they were able to prevent the induction of LTP, indicating that transmission through the GABA$_A$ receptor may be important for regulating excitability. On the other hand, one study that assessed cortical inhibition before and after PAS found no difference between the two measurements, indicating that other mechanisms may be at play (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002).

Evidence that PAS induces excitability through a mechanism similar to LTP comes from studies showing that it is impaired when participants are administered an NMDA antagonist (Katja Stefan, Kunesch, Benecke, Cohen, & Classen, 2002). These results indicate that PAS-induced plasticity is dependent on signaling through NMDA receptors, as is LTP. Furthermore, increased excitability is constrained to the stimulated area and the effect depends on the simultaneous activation of the motor cortex by the electrical wrist and cortical TMS stimuli, with longer time intervals between the two being ineffective (Stefan 2000). As PAS-induced excitability shares these important characteristics with LTP, in this paper the term used for the phenomenon is “PAS-LTP”.
While PAS is most often performed in the motor cortex, it can also be used to induce plasticity in other regions, with changes in cortical excitability being measured by EEG. In the somatosensory cortex, PAS-LTP is measured by the change in amplitude of sensory evoked potentials, which are evoked in cortical area S1 by peripheral nerve stimulation. The amplitude of the SEP after PAS is compared to the amplitude before PAS (Pellicciari, Miniussi, Rossini, & De Gennaro, 2009; Lucia, Lu, Bliem, & Ziemann, 2011). This measure has behavioural significance, as changes in SEP response to median nerve stimulation after PAS have been found to be associated with improvements on a tactile discrimination task (Litvak et al., 2007). One recent study paired TMS stimulation of area M1 with TMS stimulation of the posterior parietal cortex, which is involved in motor processing (Andersen, Snyder, Bradley, & Xing, 1997). Researchers tested two PAS protocols; M1 was stimulated either 5 ms before or 5 ms after the parietal cortex. Activity in the posterior parietal region was quantified using global mean field power, but was not found to differ after either PAS protocol. However, MEP amplitude measured during single pulses to M1 showed potentiation when M1 was stimulated first and depression when parietal cortex was stimulated first (Veniero, Ponzo, & Koch, 2013). A second study following a similar protocol replicated the potentiation of MEP after paired stimulation of posterior parietal and motor cortices (Chao et al., 2013).

While pairing of stimuli to the motor and posterior parietal cortices was only effective in enhancing MEP amplitude but not cortical evoked activity, another recent study found that potentiation of cortical activity can be effectively induced in dorsolateral prefrontal cortex (DLPFC) with PAS. Researchers paired stimulation of the median nerve with TMS to the
DLPFC, which increased the magnitude of cortical evoked activity measured by EEG. Furthermore, this potentiation was localized to the left frontal region and was greatest in electrodes overlying the DLPFC (Rajji et al., 2013).

1.3.3 Neuroplasticity in Schizophrenia

Two PAS studies have been performed in people with schizophrenia. The first examined potentiation of MEPs in controls and people with schizophrenia using PAS in the motor cortex, and found significantly less plasticity in the schizophrenia group. Additionally, impairments in plasticity were significantly associated with impairments in motor learning. There was no correlation between antipsychotic dose and PAS-LTP, suggesting that the effect was likely not due to medication (Frantseva et al., 2008). Data from a second study recently presented at an international meeting, which used PAS in DLPFC, also shows impaired plasticity in people with schizophrenia (T. Rajji, 2014). Taking into account the important role of neuroplasticity in learning and memory, these results indicate a possible cause of impaired cognition in schizophrenia, however further research is needed to expand on the aforementioned studies.

Other brain stimulation studies provide further evidence that neuroplasticity is impaired in people with schizophrenia, and that this effect is independent of medication status. A study of use-dependent plasticity – which assesses the brain's adaptation to use of a muscle – reported that both medicated and unmedicated patients showed impairments compared to controls (Daskalakis et al., 2008). A second study also found impaired plasticity in the motor cortex of people with schizophrenia, assessed using transcranial direct current stimulation. Neuroplasticity was
measured in controls, patients who had experienced a single psychotic episode and patients who had experienced multiple psychotic episodes. Impairments were evident only in the patients who had multiple psychotic episodes, indicating that disease severity or duration may be a factor in these deficits. The researchers also observed no correlation between plasticity and antipsychotic dose (Hasan et al., 2011).

1.3.4 Mechanisms of Impaired Neuroplasticity in Schizophrenia

The dopamine hypothesis of schizophrenia posits that abnormal dopaminergic transmission is an important cause of its symptoms (K. L. Davis, Kahn, Ko, & Davidson, 1991). The theory has been modified and updated since its origin in the 1970s, and in its most recent incarnation argues that dopaminergic signaling is increased in the striatum and reduced in the prefrontal cortex (Howes & Kapur, 2009). Defective regulation of striatal dopamine release causes abnormalities in the salience of stimuli, and as a result the importance of inconsequential stimuli is inflated and could contribute to delusional thinking. Frontal hypodopaminergia, on the other hand, is hypothesized to be at the root of negative symptoms. The authors also suggest that negative and cognitive symptoms are not a direct result of dopamine dysregulation, but instead due to abnormalities in other neurotransmitter systems (Howes & Kapur, 2009). Indeed, a recent review of magnetic resonance spectroscopy (MRS), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging studies in unmedicated and drug-naive patients with schizophrenia describes evidence for atypical glutamatergic and GABA-ergic transmission, in addition to the aforementioned dopaminergic abnormalities (Salavati et al., n.d.). The review reports elevated glutamate and glutamine in first-episode patients that is reduced in chronic schizophrenia, as well as elevated GABA in medial prefrontal cortex.
Dopamine, glutamate and GABA all modulate LTP, therefore abnormal neurotransmission of these compounds may impair neuroplasticity and in turn memory and other cognitive abilities.

Altered neurotransmission of glutamate and dopamine have been implicated as underlying causes for disrupted plasticity in people with schizophrenia (Ben-Shachar & Laifenfeld, 2004). Glutamine, a vital neurotransmitter for LTP induction, is also abnormally regulated in schizophrenia. Low glutamate levels have been reported in cerebrospinal fluid (Kim, Kornhuber, Schmid-Burgk, & Holzmüller, 1980) as well as decreased glutamate in the prefrontal cortex (Tsai & Coyle, 2002). Glutamate receptors distributions are also abnormal, with over- and under-expression in different brain regions (Breese et al., 2000). Of particular interest, researchers have observed impaired transmission through NMDA receptors, and NMDA antagonists can induce symptoms similar to schizophrenia (Olney, Newcomer, & Farber, 1999). Genetic studies have identified a number of genes responsible for encoding NMDA receptor proteins which are disrupted in schizophrenia (Stephan, Baldeweg, & Friston, 2006). The central role of the NMDA receptor in inducing NMDA-receptor dependent LTP suggests that impaired function of these receptors would disrupt the proper regulation of neuroplasticity. Other molecules important for LTP are also abnormal in people with schizophrenia. These include dysbindin, reelin and neuregulin (Hahn et al., 2006; Impagnatiello et al., 1998; Mei & Xiong, 2008; Talbot et al., 2004).
1.3.5 Neuroplasticity and Cognition in Schizophrenia

Altered neuroplasticity in schizophrenia has implications for cognition, and has been implicated in impaired episodic, perceptual, procedural, and social learning (Weinberger 1999, Friston 2002). One hypothesis explains that abnormal neuroplasticity can cause disruptions in connectivity. Dysregulation of LTP causes a failure to appropriately modulate synaptic strength, which in turn can cause abnormal connectivity between neuronal populations. Communication and integration between functional areas is thus impaired (Stephan, Friston, & Frith, 2009). The authors note that structures that are typically highly plastic - including the hippocampus, amygdala and prefrontal cortex - are the ones most affected in schizophrenia (Friston, 2002). Furthermore, a disconnection between motor and sensory areas can create disruptions in corollary discharge - the communication of motor areas which generate an action with sensory areas which perceive it. This in turn can lead to failures of self-monitoring and the experience of hallucinations in schizophrenia (Friston, 2002). In support of this theory, a series of EEG coherence studies have shown poor communication between motor and sensory cortices in patients (Ford & Mathalon, 2005; Ford, Roach, Faustman, & Mathalon, 2008).

1.3.6 Effect of Antipsychotics on Neuroplasticity and Cognition

Antipsychotics are important medications for treating symptoms of schizophrenia. While different antipsychotic compounds have different receptor binding profiles, they all antagonize D2 receptors to some degree (Seeman, Lee, Chau-Wong, & Wong, 1976; Philip Seeman, 2002). These medications are classified into atypical and atypical antipsychotics, distinguished by the rapidity with which they dissociate from D2 receptors. Atypical antipsychotics, which generally dissociate more quickly, have the advantage of lower motor side effects (Kapur & Seeman,
While antipsychotics alleviate psychotic symptoms, there is evidence that they may alter neuroplasticity and are detrimental to cognition.

A review of the literature on antipsychotics and LTP reveals that in wild-type animals given acute doses of antipsychotics LTP is generally impaired (Price et al., 2014). On the other hand, two studies performed in animal models of schizophrenia with impaired plasticity showed that antipsychotics improved LTP, and even restored it fully when administered chronically (Delotterie et al., 2010; Xu et al., 2009). Only two studies have been published which examine the effects of antipsychotics on LTP in humans (Korchounov & Ziemann, 2011; Monte-Silva et al., 2011). Both of these were performed in healthy people given an acute antipsychotic dose, and both reported impaired plasticity as measured using TMS paradigms. These studies are limited in their application to understanding the effects on antipsychotics in people with schizophrenia because the subjects were either animals or healthy humans and for the most part were only given a single antipsychotic dose. Numerous changes in dopaminergic transmission have been reported to result from chronic antipsychotic use, including changes in D2 receptor affinity and dopamine metabolism (Bacopoulos, Redmond, Baulu, & Roth, 1980; Chiodo & Bunney, 1983; Florijn, Tarazi, & Creese, 1997; Silvestri et al., 2000; Stockton & Rasmussen, 1996; Tarazi, Florijn, & Creese, 1997; Wilmot & Szczepanik, 1989). It is therefore unlikely that the effects of acute antipsychotics on plasticity would suitably model those of chronic treatment. The results of this review indicate that additional research is needed to understand the effects of antipsychotics on neuroplasticity in people with schizophrenia receiving long-term antipsychotic treatment (Price et al., 2014).
While the aforementioned electrophysiology animal studies provide conflicting evidence, neuroanatomical research suggests that antipsychotics may benefit synaptic plasticity. Rats treated with haloperidol exhibited changes in dendritic spine shape and density which are indicative of neuroplasticity (Dawirs, Hildebrandt, & Teuchert-Noodt, 1998; Kelley, Gao, Tamminga, & Roberts, 1997). Furthermore, haloperidol has been found to induce neurogenesis in animals (Dawirs, Hildebrandt, & Teuchert-Noodt, 1998), another indication that antipsychotics may alter neuroplasticity.

While their effect on neuroplasticity is unclear, antipsychotics have been found to be detrimental to cognition (Beuzen, Taylor, Wesnes, & Wood, 1999; Sharma, 1999; Uchida et al., 2009), with some of these deficits attributed to D2 antagonism. One cross-sectional PET study in patients with schizophrenia chronically treated with the antipsychotic risperidone found that D2 occupancy in excess of 75% is correlated with attention deficits (Uchida et al., 2009). Some behavioural studies have reported improvements in cognitive abilities in first-episode schizophrenia patients who had begun antipsychotic treatment (Davidson et al., 2009; Keefe et al., 2004), however a more recent study indicates that these results are likely due to a practice effect, and may not accurately reflect improvements in cognitive ability in patients (Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008).
Figure 1.1: Effects of Age and Illness in Older People with Schizophrenia

In older people with schizophrenia, cognitive abilities can be disrupted by schizophrenia pathophysiology, aging, and antipsychotic use.

1.3.7 Aging and Neuroplasticity in Healthy People

A limited number of PAS studies have been performed in older people to investigate the effects of aging on neuroplasticity. In one study, a group of older people of mean age 61.1 +/- 4.1 years was compared to a younger group of mean age 29.8 +/- 4.5. They found that PAS-LTP was consistent for men regardless of age, but decreased in older women. This phenomenon might be explained by changes in the women's hormone levels post-menopause (Tecchio et al, 2008). In a second study, PAS-LTP was found to decrease with increasing age in both men and women. This second group of researchers explain the difference between their results and those of the 2008 study by noting that the first group used a shorter PAS protocol, suggesting that it is therefore difficult to compare their results (Fathi et al., 2010). A third study performed in the motor cortex of 27 participants age 22-71 found that increased age was associated with decreased PAS-LTP (Müller-Dahlhaus et al., 2008). Finally, one study focused on plasticity in the somatosensory cortex, a group of 16 older people exhibited a greater increase in SEP amplitude than a group of
16 younger people – indicative of increased plasticity with age. The effect was larger in women than in men (Pellicciari, Miniussi, Rossini, & De Gennaro, 2009). The results of these four PAS studies indicate that while plasticity is altered in older people, the exact nature of these changes are unclear and may differ between brain regions.

Given the challenges of non-invasively measuring synaptic plasticity in humans, the number of studies of LTP in older people is modest. However, results from behavioural experiments provide indirect evidence that neuroplasticity is impaired in older people. Aging causes cognitive decline in healthy individuals, particularly in memory and reasoning skills. These cognitive deficits are believed to largely be the result of impairments in memory, processing speed, and attentional regulation (Jones et al, 2006, Burke & Barnes, 2006). Impairments in these three cognitive domain are likely inter-related, for example working memory impairments can be worsened by slower processing speed (Jones et al, 2006). Deficits in spatial learning and memory have been observed in older participants on tasks that require recall of spatial position and ordering, though they matched the performance of younger people on landmark recognition tasks. This impairment was found to begin around the age of 60 (Newman & Kaszniak, 2000; Uttl & Graf, 1993; Wilkniss, Jones, Korol, Gold, & Manning, 1997). Impairments are also seen on tests of executive function and working memory. Aging has been shown to be associated with impaired performance on a delayed non-matching-to-sample task for working memory (Lyons-Warren, Lillie, & Hershey, 2004). It is also associated with impaired executive function as measured by the Wisconsin card sorting task, where older people have been found to make more
perseverative errors (Rhodes, 2004). Another study has found that N-back performance but not reaction time is impaired (Verhaeghen, Cerella, Bopp, & Basak, 2005).

One phenomenon that has been observed in fMRI studies of cognition in older people is an increase in neural activation compared to younger people performing the same task. This can occur as over-activation of a brain region or as a reduction in functional asymmetry, where tasks which are normally lateralized to a region in one hemisphere instead activate both (Cabeza, 2002; Grady, 2012). This increased activation has been interpreted as either a compensatory mechanism to cope with impairments in brain function associated with age, or as an indication of less efficient neural computation (Grady, 2002). A number of studies provide support for the compensatory mechanism theory, finding that over-recruitment can be associated with improved task performance in older adults (Corbetta, Patel, & Shulman, 2008; S. W. Davis, Kragel, Madden, & Cabeza, 2012; Lee, Grady, Habak, Wilson, & Moscovitch, 2011; Vincent, Kahn, Snyder, Raichle, & Buckner, 2008). Furthermore, unilateral deactivation of brain regions with TMS impairs cognitive task performance more in younger than in older participants, suggesting that the older participants are able to recruit both hemispheres for the task whereas the younger use only one (Manenti, Cotelli, & Miniussi, 2011; Rossi et al., 2004). As a compensatory mechanism, increased activation and recruitment of cortical areas can be considered a form of neuroplasticity in older people that acts to maintain cognitive performance.

Abnormal regulation of LTP has been posited as a potential cause of plasticity impairment in older people. One suggested cause of decreased LTP is reduced signaling through NMDA
receptors at perforant path-dentate gyrus synapses (Rosenzweig & Barnes, 2003). Decreased neuronal excitability could also play a role. Researchers have identified an increase in the number of calcium channels in CA1 pyramidal cells. Signaling through these channels opens potassium channels which hyperpolarize the neurons, in theory making them less excitable, though reduced excitability has not been observed in-vitro (Rosenzweig & Barnes 2003). In humans, memory improvements have been demonstrated in people aged 65-75 who had been given a drug that increases excitability by enhancing signaling through AMPA receptors (Lynch, 2002). Furthermore, animal studies provide some evidence for impaired induction and maintenance of LTP, but these results are dependent on LTP induction protocol and vary between brain regions (Burke & Barnes 2006).

Altered dopaminergic transmission is likely another important culprit for cognitive impairments in older people, as evidence suggests that dopamine signaling is diminished with increasing age. A progressive decrease in D2 receptors by approximately 10% each decade starting at the age of 20 has been observed in PET studies (Kaasinen et al., 2000; Rinne et al., 1993). Another PET study suggests that D1 receptors are affected, as there is evidence of loss of D1 receptors with increasing age in the caudate, putamen and occipital cortex, which was correlated with decreased performance on a manual dexterity task (Wang et al., 1998). Furthermore, in a study using young and elderly monkeys, a D1 antagonist caused memory impairments in young but not elderly monkeys, whereas a partial agonist alleviated cognitive impairments associated with age (Arnsten, Cai, Murphy, & Goldman-Rakic, 1994). The role of dopaminergic transmission in neural computation has been investigated using computational modelling, which has provided
evidence to support that dopamine serves to increase the signal to noise ratio by increasing signal gain (Li, Lindenberger, & Sikstrom, 2001; Winterer & Weinberger, 2004). The implication of this research for aging is that in older people, neural signaling is less effective and memories can be less distinct.

1.4 The Dorsolateral Prefrontal Cortex

1.4.1 The Dorsolateral Prefrontal Cortex and Working Memory

The dorsolateral prefrontal cortex (DLPFC) is the region of the brain responsible for executive functions, including working memory (Curtis & D’Esposito, 2003). There is extensive signaling through dopamine in this region through both D1-type and D2-type receptors (Lewis, Foote, Goldstein, & Morrison, 1988; S. M. Williams & Goldman-Rakic, 1993). Dopaminergic afferent neurons synapse onto both pyramidal cells and interneurons, allowing for either excitation or inhibition depending on the population and receptor subtype that is stimulated (Sawaguchi & Goldman-Rakic, 1994; Winterer & Weinberger, 2004). Early evidence for the key role of dopamine in DLPFC comes from a 1979 study which revealed that depletion of this neurotransmitter in the prefrontal cortex of rhesus monkeys caused deficits in working memory (Brozoski, Brown, Rosvold, & Goldman, 1979). Reduced dopamine has also been suggested to be a cause of working memory deficits in people with Parkinson's disease (Gotham, Brown, & Marsden, 1988; Levin, Llabre, & Weiner, 1989). Dopamine has an inverted U shaped effect on cognition, with very high or very low levels causing cognitive impairment (Sawaguchi, Matsumura, & Kubota, 1988; G. V Williams & Goldman-Rakic, 1995). Thus, increased
dopaminergic transmission in people with schizophrenia could be a cause of cognitive impairment in this population.

Unilateral lesions to the left and right DLPFC provide evidence of functional asymmetry in this region. (Levy & Goldman-Rakic, 2000). More recently, the results from these lesion studies have been confirmed by fMRI. Results from imaging studies show greater activation of left DLPFC during verbal encoding working memory tasks, whereas right DLPFC was more active during pattern encoding working memory tasks (Golby et al., 2001). Internal mental calculation is lateralized to left DLPFC in right-handed subjects, and are bilaterally represented in left-handed people (Burbaud et al., 1995). This lateralization of different memory types has implications for studies of working memory and DLPFC activity, as the choice of memory task can influence which side of the frontal cortex will be most active.

1.4.2 The Dorsolateral Prefrontal Cortex in Healthy Older People

The DLPFC is of interest in aging research in part because of its role in working memory, a cognitive ability that is commonly impaired with aging. Age-related deficits in DLPFC function have been shown to occur earlier than deficits in other regions. In one study, a group of average age 56.6 years compared to a group of average age 27.1, performance was already impaired on multiple tests of prefrontal cortex function. In contrast, item recognition – primarily processed in the temporal lobe - has only been shown to be significantly impaired after the age of 71 (Daigneault, Braun, & Whitaker, 1992). The vulnerability of prefrontal function to decline during aging is supported by a neuroimaging study that found that prefrontal grey matter shows
the most significant decrease in volume as people age (Raz et al., 1997). There are few studies that directly address the neural activity that underlies cognitive changes. However, one study of working memory that incorporated an imaging component sheds some light on this area (Jones et al., 2006). Participants were divided into young and old groups, and evaluated on their ability to memorize lists of nouns before and after learning a mnemonic technique. PET imaging was also performed during the task. Behavioural results showed that young people were more successful, on average, than the older group at improving their recall by employing the mnemonic technique. Imaging results showed that the young group, but not the old, showed increased activity in the left DLPFC. Furthermore, the younger group and the subset of members of the older group who did significantly improve their performance showed increased activity in the occipito-parietal region. The subset of older participants who did not improve did not demonstrate increased activity in this area. Further studies of DLPFC have shown that while function of DLPFC is typically lateralized in healthy younger people, older people can sometimes recruit this cortical regions bilaterally. Additional evidence shows that older people who recruit both left and right DLPFC during a verbal memory task perform better than those who recruit only left DLPFC, suggesting that this abnormal recruitment pattern is compensatory mechanism (Cabeza, Anderson, Locantore, & McIntosh, 2002; Reuter-Lorenz et al., 2000). Taken together the results from these behavioural studies and one imaging study are indicative of a link between aging, plasticity and neural activity.

1.4.3 The Dorsolateral Prefrontal Cortex in Sczhiophrenia

Given its important role in executive function and working memory, it is unsurprising that abnormalities in the DLPFC have been implicated in cognitive deficits in schizophrenia. Studies
of DLPFC function in people with schizophrenia consistently show impairments in working memory task performance (Heinrichs & Zakzanis, 1998). Early physiologic evidence for abnormal DLPFC function in schizophrenia came in the 1980s from cerebral blood flow research. One research group reported that in comparing people with schizophrenia to controls performing a working memory task, there was a smaller increase in blood flow to the DLPFC of the patients. In individual schizophrenia participants, higher blood flow to DLPFC was correlated with better working memory performance (Weinberger, Berman, & Zec, 1986). The results were the same in both medicated and drug-naive patients (Berman, Zec, & Weinberger, 1986). The same group reproduced these results in 1988, and furthermore found that cerbrospinal fluid concentrations of dopamine metabolites were correlated with prefrontal blood flow during a working memory task but not on other tasks unrelated to memory, which provides evidence that dopamine plays a role in working memory impairments in schizophrenia (Weinberger, Berman, & Illowsky, 1988).

A number of fMRI studies have found further evidence that activation of the DLPFC differs between healthy subjects and patients. In one study, 12 participants with schizophrenia and 10 controls performed working memory tasks of varying loads. Activity in left DLPFC was higher in the schizophrenia group, whereas there was no difference in activity in the right DLPFC. Increased left DLPFC activation was also correlated with higher error rate on the task (Manoach et al., 1999). A later study by the same research group compared unaffected siblings of people with schizophrenia to controls. Performance on the working memory task did not differ between the two groups, however the sibling group showed increased DLPFC activation similar to
patients with schizophrenia in the previous study. These results indicate a genetic component to the observed physiologic differences during working memory, and also suggest that the results observed in schizophrenia patients were not caused by antipsychotic medication (Callicott et al., 2003).

The finding of over-activation of left DLPFC in schizophrenia has been replicated by different research groups (Callicott et al., 1999; Manoach et al., 2000). In one large study, 128 participants with schizophrenia and 128 matched controls performed working memory tasks of various loads during fMRI. The patient group was found to have higher activation of DLPFC, with the greatest difference from controls when performing moderate-load tasks. When subjects were compared with controls whose performance matched theirs, the same result of higher activity in patients was observed (Potkin et al., 2009). The conclusion drawn from these studies is that the DLPFC in people with schizophrenia is less efficient than in healthy people, and so this region exhibits high activity even at moderate memory loads as a compensatory mechanism.

Anatomical studies of DLPFC also reveal differences in the brains of people with schizophrenia. In healthy people, the left DLPFC is denser than the right, whereas in people with schizophrenia the right is denser. There are differences in cell morphology as well, as pyramidal neurons in layer 3 of the cortex have been found to be larger and more round in left DLPFC of people with schizophrenia. These findings are indicative of abnormal development and function in this region.
(Cullen et al., 2006). Changes in DLPFC function associated with both aging and schizophrenia, along with the vital role that it plays in executive function and working memory, make it an important target for future research in understanding cognition in older people with schizophrenia.

1.5 Theta-Gamma Coupling and Cognition

1.5.1 Theta-Gamma Coupling

EEG measures fluctuation in electromagnetic field caused by fields of neural activity, and these voltage changes display oscillatory activity (Ward, 2003). Neural oscillations are categorized based on their frequencies; delta (0.1 – 3 Hz), theta (4 – 7 Hz) alpha (8 – 15 Hz), beta (16 – 29 Hz) and gamma (30 – 100 Hz). These different frequencies have been associated with different cognitive processes (Cullen et al., 2006).

Theta oscillations are prominent in the hippocampus and cortex, where they are associated with learning and memory (Hasselmo, 2005). Hippocampal theta oscillations are generated the medial septal region (Ward, 2003) by GABAergic and glutamatergic signaling (Ujfalussy & Kiss, 2006). Computational models of neural networks suggest that theta oscillations may play a role in signal transmission across brain areas by timing the communication between hippocampus and prefrontal cortex (Hasselmo, 2005). Gamma oscillations occur at frequencies from 30-100 Hz and are related to sensory and cognitive processing (Canan Basar-Eroglu, Struber, Schurmann, Stadler, & Basar, 1996). These high-frequency oscillations are generated by fast-spiking
interneurons signaling through GABA_\text{A} receptors (Buzsaki & Wang, 2012). While both gamma and theta oscillations are associated with different cognitive states and functions, the coupling of their amplitude and phase has been suggested to serve a role in memory and other cognitive domains (Canolty & Knight, 2010).

The interactions between these different frequency oscillations is called cross-frequency coupling and can also have functional significance. Theta-gamma coupling is a form of cross-frequency coupling that relates the phase of theta oscillations and the amplitude of gamma oscillations. When theta-gamma coupling is high, each phase of the theta oscillation is associated with a particular gamma amplitude. When coupling is low, gamma amplitude is random with respect to theta phase.

There are several different ways to calculate the magnitude of theta-gamma coupling, one of these is the Kullback-Liebler Modulation Index (MI). MI is calculated by plotting a histogram of gamma amplitude with respect to theta phase, normalizing the plot then calculating the difference between this distribution from a uniform distribution using the Kullback-Leibler statistic; a measure of the difference between two distributions (Kullback & Leibler, 1951; Tort, Komorowski, Eichenbaum, & Kopell, 2010). The Kullback-Leibler MI has the advantage over other statistics for calculating because of its greater sensitivity to coupling (Canolty & Knight, 2010; Tort et al., 2010).
1.5.2 Neural Mechanism for Coupling

Theta-gamma coupling is believed to be generated by the dynamics of GABA in inhibitory neural networks in the cortex and hippocampus (Tort et al., 2008; Wulff et al., 2009). GABAergic parvalbumin-positive interneurons have been found to be essential for theta-gamma coupling, as demonstrated by genetically modifying the fast GABA\(_A\) receptor in mice that were then shown to have impaired coupling (Wulff et al., 2009). Furthermore, a second study performed in rats administered methylazoxymethanol acetate to model schizophrenia showed that a loss of parvalbumin-containing interneurons is associated with reduced gamma oscillation power (Lodge, Behrens, & Grace, 2009). Another subset of GABA\(_A\) receptors found in the hippocampus – the slow GABA\(_A\) receptors – have a longer time constant which allows for the generation of theta oscillations. These slow GABA\(_A\) interneurons synapse onto the fast GABA\(_A\) interneurons which contribute to the generation of gamma oscillations (White, Banks, Pearce, & Kopell, 2000). Further research into the mechanism of theta-gamma coupling, performed by computational modeling of neural signaling, suggests that acetylcholine and serotonin may enable coupling through afterdepolarization. Following generation of an action potential, neurons are typically hyperpolarized to reduce their excitability, causing a refractory period during which the cell will not fire. Afterdepolarization refers to the opposite phenomenon, when cells are depolarized after firing thus facilitating subsequent action potentials. If one assumes that memories are represented by sub-populations of neurons, then by allowing neurons to fire continuously afterdepolarization could allow for maintaining a particular item in memory. The duration of afterdepolarization is sufficient for neurons to fire on subsequent theta cycles and as such would be an effective generator of theta gamma coupling (J. E. Lisman & Idiart, 1995).
By coupling the activity of theta and gamma oscillations, theta-gamma coupling could serve as a means for transmitting information across brain regions. Theta oscillations have been hypothesized to play a role in long-range synchronization (Von Stein & Sarnthein, 2000). Gamma oscillations, on the other hand, result from local processing in inhibitory networks of GABA_A interneurons (White et al., 2000). As described in Jensen et al (2007), the slower period of theta oscillations could allow for spatial integration over larger areas, enabling communication between separate networks (Jensen et al., 2007).

The model for the theta-gamma that these researchers developed further explained that theta-gamma coupling could explain the working memory limit for maintaining 7 +/- 2 items in memory at one time. The ratio between the frequencies of gamma and theta is approximately 7:1, meaning that roughly 7 gamma cycles can be nested within one cycle of theta. Evidence from these studies suggests that coupling may serve as a mechanism for ordering and separating information. Theta waves serve to segment groups of gamma waves into groups, where each gamma cycle represents a single item of information (J. E. Lisman & Idiart, 1995).

1.5.3 Role of Theta-Gamma Coupling in Cognition

An abundance of research indicates a link between theta-gamma coupling and cognition (Canolty & Knight, 2010; J. E. Lisman & Jensen, 2013). The subject has been extensively studied as it applies to mechanisms for working memory. Place cells are neurons which fire when an animal is in a particular location associated with that cell (Fenton et al., 2008). The firing of these cells has been found to be associated with extracellular field potentials, in that a cell will fire at a
particular phase of theta oscillations of the extracellular potential (O'Keefe & Recce, 1993).

Furthermore, Jensen & Lisman (1996) used experimental evidence to support a model that describes how theta gamma coupling coordinates the firing of place cells, allowing rats to recall upcoming locations based on their position in a maze. In their model, each gamma cycle represents a location, encoded by a particular subset of neurons. Each theta cycle contains multiple gamma cycles, ordered such that the place cell encoding the animal's current location is succeeded by those encoding upcoming locations. In this way, the animal's location - along with upcoming locations in the direction of movement – can be held in memory (Jensen & Lisman, 1996). In another spatial learning experiment performed in rats, theta-gamma coupling increased as the animals explored and learned their environment (Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009).

Aside from spatial learning, other memory tasks have also been shown to increase theta-gamma coupling in humans. In one study, researchers measured activity using subdural electrodes located in parietal, temporal and frontal regions. Participants performed various sensory motor and cognitive tasks. Theta gamma coupling was increased in different electrodes depending on the task (Canolty et al., 2006). In another study, theta-gamma coupling was observed during a word recognition task. Coupling was highest in the hippocampus when participants correctly identified a target, and highest in the rhinal cortex when participants correctly rejected a word (Mormann et al., 2005).
Preliminary results from the Temerty Centre for Therapeutic Brain Intervention show a relationship between theta-gamma coupling and working memory, evaluated during the N-back task. The researchers found that coupling increased with memory load (from 0- to 3-back). On the most demanding task, coupling was higher in the high-performing group than in the low performing group (T K Rajji et al., 2014). These results further highlight the relationship between theta-gamma coupling and memory.

Finally, a TMS-EEG study by the same group has shown that theta-gamma coupling is increased following PAS. This finding suggests that the modulation of coupling after PAS could be used as a measure of neuroplasticity in further PAS experiments (Tarek K Rajji et al., 2013).

### 1.5.4 Neural Oscillations and Theta-Gamma Coupling in Schizophrenia

There is limited research on theta-gamma coupling in people with schizophrenia. One recent study found no difference in coupling between people with schizophrenia and controls, however recordings were performed during steady-state auditory stimulation and not during a memory task (Kirihara, Rissling, Swerdlow, Braff, & Light, 2012). Given the suggested importance of theta-gamma coupling in memory, it would be informative to observe coupling in people with schizophrenia during a test of this cognitive ability. From the Temerty Centre group, early results show that deficiencies in theta-gamma coupling can be observed during the N-back task. Finally, in people with schizophrenia coupling is not increased by PAS to the same extent as in controls (T. Rajji, 2014).
While research on theta-gamma coupling in schizophrenia is limited, a number of studies have observed differences in neural oscillations during memory tasks, finding both gamma and theta oscillation activity to be abnormal. The first of these studies showed that gamma power differed between people with schizophrenia and controls during the N-back working memory task (Canan Basar-Eroglu et al., 2007). In controls, gamma power increased with task difficulty, whereas in people with schizophrenia it was consistently high under all memory loads. These results indicate that people with schizophrenia may be using compensatory mechanisms to perform the task, or that neural computation is less efficient in this population. Further evidence of abnormal gamma during the N-back task comes from a study where people with schizophrenia exhibited excessive gamma power in frontal regions, with the greatest difference from healthy controls seen during the most cognitively demanding task (Barr et al., 2010). A third working memory study found that while gamma power in people with schizophrenia increased during maintenance of items in memory as it did in controls, it was deficient during retrieval (Haenschel et al., 2009). Finally, a TMS-EEG study reported that TMS-evoked gamma activity was reduced in people with schizophrenia compared to healthy controls (Ferrarelli et al., 2008).

There is evidence that theta oscillations are disrupted during memory in people with schizophrenia. In one study, theta band activity during a working memory task increased in the fronto-central region of healthy controls, but not in people with schizophrenia (Schmiedt, Brand, Hildebrandt, & Basar-Eroglu, 2005). Another research group observed increased theta amplitude during steady-state auditory stimulation, which was associated with verbal memory deficits (Kirihiara et al., 2012). Deficiencies in theta coherence have also been observed, indicating that connectivity and/or transmission across cortical regions mediated by theta oscillations may be
defective (Ford, Mathalon, Whitfield, Faustman, & Roth, 2002). Finally, another group of researchers observed differences in the topography of theta power increases between schizophrenia and healthy participants during a visual oddball task (C Basar-Eroglu, Schmiedt-Fehr, Marbach, Brand, & Mathes, 2008).

Further support for impaired theta-gamma coupling in schizophrenia comes from research on the pathophysiology of the disease. As previously mentioned, parvalbumin-containing cells are necessary for normal theta rhythm generation and for theta-gamma coupling (Lodge et al., 2009). Mutant mice with reduced populations of these cells also had reduced gamma oscillation power. Research into the neuroanatomy of people with schizophrenia has shown that the numbers of these cells are reduced in multiple brain regions, including in the prefrontal cortex (Beasley & Reynolds, 1997; Zhang & Reynolds, 2002). The expected result of this reduced cell population could be impaired coupling and decreased theta oscillation power. It has been proposed that deficits in theta oscillations could cause impairments in segmenting and ordering information, potentially leading to the kinds of disordered thoughts that are seen in schizophrenia (J. Lisman & Buzsaki, 2008).

Evidence from research in neural oscillations and neuroanatomy in schizophrenia suggest that theta-gamma coupling may be impaired in people with this mental illness. At this time, there is insufficient research investigating coupling in patients to support or refute this hypothesis. Given the more abundant evidence that theta-gamma coupling serves to order and segment items in memory, deficiencies in coupling could be a possible explanation for some of the cognitive
deficits seen in people with schizophrenia. Measuring theta-gamma coupling during PAS can also serve as a measure of neuroplasticity. Single TMS pulses cause a transient increase in theta-gamma coupling, and this response is potentiated by PAS. The potentiation of coupling by PAS – like potentiation of cortical excitability – is a measure of neuroplasticity that can provide insight into cortical function in schizophrenia (Tarek K Rajji et al., 2013).

1.5.5 Theta-Gamma Coupling in Healthy Older People

There have been few studies that directly address theta-gamma coupling in healthy elderly people. One such study was performed in a group of 31 people with average age 66. The subjects performed a variety of cognitive tasks while EEG was recorded. Researchers observed that theta-gamma coupling increased in the parietal cortex, and this increase was correlated with better performance on delayed match to sample and delayed figure recall but not delayed verbal recall. This study did not include a younger subject group to compare effect of age on coupling, however the increase in coupling in older participants is consistent with what is seen in previous studies in younger subject groups (Park, Lee, & Lee, 2011). Other studies have investigated the possibility of measuring theta-gamma coupling to aid in diagnosing dementia, however little research has been performed in healthy older people. (Jackson & Snyder, 2008; Jelic & Kowalski, 2009). Further investigation in this area would provide insight into the changes in brain function and cognition associated with aging.

EEG studies in healthy older people provide evidence that theta oscillation power is decreased in this population. In one study that compared 14 older (age 60-80) and 14 younger (age 18-27)
people performing a working memory task, the researchers observed reduced theta power in the older group during retention and recognition phases. The largest discrepancy in theta power between young and old participants was observed in the frontal region. Lower theta power was also observed in older people during resting EEG, however the difference was less pronounced (Cummins & Finnigan, 2007). In a much larger study of 471 people ranging in age from 21 to 82, EEG was recorded while participants performed category and letter verbal fluency tasks. Consistent with the previous study, increased age was associated with a reduction in theta power (Brickman et al., 2005).

Gamma band power has also been found to be reduced in older people. In one study that compared children, young adults (20-26yrs) and older adults (70-76 yrs) performing a visual choice-reaction task. Older adults demonstrated lower gamma power than younger adults during the task, while gamma power in children was lower still. The authors interpreted these results as evidence that the firing or neurons in young adults is better entrained and synchronized, whereas neuron loss in older adults may impair this experience-dependent ability and results in neural activity more similar to children (Werkle-Bergner, Shing, Müller, Li, & Lindenberger, 2009). A smaller EEG study comparing a group of younger individuals with average age 36.6 and an older group of average age 47.6 performing a visual discrimination task. The results agreed with previous studies, finding lower gamma power in the older age group (Böttger, Herrmann, & von Cramon, 2002).

Despite a lack of evidence for impaired theta-gamma coupling in older people, altered theta and gamma band activity associated with aging suggests that theta-gamma coupling might also be
altered in older people. Further studies on cross-frequency coupling are needed in this population.

1.6 Conclusion
A review of the literature indicates that plasticity of cortical activity and theta-gamma coupling have not been extensively studied in older people, and in particular older people with schizophrenia. Altered neurplasticity may play a role in cognitive impairments that are observed in people with schizophrenia and worsen with increasing age, thus assessing these two measures of neuroplasticity in healthy and patient groups throughout the lifespan may lead to a better understanding of the cognitive changes that occur in people with schizophrenia as they age. The DLPFC is a region of particular interest for this research, given that it plays a vital role in working memory and executive function and thus contributes to functional outcome. Furthermore, age and schizophrenia pathophysiology have both been found to cause abnormal patterns of activity in this region. It is hoped that a better understanding of plasticity, cross-frequency coupling and aging can contribute to treatments that maximize cognition in older people with schizophrenia, preserving quality of life as they age.
2 Aims and Hypotheses

2.1.1 Neuroplasticity in the Dorsolateral Prefrontal Cortex of Older People with Schizophrenia: A Paired Associative Stimulation – Electroencephalography Study

Aim 1: Compare neuoplasticity in the DLPFC as assessed by PAS-LTP and potentiation of theta-gamma coupling in healthy older people and older people with schizophrenia.

Hypothesis 1a: People with schizophrenia will demonstrate reduced PAS-LTP in the DLPFC.

Hypothesis 1b: People with schizophrenia will demonstrate reduced potentiation of theta-gamma coupling in the DLPFC.

Aim 2: To assess the affect of age on neuroplasticity in the DLPFC in healthy controls and people with schizophrenia.

Hypothesis 2a: A regression analysis will reveal decreased PAS-LTP in the DLPFC with increased age in both participant groups.

Hypothesis 2b: A regression analysis will reveal decreased potentiation of theta-gamma coupling in the DLPFC with increased age.
Exploratory Aim 1: Examine the relationship between N-back task memory load and theta-gamma coupling.

Exploratory Hypothesis 1: Theta-gamma coupling will increase with increasing memory load. On more difficult tasks, higher coupling will be associated with higher performance.

Exploratory Aim 2: Compare theta-gamma coupling in healthy older people and older people with schizophrenia.

Exploratory Hypothesis 2: Across all difficulty levels of n-back, people with schizophrenia will exhibit lower theta-gamma coupling compared to controls.

2.1.2 Effect of Antipsychotic Dose Reduction on Neuroplasticity in the Dorsolateral Prefrontal Cortex of Older People with Schizophrenia: A Paired Associative Stimulation – Electroencephalography Study

Aim 1: Examine the relationship between PAS-LTP, theta-gamma coupling and working memory on a high antipsychotic dose.

Hypothesis 1: PAS-LTP and potentiation of theta-gamma coupling will be associated with higher n-back accuracy.
Aim 2: Examine the relationship between PAS-LTP, theta-gamma coupling and working memory on a high antipsychotic dose.

Hypothesis 2: PAS-LTP and potentiation of theta-gamma coupling will be associated with higher n-back accuracy. These measures will be increased compared to the high antipsychotic condition.

2.1.3 Cortical Response to Median Nerve Stimulation and Relationship to PAS

Aim 1: Determine whether the use of a 25 ms interstimulus interval for PAS in the DLPFC of healthy participants is supported by the SEP pattern evoked by median nerve stimulation.

Hypothesis 1: SEP analysis will show a potential in the frontal cortex close to 25 ms latency.

Aim 2: Determine whether there are differences in median nerve SEP between controls and people with schizophrenia.

Hypothesis 2: SEP timing will not differ significantly between controls and people with schizophrenia.

Aim 3: Assess whether PAS-LTP and potentiation of theta-gamma coupling are influenced by SEP timing.
Hypothesis 3: Individuals with SEP times closer to the 25ms interval used for PAS will be associated with higher PAS-LTP and potentiation of theta-gamma coupling.
3 General Methods

3.1 Localization of DLPFC

DLPFC was localized using neuronavigation techniques with the MRIcro/reg software and a T1-weight MRI scan obtained for each subject with seven fiducial markers in place and MINIBIRD system, which has a resolution of 0.5mm in position and 0.11 in orientation, with a static accuracy of 1.8mm root mean square (RMS) in position and 0.5° RMS in orientation (Ascension Technologies, USA). Stimulation was directed at the junction of the middle and anterior one-third of the middle frontal gyrus (Talairach coordinates (x, y, z)=(-50, 30, 36)), corresponding with posterior regions of Brodmann area (BA) 9, which overlap with the superior section of BA 46.

3.2 TMS-EEG Recording

To measure TMS induced cortical evoked activity, EEG was recorded concurrently with EMG recordings. To assess PAS-LTP in the DLPFC, the recording electrode that corresponds to the overlap of BA 9 and 46 of the DLPFC - as determined by DLPFC localization detailed above – was used as the site of stimulation and considered to represent activity in DLPFC. EEG recordings were acquired through a 64-channel Synamps 2 EEG system. A 64-channel EEG cap was be used to record the cortical signal, and four electrodes placed on the outer side of each eye, and above and below the left eye to monitor the eye movement artefact. All electrodes were referenced to an electrode placed on the vertex positioned posterior to the Cz electrode. EEG signals were recorded DC at a 20 kHz sampling rate and with a low pass filter of 100 Hz, which in pilot experiments was shown to avoid saturation of amplifiers and minimize the TMS related artefact.
3.3 Electromyography (EMG)

EMG was recorded from the abductor pollicis brevis (APB) with Ag-AgCl electrodes placed over the muscle belly. The EMG signal was displayed on a computer screen. The signal was amplified (Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada) filtered (band pass 2Hz – 5kHz), digitized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, UK) and stored in a laboratory computer. The subjects were instructed to relax the muscles of their arms and hands throughout the study.

TMS was delivered to the hand area of the motor cortex to evoke an EMG response in the abductor pollicis brevis muscle. Motor threshold was determined as the TMS stimulation intensity that elicited an EMG response of at least 50 microV in five out of ten trials with a gain of 10 kdB. A 1 mV peak-to-peak value was determined for each participant as the TMS stimulation intensity which elicited an average MEP response of approximately 500 microV 5 kdB.

3.4 Peripheral Nerve Stimulation

The right median nerve was stimulated at the wrist with standard bar electrodes (0.5 ms square wave constant current pulses), with cathode positioned proximally. A conditioning stimulus was delivered with a pulse width of 200 μs and intensity adjusted to three times the sensory threshold; defined as the minimum stimulation intensity for which the participant can detect a pulse. Median nerve stimulation took place during PAS, preceding the TMS pulse by 25 ms.
3.5 Transcranial Magnetic Stimulation

Monophasic TMS pulses were administered to the left DLPFC (BA 9/46) using a 7 cm figure-of-eight coil, and two Magstim 200 stimulators (Magstim Company Ltd., UK) connected via a Bistim module and MEP data was collected using commercially available software, Signal (Cambridge Electronics, UK). A train of 100 TMS pulses at 0.2 Hz at an intensity equal to 1 mV peak-to-peak value was administered before PAS (pre-PAS), immediately after (post-0), and at 15 (post-15) and 30 (post-30) minutes after finishing PAS.

3.6 Paired Associative Stimulation

PAS consisted of 180 PNS delivered to the right median nerve, each followed by a single TMS pulse delivered to the left DLPFC. The median nerve stimulation preceded TMS by 25 ms. This interstimulus interval was selected to result in approximately simultaneous arrival of both stimuli in M1 and has been reported on previous publications to markedly enhance the TMS-induced MEP following PAS (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). The same interval is used in this experiment for stimulation of DLPFC because both M1 and DLPFC are precentral. Pairs of electrical stimuli and TMS were delivered at 0.1 Hz during a 30 min period for a total of 180 pairs. Electrical median nerve stimulation was delivered at three times the sensory threshold and TMS was be delivered at an intensity equal to the 1 mV peak-to-peak intensity determined for each participant. As it has been previously shown that the effects of attention play an important role in LTP, participants were instructed to direct their attention to the right hand and to count the number of pulses felt over the duration of PAS. Participants’ count were reported several times throughout the PAS session and compared to the actual number of pulses in order
to assess attention. Although all subjects were aware that the duration of PAS would be roughly 30 min, they were not be aware of the frequency of the paired stimuli nor of their total number.

### 3.7 TMS-EEG Pre-Processing

The EEG recordings were first processed offline using commercially available software, Neuroscan (Compumedics, USA). The TMS-EEG data was down-sampled to a 1 kHz sampling frequency, and segmented with respect to the TMS stimulus such that each epoch included 1000 ms pre-stimulus baseline recording and 1000 ms post-stimulus recording. Epochs were baseline corrected with respect to the TMS-free pre-stimulus interval (1000 msec to 110 msec prior to the stimulus). The baseline corrected post stimulus intervals (25ms-1000ms) were extracted and digitally filtered by using a zero-phase shift 1-100 Hz band pass filter (48dB/Oct).

### 3.8 Cleaning TMS-EEG Recordings

In MATLAB (The MathWorks. Inc. Natick, MA, USA), the 60-Hz powerline artifact was removed using a previously described spectral estimate technique (Percival & Walden, 1993). Statistical testing was performed using the Thomson F-test. Next, trials and channels that were consistently contaminated with noise or other artifacts were selected based on extreme amplitude, signal power and standard deviation of samples. Once these channels and trials have been removed, Independent Component Analysis was run using the EEGLAB toolbox. Components representing eye movements, eye blinks and high-frequency noise were identified visually and removed. Each condition (pre-PAS, post 0, post 15, post 30, post 60) was cleaned
separately. An average CEA signal was calculated for each of these conditions from time 25 ms to 275 ms post-TMS stimulus.

3.9 Calculating PAS-LTP

In MATLAB, cleaned EEG data for every remaining trial was averaged to obtain a cortical evoked potential (CEP) for each condition (pre-PAS, post 0, post 15, post 30, post 60). The area under the rectified curve was calculated for each condition from time 25ms-275ms post-stimulus. This cutoff (25 ms post-stimulus) was chosen as it represents the earliest TMS artifact free data that can be recorded post-stimulus. Potentiation for post 0, 15, 30 and 60 were determined using the following formula:

$$\text{PAS-LTP} = \frac{\text{Area under rectified curve Post-PAS}}{\text{Area under rectified curve Pre-PAS}}$$

Thus, a value greater than one represents potentiation of the TMS cortical evoked potential. Potentiation values are calculated separately for each of the four post-PAS conditions (post 0, post 15, post 30, post 60). The values reported here are the maximum potentiation values of these four conditions.

3.10 Calculating MI in PAS Recordings

Theta-gamma coupling was evaluated using the K-L Modulation Index to measure of the modulation of gamma oscillation amplitude by theta oscillation phase (Tort et al., 2010). Pre-processed and cleaned EEG recordings were averaged across trials for each participant using MATLAB. Signals were filtered into theta (3-7 Hz) and gamma (30-50 Hz) waveforms using a zero-phase shift filter. The Hilbert transform was applied to separate phase and amplitude for
each frequency band. Theta phase was divided into six bins, each consisting of 60 degrees, with zero degrees corresponding to the waveform peak.

A distribution of gamma amplitude with respect to theta phase was created by dividing gamma amplitude into the six theta phase bins as described in detail in Tort et al (2010). This distribution was then normalized by dividing the amplitude of each bin by the sum of the amplitudes of all bins. Maximum entropy occurs when the distribution is uniform, which is when the amplitude of each bin is 1/N. Increased MI represents increased order, or lower entropy H(P).

The K-L modulation index is calculated using the following formula (Tort et al 2010):

\[
\text{MI} = \frac{\log(N)H(P)}{\log(N)}
\]

N is the number of phase bins. Log(N) is entropy of a uniform distribution, P is the relative amplitude distribution sorted according to phase bins, and H(P) is the entropy of the P distribution, which is calculated as:

\[
H(P) = \sum_{j} P(j) \log[P(j)]
\]

Potentialiation of MI is reported as the ratio of the MI at the time of maximum gamma power after PAS to the MI during pre-PAS. Recordings are MI is calculated during the 250ms interval from 25ms post-TMS stimulus to 275 ms post-TMS stimulus. Gamma power is found for times post-0,
post-15, post-30 and post-60 by bandpass filtering averaged waveforms for each condition for gamma oscillations (30 Hz-50 Hz).

### 3.11 N-Back EEG Recording

EEG recordings were acquired through a 64-channel Synamps 2 EEG system. A 64-channel EEG cap was used to record the cortical signal, and four electrodes placed on the outer side of each eye, and above and below the left eye to monitor the eye movement artefact. All electrodes were referenced to an electrode placed on the vertex positioned posterior to the Cz electrode. EEG signals were recorded DC at a 1 kHz sampling rate.

Participants performed a computerized version of the N-back task for verbal working memory. Stim2 software (Neuroscan) was used for displaying stimuli and recording participant responses. 0-, 1-, 2- and 3-back tasks were performed in randomized order for the majority of subjects. The last three participants performed the tasks in order of increasing difficulty (0, 1, 2 then 3-back) so that participants could more easily understand task instructions. Stimuli were the first 13 letters of the alphabet displayed in random order black font on a time on a white background. Letters were presented one at a time for 250 ms, followed by a 3000 ms delay period. Participants were instructed to indicate whether each letter matched the target “n” letters ago using their right hand on a response pad. When these letters matched, it is referred to as a target condition. When they did not match the trial is termed a non-target condition. The 3-back was performed for 30 min while the other tasks lasted 15 min each.
EEG recordings were acquired through a 64-channel Synamps 2 EEG system. A 64-channel EEG cap was used to record the cortical signal, and four electrodes placed on the outer side of each eye, and above and below the left eye to closely monitor the eye movement artefact. All electrodes were referenced to an electrode placed on the vertex positioned posterior to the Cz electrode. EEG signals were recorded DC at 1 kHz.

3.12 Pre-Processing and Cleaning

N-back EEG data recorded at 1kHz was imported into MATLAB. Html files recorded by STIM2 software of participant responses was used to segment EEG data and sort trials into target correct, non-target correct, target non-correct and non-target non-correct conditions. Data from STIM2 files was also used to calculate participant performance for each of the four conditions. The 60-Hz powerline artifact was removed using a previously described spectral estimate technique (Percival & Walden, 1993). Statistical testing was performed using the Thomson F-test. Trials and electrodes which were consistently contaminated with noise were removed based on extreme amplitude, standard deviation, skewedness and kurtosis of samples.

After pre-processing, one round of Independent Component Analysis was used to remove eye blink and muscle artifacts. Characteristic components of these artifacts were identified visually and selected for removal. Average CEA waveforms were calculated for target correct, target non-correct, non-target correct and non-target non-correct conditions.
3.13 N-Back Theta-Gamma Coupling Analysis

Pre-processed and cleaned EEG recordings were averaged across trials for each participant using MATLAB to generate waveforms for target correct, target non-correct, non-target correct and non-target non-correct conditions. Waveforms were 1000 ms long, with the starting point being at the time of test stimulus presentation. The duration was chosen as the average reaction time for participants, which was approximately 1000 ms. MI for this 1000 ms interval was calculated using the formulae described in section 3.10.
Neuroplasticity refers to the brain's ability to alter itself to adapt to new stimuli. One form of plasticity is synaptic plasticity, or the modification of synaptic strength to strengthen or weaken connections between neurons. LTP is the most extensively studied form of synaptic plasticity. It occurs when pre and post-synaptic neurons are stimulated simultaneously, and leads to a strengthening of the synapse between them that can last for over 24 hours (T. V. P. Bliss & Lømo, 1973; Davis, Bliss, Dutrieux, Laroche, & Errington, 1997; Malenka & Bear, 2004). LTP and synaptic plasticity have been linked to cognition, namely memory and learning. In an early experiment researchers showed that rats given a compound that abolishes LTP causes impairments in spatial learning/memory, and mice lacking NMDA receptors in the hippocampus demonstrate impaired spatial memory (Morris, 1989; Tsien, Huerta, & Tonegawa, 1996).

Impaired neuroplasticity has been observed in people with schizophrenia. Evidence comes from brain stimulation studies, which report that both medicated and unmedicated people with schizophrenia exhibit impairments (Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Frantseva et al., 2008). These findings have implications for cognition, as dysregulation of synaptic plasticity has been proposed to disrupt connectivity and impair communication between cortical areas (Friston, 2002; Stephan, Friston, & Frith, 2009). Cognitive impairments seen in people with schizophrenia are seen in a number of domains, including working memory, and
abnormal plasticity is a mechanism through which these cognitive abilities may be impaired (Goldman-Rakic, 1994).

Aging also causes declines in neuroplasticity and cognition. Its effect on neuroplasticity has been observed in brain stimulation studies, where older people show reduced plasticity in the motor cortex (Fathi et al., 2010; Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Tecchio et al., 2008). Cognitive abilities like memory, processing speed, and attentional regulation are susceptible to decline with aging in healthy people (Burke & Barnes, 2006; Jones et al., 2006). In people with schizophrenia, aging is associated with increased stability of positive symptoms but decline in cognitive skills (Jeste et al., 2003; Tarek K Rajji & Mulsant, 2008). Cognitive skills in this population have been repeatedly found to be strongly linked to functional outcome (J. F. Green & King, 1996; Michael F Green et al., 2004; Michael Foster Green et al., 2000), therefore maximizing cognitive abilities is a key target for improving quality of life in older people with schizophrenia.

PAS is a TMS paradigm used to assess LTP-like activity through non-invasive brain stimulation. Conventionally, PAS has been performed by measuring the amplitude of motor evoked potentials that are elicited by TMS stimulation of the motor cortex. Single TMS pulses are delivered to the motor cortex while the resulting motor evoked potentials (MEP) are measured. Then, paired stimulation of the median nerve with TMS stimulation of area M1 is performed to induce an increase in excitability of this brain region. Following paired stimulation, MEPs are
once again recorded in response to single TMS pulses. The increase in MEP amplitude after paired stimulation gives a measure of LTP-like plasticity (Stefan et al., 2000).

It has recently been shown that PAS is effective for increasing excitability in the dorsolateral prefrontal cortex (DLPFC). By pairing median nerve stimulation with TMS stimulation of DLPFS, cortical evoked activity (CEA) measured by EEG can be increased. Furthermore, this effect is localized to DLPFC (Rajji et al., 2013). PAS in DLPFC is of particular use for studying plasticity in people with schizophrenia as the DLPFC is responsible for executive functions and working memory, and so abnormal plasticity in this could be at the root of impairments in these important cognitive abilities.

Another physiologic measure related to cognition is cross-frequency coupling, which describes the relationships between neural oscillations of different frequencies. Of particular relevance is theta-gamma coupling, the modulation of the amplitude of gamma oscillations (30-50Hz) by the phase of theta oscillations (4-7 Hz). This measure has been found to be potentiated by PAS and has been linked to memory and other cognitive abilities (Canolty & Knight, 2010; J. E. Lisman & Idiart, 1995; J. E. Lisman & Jensen, 2013).

In order to better understand neuroplasticity and the neural mechanism underlying deficits in working memory in older people with schizophrenia, this study investigates PAS-LTP, N-back performance, and theta-gamma coupling in people with schizophrenia across the lifespan.
4.2 Methods

4.2.1 Study Design

Patients and healthy controls were assessed at baseline using clinical and cognitive assessments, including the N-back task. Then they received pre-PAS, consisting of 100 single TMS pulses delivered at 0.1 Hz. Pre-PAS was followed by PAS for 30 min. After PAS, cortical evoked potentials (CEPs) in response to single pulse TMS were assessed at Time 0, 15 min, 30 min and 60 min. EEG was recorded throughout.

4.2.2 Participants

Assessments were performed on right-handed clinically stable patients and healthy controls age 49 or older. For this study clinical stability was defined as (1) not having been hospitalized within 3 or more months prior to assessment, and (2) having had no change in antipsychotic medication dosage within the 4 weeks prior to assessment. Detailed inclusion and exclusion criteria as well as baseline cognitive assessments are outlined in Appendix A.

14 participants were included in the healthy and schizophrenia groups. This sample size participants of is comparable to that used in Frantseva et al. (2008) for a similar PAS study. Using 15 participants with schizophrenia and 15 controls, Frantseva et al. (2008) found a statistically significant difference in PAS-LTP between the two groups at α-level of 0.05 and with a Cohen’s effect size of 1.01.
PAS protocol, N-back, data collection and analysis of PAS-LTP and Theta-Gamma Coupling are detailed in Section 3: General Methods.

4.2.3 N-Back Frequency Analysis

Cleaned, segmented recordings were averaged for target correct and non-target correct conditions to obtain an average waveform for each condition in each participant. These recordings were bandpass filtered for their theta (4 Hz – 7 Hz) and gamma (30 Hz – 50 Hz) bands, and the power signal was obtained for each participant. Power is reported for the 1000 ms interval following test stimulus presentation, as this represents the average response time of participants across all N-back conditions.

4.2.4 Statistical Analysis

PAS-LTP was analyzed using univariate ANOVA. Potentiation of theta-gamma coupling was evaluated using a repeated-measures ANOVA. Differences in N-back performance, theta and gamma power, and MI were assessed using t-tests.

4.3 Results

4.3.1 Participants

14 participants with schizophrenia over 50 years old and 14 controls participated in the study. Pre-PAS EEG recorded from one schizophrenia participant was unusable due to noise and artifacts that could not be successfully removed. One of the control participants was unable to
tolerate TMS stimuli during PAS and as a result withdrew from the study, thus 13 participants from each group were included in these analyses. An additional 18 younger schizophrenia participants and 22 younger controls were included to examine the effects of aging.

Demographics for the older participant group and the group of all ages is presented in Table 4.1.

<table>
<thead>
<tr>
<th>Older Participants</th>
<th>Control</th>
<th>Schizophrenia</th>
<th>Sex Distribution $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13 (5 male)</td>
<td>13 (11 male)</td>
<td>$\chi^2 = 3.94; P = 0.047$</td>
</tr>
<tr>
<td>Mean Age</td>
<td>67.2</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>11.3</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All participants</th>
<th>Control</th>
<th>Schizophrenia</th>
<th>Sex Distribution $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35 (19 male)</td>
<td>31 (23 male)</td>
<td>$\chi^2 = 2.62; P = 0.11$</td>
</tr>
<tr>
<td>Mean Age</td>
<td>44.6</td>
<td>45.7</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>20.8</td>
<td>17.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Study Participants

4.3.2 Potentiation of Cortical Evoked Activity and Coupling in Older Participants

Averaged CEP waveforms for pre-PAS and post-PAS conditions were calculated for each participant and the ratio of area under the curve post-PAS/pre-PAS was evaluated.

Figure 4.1 shows averaged pre-PAS and post-PAS CEP waveforms in the left frontal region for the healthy and schizophrenia groups, and results for potentiation in the left frontal cortex of the two groups are displayed in Figure 4.2. A univariate ANOVA controlling for age and sex indicates a significant deficit in PAS-LTP in left frontal electrodes (F3, F5, F7, AF3) in schizophrenia ($F = 4.56, P = 0.043$). The effects of age and sex were not found to significantly affect PAS-LTP (sex: $F = 0.143, P = 0.71$; age: $F = 0.06, P = 0.81$). Consistent with previous
PAS-DLPFC results (Rajji et al., 2013), potentiation is localized to the left DLPFC in both control and schizophrenia participants. This is apparent in the topographic projections in Figure 4.3, showing spatial distribution at the time of maximum potentiation.

Figure 4.1: CEA Waveforms for Control and Schizophrenia Pre-PAS and Post-PAS

Averaged TMS-evoked potential pre- and post-PAS in older healthy controls (top) and schizophrenia participants (bottom).
PAS-LTP is impaired in older people with schizophrenia (F = 4.56, P = 0.043). Included are 13 controls (HC) and 13 schizophrenia participants (SCZ). Results are displayed for left frontal region (electrodes F3, F5, F7, AF3). Error bars +/- 1 SEM.

**Figure 4.2: Potentiation of Cortical Evoked Potential by PAS**

Averaging potentiation across participants for each electrode shows that the potentiation of CEA is localized to the left frontal region in both control and schizophrenia participants.

Group MI in the left frontal region pre- and post-PAS at the time of maximum gamma power potentiation are shown in **Figure 4.4**. Pre-PAS MI did not differ between the two older
participant groups (F = 0.615, P = 0.441). After PAS, MI was significantly increased in the control group (F = 7.504, P = 0.018) and in the schizophrenia group (F = 8.685, P = 0.012). A repeated-measures ANOVA indicates that the effects of schizophrenia (time*group) are not significant (F = 2.147, P = 0.156) Neither time*age nor time*sex were found to have a significant effect on potentiation of MI (age: F = 0.228, P = 0.638; sex: F = 1.973 P = 0.175).

Unlike PAS-LTP, potentiation of MI is not limited to the left frontal cortex (figure 4.5).

![Figure 4.4: Potentiation of Theta-Gamma Coupling by PAS in Older People](image)

**Figure 4.4: Potentiation of Theta-Gamma Coupling by PAS in Older People**

Modulation Index pre-PAS and post-PAS at the time of maximum gamma power potentiation for left frontal electrodes (F7, F5, F3, AF3) for schizophrenia participants (N = 13) and controls (N = 13). Significant potentiation is observed in both groups (HC: F = 7.504, P = 0.018; SCZ:F = 8.685, P = 0.012), but does not differ between the two (F = 0.615, P = 0.441). Error bars +/- 1 SEM.
Figure 4.5: Topographic Distribution of MI Potentiation

Potentiation of theta-gamma coupling by PAS is not confined to the left frontal cortex.

4.3.3 Potentiation of Cortical Evoked Activity and Coupling Across the Lifespan

In order to examine the effect of aging on PAS-LTP, the older subject group was joined with a group of 22 younger controls and 18 younger schizophrenia participants. The resulting group of controls consisted of 35 people (mean age = 44.6, SD = 20.8, 19 male) and the schizophrenia group consisted of 31 people (mean age = 45.7, SD = 17.05, 23 male). Neither age nor sex were significantly different between groups. As was the case with the older subject group, plasticity is impaired in left frontal electrodes in the schizophrenia group (F = 15.34 P<0.001) (figure 4.6), and a univariate ANOVA reveals no effect of age or sex on PAS-LTP (age in HC: F = 1.612, P = 0.213; age in SCZ: F = 0.285, P = 0.598; sex in HC: F = 0.02, P = 0.889; sex in SCZ: F = 2.455, P = 0.128) (figure 4.7).
Figure 4.6: PAS-LTP in All Ages

Ratio of post-PAS/pre-PAS CEA area in left frontal electrodes (F7, F5, F3, AF3) in healthy participants (N = 35) and schizophrenia participants (n = 31). Potentiation is impaired in schizophrenia (F = 15.34, P<0.001). Error bars +/- 1 SEM.

Figure 4.7: PAS-LTP Across the Lifespan

For both healthy control and schizophrenia participants there was no effect of age on PAS-LTP (N = 35 HC and 31 SCZ). (HC: F = 1.612, P = 0.213; SCZ: F = 0.285, P = 0.598). Results are displayed for left frontal electrodes (F7, F5, F3, AF3).
An analysis of the pooled group of older and younger participants found that MI is significantly potentiated by PAS in the schizophrenia and control groups in left frontal electrodes (HC: $F = 35.76, P < 0.001$; SCZ: $F = 29.43, P < 0.001$) (figure 4.8). MI is reported at the time of maximum gamma power potentiation. Repeated measures ANOVA reveals a significant effect of time*group ($F = 5.281, P = 0.025$) but not time*sex ($F = 0.694, P = 0.408$) on MI potentiation. Schizophrenia is associated with a decrease in pre-PAS and post-PAS MI (SCZ: $F = 4.38, P = 0.04$), and both pre-PAS and post-PAS MI decrease with increasing age as shown in figure 4.9 (pre-PAS in HC: Pearson's $R = -0.399$, $P = 0.017$, post-PAS in HC: Pearson's $R = -0.488$, $P = 0.003$ pre-PAS in SCZ: Pearson's $R = -0.473$, $P = 0.007$, post-PAS in SCZ: Pearson's $R = -0.659$, $P < 0.001$). However, the ratio of post-PAS MI/pre-PAS MI is unaffected by age (figure 10, HC: Pearson's $R = -0.199$, $P = 0.25$ SCZ: Pearson's $R = -0.281$, $P = 0.126$). Finally, there was a significant inverse correlation between pre-PAS MI and potentiation of MI in left frontal electrodes (figure 4.11). This effect is significant for both schizophrenia and control participants (HC: Pearson's $R = -0.344$, $P = 0.043$; SCZ: Pearson's $R = -0.412$, $P = 0.021$).

Figure 4.8: Potentiation of Theta-Gamma Coupling by PAS in All Ages

Modulation Index pre-PAS and post-PAS at the time of maximum gamma power potentiation for left frontal electrodes (F7, F5, F3, AF3) for 31 schizophrenia participants and 35 controls. There is a significant effect of group*time on potentiation of MI ($F = 5.281$, $p = 0.025$). Error bars +/- 1 SEM.
Healthy Controls (n = 35)

Schizophrenia (n = 31)

Figure 4.9: Theta-Gamma Coupling Across the Lifespan

In both schizophrenia and healthy controls, pre-PAS and post-PAS MI decrease with aging (pre-PAS in HC: Pearson's R = -0.399, P = 0.017, post-PAS in HC: Pearson's R = -0.488, P = 0.003 pre-PAS in SCZ: Pearson's R = -0.473, P = 0.007, post-PAS in SCZ: Pearson's R = -0.659, P < 0.001). The plots above show MI values for pre- and post-PAS in electrodes F7, F5, F3, and AF3. Post-PAS is reported at the time of maximum gamma potentiation.
Figure 4.10: Potentiation of Theta-Gamma Coupling Throughout the Lifespan

Ratio of post-PAS MI/pre-PAS MI in left frontal electrodes is not significantly affected by age (HC: Pearson's R = -0.199, P = 0.25 SCZ: Pearson's R = -0.281, P = 0.126).

Figure 4.11: Relationship Between Pre-PAS MI and MI Potentiation in All Ages

In both control and schizophrenia there is a significant negative correlation between prePAS MI and potentiation of MI in left frontal electrodes (HC: Pearson correlation = -0.344, p = 0.043; SCZ: Pearson correlation = -0.412, p = 0.021).
4.3.4 N-Back Performance

N-back performance results were omitted from one schizophrenia participant and one control participants whose low 0-back performance indicated poor attention or non-compliance with instructions. For the remaining 13 controls and 13 schizophrenia participants, N-back performance on the 1-, 2-and 3-back tasks was significantly impaired in people with schizophrenia (0BACK: $t = 0.779$, $P = 0.444$; 1BACK: $t = 2.231$, $P = 0.035$ 2BACK: $t = 2.407$, $P = 0.025$ 3BACK: $t = 2.114$, $P = 0.045$). Percentage of target correct responses for the two participant groups are shown in Figure 4.12.

![Figure 4.12: Performance on N-back Task](image)

Percentage of correct responses for the control and schizophrenia participants on the N-back task. Schizophrenia participants performed significantly lower on the 1-, 2- and 3- back tasks. (0BACK: $t = 0.779$, $P = 0.444$; 1BACK: $t = 2.231$, $P = 0.035$ 2BACK: $t = 2.407$, $P = 0.025$ 3BACK: $t = 2.114$, $P = 0.045$). Error bars +/- 1 SEM.

4.3.5 Gamma and Theta Power During N-Back

Gamma power in left and right frontal electrodes is shown in figure 4.13. In controls, power on non-target correct trials did not differ between N-back conditions (0- to 3-back) in either the left
or right hemisphere. In right frontal electrodes, gamma power on target correct trials was significantly higher than power during non-target correct trials on the 1-, 2-, and 3-back (1BACK: t = 2.78, P = 0.027; 2BACK t = 8.576, P = 0.001; 3BACK T= 7.175, P <0.001). In the right hemisphere, gamma power on target-correct trials increased significantly from between 0- to 1-back and 1- to 2-back (0-1BACK: t = -5.75, P = 0.001; 1-2BACK: t = -4.593 p = 0.01), and did not differ significantly between 2-back and 3-back (t = 2.818 p = 0.67). In the left frontal region, gamma power on target-correct trials did not differ between 1-, 2-, and 3-back. There were also no differences between the left and right hemispheres.

In the schizophrenia group, gamma power on non-target correct trials did not differ between N-back conditions (0- to 3-back) in either the left or right hemisphere. In right frontal electrodes, gamma power on target correct trials was significantly higher than power during non-target correct trials on the 1-, 2-, and 3-back (1BACK: T=2.375 P =0.49, 2BACK: T=2.513 P = 0.04 3BACK: t = 2.494 P = 0.042 ). This was also the case in the left hemisphere for the 1- and 3-back (1BACK: t = 2.424 P = 0.046 3BACK: T= 2.377 P =0.49). Results were not significantly different between left and right hemispheres.

Theta power in left and right frontal electrodes is shown in figure 4.14. In the healthy control group, theta power did not differ between left and right hemispheres or between conditions (1-,2- and 3-back). There was no difference between theta power on target correct vs non-target correct trials, with the exception of the 3-back condition in the right hemisphere (t = 2.566 P = 0.033).
In the schizophrenia group, theta power did not differ between left and right hemispheres or between conditions (1-, 2- and 3-back). There was no difference between theta power on target correct vs non-target correct trials, with the exception of the 2-back in the left hemisphere ($t = 2.56, P = 0.05$).

Compared to controls, schizophrenia participants exhibited reduced theta power in the right hemisphere on the 0-back and 2-back non-target correct conditions (0BACK: $t = 2.41, P = 0.036$, 2BACK: $t = 2.64, P = 0.018$).

**Figure 4.13: Gamma Power During N-Back Task**

Gamma power during N-back task in left and right frontal electrodes (left: AF3, F3, F5, F7; right: AF4, F4, F6, F8). TC = Target Correct, nTC = non-Target Correct. Error bars +/- 1 SEM.
4.3.6 Theta-Gamma Coupling During N-Back Task

Figure 4.15 shows theta-gamma coupling in 14 participants with schizophrenia and 14 controls on target correct and non-target correct tasks. There were no significant differences between conditions (0-,1-,2- or 3-back), trials (target correct and non-target correct) or group (control or schizophrenia).
a) Healthy Controls

b) Schizophrenia

Figure 4.15: Theta-Gamma Coupling During N-Back Task

Theta-gamma coupling during N-back task in left frontal electrodes (AF3, F3, F5, F7). TC = Target Correct, nTC = non-Target Correct. Error bars +/- 1 SEM.
4.4 Discussion

The results from this research indicate that older people with schizophrenia experience impairments in PAS-LTP and in potentiation of theta-gamma coupling. PAS-LTP was localized to the left frontal region, demonstrating specificity that parallels LTP. This measure was significantly lower in people with schizophrenia of all ages, and was not affected by aging. Potentiation of theta-gamma coupling did not differ significantly between older controls and older schizophrenia participants, however when pooled with the younger participants, a significant impairment was, in fact, observed in the schizophrenia group. It seems possible that with a sample size of 13 participants in each older group, statistical power was too small to detect a difference in potentiation of MI. While potentiation of MI also did not decrease with increasing age, pre-PAS and post-PAS MI decreased. As the distribution of sexes differed between the two older subject groups, the effect of sex was assessed for all of the measures presented here. There was no significant effect on any of the outcomes.

The finding that PAS-LTP was independent of age runs counter to other PAS experiments performed in the motor cortex, which have reported significant age-related impairments (Fathi et al., 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008). Sample sizes from these studies ranged from 27 (Florian et al 2008) to 50 (Tecchio et al 2008), which match well with our sample of 31 healthy and 35 schizophrenia and with 14 members of each group being over the aged 50 or older. Each of the three previous studies included participants from early 20s to 70s. Similarly, the participants included in this analysis ranged in age from 18 to 83 years. Whereas it is possible that an age effect on PAS-LTP was not detectable with the sample size used for this
analysis, the result may also point to heterogeneous effects of aging throughout different brain regions. This is the first study to examine the effect of age of PAS-LTP in the dorsolateral prefrontal cortex, however one PAS study performed in the somatosensory cortex found that PAS-LTP did not decrease in older people and in fact was significantly higher than the younger participant group (Pellicciari, Miniussi, Rossini, & De Gennaro, 2009). Furthermore, one study which tested PAS-LTP in the motor and somatosensory cortices in a younger subject group found that within subjects there was no correlation between PAS-LTP in the two brain regions, highlighting that responses to PAS can differ between cortical regions (Lucia, Lu, Bliem, & Ziemann, 2011). Taken together, these results provide evidence that aging does not necessarily cause decline in all brain regions at the same rate. Imaging studies have reported that older people exhibit greater DLPFC activation during working memory tasks as a compensatory mechanism (Cabeza, Anderson, Locantore, & McIntosh, 2002; Cabeza, 2002; Grady, 2012). Similar compensatory mechanisms could potentially mitigate the effects of age on plasticity in the DLPFC, resulting in the lack of effect of age observed in this subject group.

As predicted, potentiation of MI was significantly impaired in schizophrenia, however potentiation of MI was not. Also, in the pooled schizophrenia group and the pooled control group there was a significant inverse correlation between pre-PAS MI and potentiation of MI. Participants whose pre-PAS MI was lower experienced a greater potentiation of MI than those whose pre-PAS MI was already high. This may be explained by a ceiling affect, where MI is unable to increase above a certain threshold. A similar effect has previously been demonstrated for PAS-LTP in the motor cortex in an experiment that had participants received two sessions of
PAS. During the first session participants received PAS with interstimulus intervals chosen to induce either PAS-LTP or PAS-LTD. In the second PAS session, which used a 25 ms interval to induce potentiation, participants whose motor cortex excitability had been elevated by PAS-LTP experienced a smaller increase in excitability than those whose excitability had been decreased (Muller, Orekhov, Liu, & Ziemann, 2007). A similar homeostatic mechanism may play a role in maintaining a ceiling for theta-gamma coupling in the DLPFC.

The impairments seen in schizophrenia in PAS-LTP and potentiation of MI have implications for cognition. Impaired plasticity in schizophrenia has been hypothesized to be associated with impaired connectivity and communication between cortical areas (Stephan et al., 2009), and also to some of the positive symptoms of schizophrenia (Friston, 2002). Impaired theta-gamma coupling has been suggested to result in impairments in segmenting and ordering information (J. Lisman & Buzsaki, 2008). Results from this analysis show that baseline MI is decreased with increasing age in schizophrenia as well as in healthy controls. Potentiation of MI by PAS is also deficient in people with schizophrenia. The significant effects of both age and schizophrenia indicate that older individuals with this mental illness may be at an increased risk of cognitive impairment.

In healthy people, previous behavioural research has found that working memory declines begin as early as mid-fifties, however in this analysis there was no observed effect of age on PAS-LTP in the DLPFC of people in this age group (Daigneault, Braun, & Whitaker, 1992). The age-related working memory impairments previously observed may not be a result of impaired
plasticity of cortical evoked activity, but rather caused by deficits in other cognitive abilities such as processing speed, which has been found to slow in older people (Salthouse, 1996), or in theta-gamma coupling.

Working memory as measured by the N-back task was impaired in schizophrenia. Performance was significantly lower in this group on the 1-, 2-, and 3-back tasks. This matches with previous behavioural research which has also found working memory to be impaired in people with schizophrenia (Goldman-Rakic, 1994). Frequency analysis of EEG recorded during the N-back showed that gamma power was generally higher on target correct compared to non-target correct tasks. A related EEG study has been performed in younger schizophrenia participants by another research group using the N-back to engage working memory. These researchers found that healthy controls displayed increased gamma power as the memory load of the task increased, whereas gamma power in people with schizophrenia was consistently high regardless of task difficulty. This indicates that the schizophrenia participants were engaging complex processing even during less demanding tasks (Canan Basar-Eroglu et al., 2007). The participants of this study show a similar trend, however differences are not significant with this sample size.

Theta-gamma coupling did not show significant modulation during different N-back conditions. Pilot data from healthy younger participants in a related study found that coupling increased from 0-back to 2-back, (T K Rajji et al., 2014). Additionally, coupling increased from the 2- to 3-back conditions in high-performing participants, whereas it decreased in low-performing participants. Further research in this area requires a larger subject group. Theta-gamma coupling
on the 3-back is related to performance, and so a more thorough analysis of theta-gamma coupling results taking into account each participant's success on the task may elucidate a pattern between coupling and working memory.

One important limitation of this study is small sample size. While the 13 participants in each group was sufficient for analysis of PAS-LTP and potentiation of coupling, and is consistent with the sample size used in similar research (Fathi et al., 2010; Frantseva et al., 2008; Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Tecchio et al., 2008), it was not adequate for frequency analysis or for examining theta-gamma coupling during the N-back task. Another limitation is that the average age of our older participants was in the early to mid-sixties, which may not be old enough to detect an age-related impairment of PAS-LTP in the DLPFC. Furthermore, while this study did include a control group there was no control PAS condition. Finally, all participants were treated with antipsychotic medication. Previous studies have not found an effect of antipsychotic dose on plasticity, and in one study plasticity was impaired equally in antipsychotic-naive and antipsychotic-treated patients (Daskalakis et al., 2008; Frantseva et al., 2008; Hasan et al., 2011). Nonetheless, chronic antipsychotic treatment has been found to alter dopaminergic transmission (Bacopoulos, Redmond, Baulu, & Roth, 1980; Chiodo & Bunney, 1983; Florijn, Tarazi, & Creese, 1997; Silvestri et al., 2000; Stockton & Rasmussen, 1996; Tarazi, Florijn, & Creese, 1997; Wilmot & Szczepanik, 1989), and as such may have confounded PAS-LTP results, in particular in the older age group, as participants had received many years of antipsychotic treatment.
Overall, the results presented here indicate that plasticity of cortical excitability and of theta-gamma coupling in the DLPFC are impaired in schizophrenia but unaffected by aging. Further work along this line of research could investigate PAS-LTP in other regions of the brain, to identify whether changes in neuroplasticity in other cortical regions are detectable at different points in the aging process. The outcome measures of this study that relate to behaviour during the N-back test could benefit from further investigation using a larger sample size. This could allow for better understanding of cognitive correlates of neuroplasticity in older people with schizophrenia.
5 Effects of Antipsychotic Dose Reduction on Plasticity in the Dorsolateral Prefrontal Cortex: a Paired Associative Stimulation Study

5.1 Introduction

People with schizophrenia face cognitive impairments in addition to the positive and negative symptoms of their illness (Friston, 2002; Heinrichs & Zakzanis, 1998; Weickert et al., 2000). Aging in schizophrenia is associated with additional cognitive impairments in a multitude of domains, including in working memory (Friedman et al., 2001; Tarek K Rajji & Mulsant, 2008). Cognitive ability has been strongly linked to functional outcome in people with schizophrenia, and as such it is an important target for improving quality of life (J. F. Green & King, 1996; Michael F Green et al., 2004; Michael Foster Green et al., 2000).

Antipsychotic medications, while effective for treating the positive symptoms of schizophrenia, have been implicated in impairing cognition (Beuzen, Taylor, Wesnes, & Wood, 1999; Sharma, 1999; Uchida et al., 2009). This effect may be through the antagonism of dopamine D2 receptors. Dopamine has been found to have an inverted U shaped effect on cognition and plasticity, with impairments seen when dopaminergic transmission is either very high or very low (Cools & D’Esposito, 2011; Monte-Silva et al., 2009). In one study, the blockade of D2 receptors by risperidone was correlated with cognitive impairments in people with schizophrenia (Uchida et al., 2009). Dopamine acts as one of the many modulators of LTP, and by altering dopaminergic transmission antipsychotics might cause disregulation of neuroplasticity (Iversen, 1975; Kebabian & Calne, 1979). LTP is understood to be an important neural mechanism for
learning and memory, thus its disregulation may contribute to the cognitive impairments (Collingridge & Bliss, 1995).

Theta-gamma coupling, which refers to the modulation of gamma oscillation amplitude by theta oscillation phase, is also of interest in older people with schizophrenia. It has been shown to be increased during cognitive tasks and impaired in schizophrenia (Canolty & Knight, 2010; J. E. Lisman & Jensen, 2013; T. Rajji, 2014). Theta-gamma coupling can also be potentiated by PAS, and is therefore a measure of neuroplasticity (Rajji et al., 2013).

While there have been a number of studies investigating LTP and antipsychotics, the majority of these have been performed in animals given acute doses of antipsychotics (Price et al., 2014). Similarly, the two brain stimulation studies of antipsychotics that were performed in humans used healthy volunteers given a single dose of antipsychotic (Korchounov & Ziemann, 2011; Monte-Silva et al., 2011). There is a dearth of studies performed in people with schizophrenia receiving chronic antipsychotic treatment. In order to better understand the effect of antipsychotics in older people with schizophrenia, this study presents pilot data that provides some direction for assessing neuroplasticity in participants on a high dose, then 6 to 8 weeks later after a gradual dose reduction.

5.2 Methods
5.2.1 Study Design
This research was conducted as an addition to a larger parent dose reduction study. Patients with schizophrenia or schizoaffective disorder who met eligibility criteria completed a baseline
session of PAS. Patients were be assessed at baseline using clinical and cognitive assessments including the N-back task. They underwent a brain MRI as per parent protocol. Then, they received a session of PAS before antipsychotic dose reduction. Following PAS, cortical evoked potentials (CEPs) in response to single pulse TMS were be assessed at Time 0, 15, 30, and 60 min. One week after reaching the final reduced antipsychotic dose, subjects underwent a second session of PAS-25 and the N-back as before the dose reduction.

5.2.2 Participants

Participants were recruited for the study who were 50 years or older and treated with a daily dose of ≥2 mg oral risperidone, or ≥ 10 mg olanzapine, and had been on a stable dose for of medication for at least 12 months at time of screening. Participants were recruited following referral by the treating physician or self-referral in response to advertisements. Detailed inclusion and exclusion criteria are listed in Appendix B.

PAS protocol, N-back task protocol, data collection and analysis are detailed in section 3: General Methods.

5.2.3 Antipsychotic Dose Reduction

Antipsychotic dose reduction was performed under the parent protocol. The daily dose of risperidone or olanzapine was reduced on a weekly basis to reach the goal of 40% reduction. For risperidone, the daily dose was reduced by 0.5 mg on a weekly basis not exceeding a dose lower
than 1.5 mg per day. For olanzapine, the daily dose was reduced by 2.5 mg per week (lowest possible dose is 7.5 mg). Psychopathology and extrapyramidal symptoms were assessed weekly. All psychotropic agents other than risperidone or olanzapine were be kept constant throughout the study.

5.2.4 Cognitive and Clinical Assessments

Schizophrenia or related disorder was confirmed through the use of the Structured Clinical Interview for DSM-IV Disorders (SCID). Information about the onset, course, and severity of illness was obtained by interviewing the patients, performing a detailed review of the subjects’ charts, and obtaining information from informants when possible.

Prior to cognitive testing, symptoms were assessed with the Positive And Negative Syndrome Scale (PANSS), Brief Psychiatric Rating Scale (BPRS), Clinical Global Impression (CGI), Targeted Inventory on Problems in Schizophrenia (TIP-Sz), Functional Assessment for Comprehensive Treatment of Schizophrenia (FACT-Sz), Subjective Well-being on Neuroleptic Medications (SWN), Udvalg for Kliniske Undersøgelser (UKU) Side Effect Rating Scale, Abnormal Movement Involuntary Scale (AIMS), Simpson-Angus Scale (SAS), and Barnes Akathesia Scale (BAS). Descriptions of cognitive assessments are provided in Appendix C.
5.3 Results

5.3.1 Participants

7 participants were recruited for this study, however one withdrew during the dose reduction phase (subject ID PAS05) and another withdrew before the first testing session (subject ID PAS06). The pre-PAS EEG data for the first dose-reduction participant was contaminated with noise and artifacts that could not be removed, and as a result it was not possible to obtain a value for PAS-LTP for this participant. Demographic information and antipsychotic doses are summarized in Table 1.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Antipsychotic</th>
<th>Initial Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>M</td>
<td>olanzapine</td>
<td>20 mg/day</td>
</tr>
<tr>
<td>52</td>
<td>M</td>
<td>olanzapine</td>
<td>25 mg/day</td>
</tr>
<tr>
<td>66</td>
<td>M</td>
<td>olanzapine</td>
<td>17.5 mg/day</td>
</tr>
<tr>
<td>68</td>
<td>F</td>
<td>risperidone</td>
<td>2 mg/day</td>
</tr>
</tbody>
</table>

Table 6.1: Dose Reduction Study Participants

5.3.2 PAS-LTP on High and Low Antipsychotic Dose

Potentiation of cortical evoked potential in left frontal electrodes (F3, F7, F7, AF3) on high and low antipsychotic doses are shown in figure 5.1 (group averages) and figure 5.2 (individual participants). Potentiation was significantly greater than 1 in the high dose but not low dose condition (high $t = 3.30, P = 0.046$; low $t = 1.85, P = 0.161$). There was no difference between potentiation in the high-dose and low-dose conditions ($t = 0.903, P = 0.433$). Additionally, participants showed no observable pattern of potentiation between the high- and low-dose...
conditions, as two participants demonstrated increased potentiation after dose reduction and two demonstrated decreased potentiation.

**Figure 5.1: PAS-LTP at High and Low Antipsychotic Dose**

There is no difference between potentiation in the high-dose and low-dose conditions ($t = 0.903$, $P = 0.433$). Error bars +/- 1 SEM.

**Figure 5.2: Individual Participant Responses to PAS**

There is no difference between potentiation in the high-dose and low-dose conditions ($t = 0.903$, $P = 0.433$). Error bars +/- 1 SEM.
5.3.3 Potentiation of Theta-Gamma Coupling

Theta-gamma coupling in left frontal electrodes during pre-PAS and post-PAS for the high dose and low dose condition are shown in figures 5.3 and 5.4. There was no significant potentiation of MI in either condition (high: t = 2.09 P = 0.128; low: t = 1.60 P = 0.21). Additionally, there was no different between potentiation in the high and low dose conditions.

Figure 5.3: Potentiation of Theta-Gamma Coupling at High and Low Antipsychotic Dose

Modulation index pre- and post-PAS in high dose condition (left) and low dose condition (right). There was no potentiation in the high or low dose conditions (high: t = 2.09 P = 0.128; low: t = 1.60 P = 0.21). Error bars +/- 1 SEM.
Figure 5.4: Potentiation of Coupling in Individual Participants

The difference between potentiation of MI is not significant, however there is a trend toward decreased potentiation of theta-gamma coupling in the low dose condition in most of the participants.

5.3.4 N-Back Performance

Performance of the N-back was generally low, even on the 0-back control condition. Performance did not differ between the high dose and low dose conditions.

Figure 5.5: N-Back Performance on Target Correct Tasks

There was no significant difference between N-back performance on the high- and low-dose conditions. Error bars +/- 1 SEM.
5.4 Discussion

Of the four people who completed this study, two experienced an increase in potentiation following dose reduction and two experiences a decrease in coupling. There was no significant effect of antipsychotic dose on PAS-LTP. The effect of antipsychotic dose on theta-gamma coupling was also not significant, however there was a trend towards decreased potentiation of coupling on the lower antipsychotic dose. Performance on the N-back task was low overall, and did not differ between the high-dose and low-dose conditions.

The most important limitation of this study is the low number of participants, which presents difficulties for drawing conclusions from the data. Another limitation is the absence of evidence for strong test-retest reliability in PAS. While studies have been performed to examine the reliability of PAS in the motor cortex, none of them yet have found a strong correlation between repeated measurements of PAS-LTP (Quartarone et al., 2003, 2006; Sale, Ridding, & Nordstrom, 2007; Stefan et al., 2000; Stinear & Hornby, 2005). At this time, no studies on test-retest reliability have been published for PAS in the DLPFC.

Future research with a larger sample size can take advantage of PET data that was collected at high and low antipsychotic doses in order to examine the correlation between D2 receptor occupancy and PAS-LTP, potentiation of MI and N-back performance.
6 Cortical Response to Median Nerve Stimulation and Application to PAS

6.1 Introduction

When the median nerve of the wrist is electrically stimulated, the impulse is propagated through afferent nerve fibers to reach the somatosensory cortex, before spreading to other cortical areas (T Allison, McCarthy, Wood, & Jones, 1991; Jones et al., 2006; Nuwer, 1998). Electroencephalography (EEG) can be used to measure the resulting fluctuations in voltage at the scalp, called sensory evoked potentials (SEPs).

The temporal distribution of median nerve SEP in healthy people have been well-characterized (T Allison et al., 1991; Nuwer, 1998). Among these findings, the earliest SEP observed in the frontal region is a positive deflection with latency from 18-24 ms (T Allison et al., 1991; García Larrea, Bastuji, & Mauguière, 1992; Valeriani, Restuccia, Barba, Tonali, & Mauguiere, 2000; Yamada, Kayamori, Kimura, & Beck, 1984). At the central sulcus – near the motor cortex – the earliest SEP occurs at 25 ms.

During Paired Associative Simulation (PAS), 25 ms delay between median nerve stimulation and TMS stimulation of the cortex allows the two stimuli to reach the motor cortex simultaneously, resulting in an increase in excitability in this region. Similarly, PAS can be performed in the dorsolateral prefrontal cortex (DLPFC). In this case, TMS stimulation is applied to the DLPFS and PAS-LTP is quantified by comparing the area under cortical evoked potentials measured
using EEG (Tarek K Rajji et al., 2013). Given that TMS and peripheral stimulation must reach the DLPCF at the same time to achieve potentiation of cortical evoked activity, the timing of the two stimuli is essential.

PAS studies performed in both the DLPFC and motor cortex in people with schizophrenia have shown that PAS-LTP is impaired in this population (Frantseva et al., 2008; T. Rajji, 2014). While median nerve stimulation in schizophrenia has been previously studied, it has not been explored in the context of its application to PAS (Gulmann, Wildschioedtz, & Ørbæk, 1982; Roemer, Shagass, Straumanis, & Amadeo, 1979; C Shagass, Straumanis Jr, Roemer, & Amadeo, 1977; Charles Shagass, Roemer, Straumanis, & Amadeo, 1979). Thus, one goal of this research is to determine whether a portion of the plasticity deficits observed in people with schizophrenia undergoing PAS might be due to transmission of the median nerve stimulus by comparing SEP timing between healthy and schizophrenia participants.

Theta-gamma coupling refers to the modulation of the amplitude of gamma neural oscillations by theta phase and has been linked to cognition (Canolty & Knight, 2010; J. E. Lisman & Jensen, 2013). Coupling is evoked by TMS pulses, and is potentiated during PAS by pairing TMS with peripheral nerve stimulation (Tarek K Rajji et al., 2013). As PNS plays a role in potentiating coupling, theta-gamma coupling was investigated in response to PNS alone and compared between the healthy and schizophrenia groups.

Therefore, we analyzed EEG recordings from healthy adults and those with schizophrenia in order to better understand the spatial and temporal propagation of the SEPs that result from
median nerve stimulation and to validate the use of the 25 ms interval for PAS in the DLPFC. Further analysis consisted of calculating theta-gamma coupling in frontal, parietal, temporal and occipital electrodes and examining whether SEP potential time is related to PAS-LTP and potentiation of coupling.

6.2 Methods

6.2.1 Participants

Participants were right-handed healthy controls and people with schizophrenia aged 18 and older who were recruited to participate in a PAS study. The self-administered Personality Assessment Screener was used to screen healthy participants for psychopathology. This screener is an objective inventory for personality and psychopathology corresponding to DSM-IV categories. Schizophrenia participants were required to be clinically stable for inclusion in the study. Clinical stability was defined as (1) not having been hospitalized within 3 or more months prior to assessment, and (2) having had no change in antipsychotic medication dosage within the 4 weeks prior to assessment. Detailed inclusion and exclusion criteria as well as baseline cognitive assessments are outlined in Appendix A.

6.2.2 Stimulation and Data Collection

For each participant, PNS-EEG was recorded at the end of a PAS session. The right median nerve was stimulated at the wrist with standard bar electrodes (0.5 ms square wave constant current pulses), with cathode positioned proximally. A conditioning stimulus (200 µs) intensity
wave was delivered at three times the motor threshold necessary for the participant to sense a pulse in the wrist.

EEG recording was performed through a 64-channel Synamps 2 EEG system with reference electrode positioned posterior to electrode Cz. The signal was recorded DC at a sampling rate of 20 kHz.

6.2.3 ERP Analysis

EEG recordings were imported into MATLAB and segmented into 1 s long trials with respect to PNS pulse time. The 100 trials were averaged to obtain a SEP for each participant. For spatial SEP analysis, averages were calculated for frontal electrodes (F3, F5, F7), parietal electrodes (CP3, CP5, CP7), temporal electrodes (FT7, T7, TP7), and occipital electrodes (PO3, PO7, O1) in the left hemisphere. Group SEPs for each of these regions were obtained by averaging recordings from each participant in the healthy and schizophrenia groups.

6.2.4 Identification of Individual P20 SEP Peaks

To identify the frontal P20 potential, the SEP was computed for electrode F5 in each participant, as this was most often found to be the electrode overlying DLPFC. P20 was identified visually as the highest peak from time 17ms to 25ms post-PNS, as previous studies have identified this as the range for P20 in frontal electrodes.
6.2.5 Temporal and Spatial Analysis of Theta-Gamma Coupling

EEG recordings were cleaned according to the procedure outlined in Section 3: General Methods. Trials were averaged for each participant and theta-gamma coupling in response to PNS stimulation was determined for successive 250-ms windows from -250 ms until 750 ms post-stimulus using the method for calculating modulation index described in Tort et al 2010.

Signals were filtered into theta (3-7 Hz) and gamma (30-50 Hz) waveforms using a zero-phase shift filter. The Hilbert transform was applied to separate phase and amplitude for each frequency band. Theta phase was divided into six bins, each consisting of 60 degrees, with zero degrees corresponding to the waveform peak.

A distribution of gamma amplitude with respect to theta phase was created by dividing gamma amplitude into the six theta phase bins as described in detail in Tort et al (2010). This distribution was then normalized by dividing the amplitude of each bin by the sum of the amplitudes of all bins. Maximum entropy occurs when the distribution is uniform, which is when the amplitude of each bin is 1/N. Increased MI represents increased order, or lower entropy H(P).

The K-L modulation index is calculated using the following formula (Tort et al 2010):

\[ MI = (\log(N)H(P))/\log(N) \]
N is the number of phase bins. Log(N) is entropy of a uniform distribution, P is the relative amplitude distribution sorted according to phase bins, and H(P) is the entropy of the P distribution, which is calculated as:

\[ H(P) = PN \sum j \frac{1}{P(j)} \log[P(j)] \]

Coupling was also calculated in surrogate data to establish a baseline of coupling that occurs by chance in data where there is no relationship between theta phase and gamma amplitude. Surrogate data was generated from EEG recordings of median nerve stimulation in each subject by randomly shuffling the phase of the theta band. Coupling was then calculated using the same procedure as the original EEG recordings. For each recording, this procedure was repeated 10 times to generate average MI of surrogate data.

6.2.6 Calculation of PAS-LTP and Potentiation of Theta-Gamma Coupling

PAS-LTP had been previously calculated in these participants for an earlier publication (Tarek K Rajji et al., 2013). EEG recordings were cleaned, PAS-LTP and potentiation of Theta-Gamma coupling was evaluated for each participant using the methods described in **General Methods**.
6.3 Results

6.3.1 Participants

Participants were 22 healthy controls (mean age = 36.5, SD = 16.4, 12 male) and 17 people with schizophrenia (mean age = 35.4, SD = 13.3, 13 male) who received PAS with a 25 ms time interval (PAS 25) followed by median nerve stimulation.

6.3.2 Spatial Analysis of Sensory Evoked Potentials

SEPs averaged for 18 healthy subjects are shown in Figure 6.1. In the left frontal region the P20 and N30 potentials are evident, consistent with other studies of median nerve stimulation (Allison, McCarthy, Wood, & Jones, 1991; García Larrea, Bastuji, & Mauguière, 1992; Valeriani, Restuccia, Barba, Tonali, & Mauguiere, 2000; Yamada, Kayamori, Kimura, & Beck, 1984). In post-central electrodes of the left parietal area, the SEP polarities are reversed to N20 and P30 potentials, also consistent with the literature. SEPs are not evident in the temporal or occipital regions, however previous studies have identified N20 and P30 potentials in occipital electrodes. Figure 6.2 shows topographic representations of positive and negative deflections at 10 ms, 18 ms, 22 ms and 24ms., as these times represent the peaks of SEPs in different brain regions. These plots illustrate that the P20 visible in the frontal region reverses polarity at the central sulcus.

For each participant, P20 times were identified by inspection as the most prominent peak between 17ms and 25 ms. SEP results were omitted for 2 healthy participant and 2 participants with schizophrenia for which a P20 peak could not be identified with confidence. For controls,
the average P20 time was 21.19 ms (SD = 1.95) and for schizophrenia was 21.38 (SD = 1.36). These figures are not significantly different between the two participant groups (t = 0.342, P = 0.73).

a) left frontal (F3, F5, F7)  
b) left parietal (CP3, CP5, CP7)  
c) left temporal (FT7, T7, TP7)  
d) left occipital (PO3, PO7, O1)

Figure 6.1: Sensory Evoked Potentials in Frontal, Parietal, Occipital and Temporal Electrodes

a) SEP in frontal electrodes, showing P20 and N30 SEPs  
b) SEP in parietal electrodes, showing N20 and P30 SEPs  
c) SEP in temporal electrodes  
d) SEP in occipital electrodes (N = 18)
Figure 6.2: Topographic Distribution of Sensory Evoked Potentials

Topographic distribution of median nerve SEP in healthy participants. Red represents positive voltage deflections and blue represents negative deflections. (N = 18).

Figure 6.3 shows theta-gamma coupling in successive 250 ms windows from 250 ms before stimulus to 600 ms after stimulus. An increase in coupling lasting approximately 300 ms is evident in the left frontal and parietal cortices, with a much smaller increase in the temporal and occipital cortices. In order to ensure that the increase in coupling was not a result of participants having recently received PAS, a separate analysis was performed of participants who had received PAS with a 100 ms interval as a control condition. Results of the control group are shown in figure 6.4. Like the PAS25 group, the control groups also showed a visible increase in coupling, indicating that this effect occurs regardless of PAS condition. The same analysis was performed in people with schizophrenia who had received PAS 25, and results are shown in
Figure 6.5. Theta-gamma coupling increased in people with schizophrenia following PNS stimulation, however by inspection it appears to show a smaller increase in the frontal region compared to controls.

a) frontal (F3, F5, F7)

b) parietal (CP3, CP5, CP7)

c) temporal (FT7, T7, TP7)

d) occipital (PO3, PO7, O7)

Figure 6.3: Theta Gamma Coupling in Healthy Participants During Median Nerve Stimulation

The earliest time point in each interval is plotted with the MI for that interval in dark blue, with dashed lighter lines indicating standard error. Red line represents coupling in surrogate data. (N = 18).
Figure 6.4: Theta-Gamma Coupling During Peripheral Nerve Stimulation After PAS-100

The earliest time point in each interval is plotted with the MI for that interval in dark blue, with dashed lighter lines indicating standard error. Red line represents coupling in surrogate data. (N = 10).

Figure 6.5: Theta Gamma Coupling in Participants with Schizophrenia after Median Nerve Stimulation

The earliest time point in each interval is plotted with the MI for that interval in dark blue, with dashed lighter lines indicating standard error. Red line represents coupling in surrogate data. (N = 11).
6.3.3 P20 Latency and Relationship to PAS-LTP and Potentiation of Theta-Gamma Coupling

The relationship between P20 time and PAS-LTP was investigated in the control and schizophrenia groups. For each participant, the difference between the observed P20 time and the time interval used for PAS (25 ms) was calculated. A graph of PAS-LTP with respect to the calculated time difference is shown in Figure 6.6. A linear regression indicates a significant effect of P20 time difference ($t = -2.79, \ P = 0.009$) and group ($t = -2.98, \ P = 0.007$), but no effect of age ($t = 0.352 \ p = 0.727$). There is a significant correlation between P20 time and PAS-LTP (Pearson's $R = -0.393, \ P = 0.016$) These results indicate that when the P20 time closely matches the time interval used for PAS (in this case, 25 ms), potentiation is greater. While potentiation in schizophrenia is lower overall, both the healthy and schizophrenia group show the same trend toward increased potentiation when the discrepancy between P20 and TMS interval is small.

The relationship between the timing of the P20 and potentiation of theta-gamma coupling was also investigated, as presented in figure 6.7. No significant effect of P20 time was observed.
Figure 6.6 : PAS-LTP is Correlated with P20 Time In Healthy and Schizophrenia Participants

As the difference between TMS stimulus time and P20 time decreases, PAS-LTP is greater. \( t = -2.79, \ P = 0.009 \), Pearson's \( R = -0.394, \ P = 0.016 \). Potentiation is also significantly lowered in schizophrenia \( t = -2.98, \ P = 0.007 \). \( N = 22 \) HC, 17 SCZ

Figure 6.7: No Relationship Between P20 time and Potentiation of Coupling

In both control and schizophrenia there is no relationship between P20 time and potentiation of coupling by PAS. \( N = 22 \) HC, 17 SCZ. \( \text{Pearson's } R = 0.387, \ P = 0.125 \).
6.4 Discussion

The results of this research agree with many previous SEP studies, and provide further insight into the contribution of median nerve stimulation to PAS. For the most part, SEPs in the frontal and parietal regions were consistent with previous research, with exception of occipital SEPs which likely could not be identified because of noise in those electrodes (T Allison et al., 1991; García Larrea et al., 1992; Valeriani et al., 2000; Yamada et al., 1984).

In healthy participants and participants with schizophrenia there was a significant correlation between P20 time and PAS-LTP, as participants whose P20 time was closer to the 25ms PAS interval displayed greater PAS-LTP. This finding highlights the importance of selecting an appropriate time interval between median nerve stimulation and TMS stimulation for the PAS protocol. It could also explain some of the variability in responses to PAS, including why certain individuals do not show potentiation after PAS. While certain research groups – including this one – have selected a fixed interval to use for all PAS participants, others have instead opted to identify SEPs in participants and select a time interval based on peak times. For example, for PAS in the motor cortex certain research groups have used N20 time, or the N20 + 2 ms as a PAS interval (Jung & Ziemann, 2009; Müller-Dahlhaus et al., 2008; Ziemann, Ilic, Pauli, Meintzschel, & Ruge, 2004). The results of this research suggest that this latter approach may be preferable for achieving maximum potentiation.

There was no observable difference in early frontal P20 time between control and schizophrenia participants. Thus, there is no evidence that peripheral transmission of the median nerve stimulus
is disrupted in people with schizophrenia. Any impairments in PAS-LTP in this population cannot be explained by poor transmission of the median nerve electrical stimulus, suggesting that there is defective mechanism in the cortex and lending support to the theory that the PAS protocol is effectively measuring LTP.

Potentiation of theta-gamma coupling was not correlated with P20 time, indicating that the mechanism for enhancement of coupling differs from that of PAS-LTP. Further research is required to determine if any aspect of peripheral nerve transmission is associated with potentiation of theta-gamma coupling.

Theta-gamma coupling was increased for approximately 300 ms following PNS pulses in healthy participants who had received PAS with either a 25 ms interval or a 100 ms control interval. The effect was observed in frontal and parietal regions, with much smaller changes in temporal and parietal regions. Participants with schizophrenia also demonstrated this increased coupling. Previous research on theta-gamma coupling has focused on coupling during cognitive tasks (Canolty & Knight, 2010; J. E. Lisman & Jensen, 2013), however the coupling shown here was induced by peripheral nerve stimulation alone. The significance of coupling in this case is not well understood at this time, however it could be that the electrical stimulus delivered to the wrist causes a “reset” of theta and gamma cycles, creating a transient increase in coupling.

One limitation of this study is the relatively low number of trials compared to other ERP experiments. The protocol used to deliver PNS pulses was intended to match them in intensity
and frequency with those delivered during PAS, thus 100 pulses were delivered at a frequency of 0.1 Hz. As a result, there was significant noise in the averaged SEPs and for a small number of individuals it was not possible to visually identify the P20 SEP. Furthermore, PNS-EEG was recorded at the end of PAS sessions that participants had completed as part of another study. There is therefore a possibility that PAS might have altered the cortical response to PNS.

Comparison of theta-gamma coupling from PAS 25 to the PAS 100 control condition provide reassurance that coupling results were unaffected, however it is nonetheless possible that PAS may have a small effect.

Another important limitation of this research is that EEG recordings were collected using a reference electrode positioned posterior to CZ. The majority of ERP studies use a non-cephalic reference located at the earlobe in order to minimize the contributions of voltage fluctuations near the reference electrode (Yao et al., 2005). The choice of reference in our study may be the reason for the discrepancy between some of the observed SEP peak times in the occipital lobe, which were not visible.

In conclusion, the results from this research point to ways that the PAS protocol can be expanded to other brain regions by identifying SEP peaks in the areas of interest. Furthermore, they suggest that PAS experiments could benefit from individually tailoring PAS interval times based on the participants' SEP peak times in order to maximize PAS-LTP.
7 General Discussion

Through analysis of the cortical responses to TMS and median nerve stimulation as well as the potentiation of cortical excitability resulting from PAS, this research has provided insight into the methodology of PAS and its application in understanding neuroplasticity in older people with schizophrenia. Plasticity of cortical evoked activity in the DLPFC of people with schizophrenia was found to be impaired throughout the lifespan. On the other hand, age had no effect on this form of plasticity. Potentiation of theta-gamma coupling by PAS was also impaired in people with schizophrenia and unaffected by age, though pre-PAS and post-PAS MI decreased with aging. An investigation of the contribution of antipsychotics to these results showed no evidence of an effect of antipsychotic dose on PAS-LTP, however due to a small sample size these results are inconclusive. A study of cortical response to peripheral nerve stimulation and its relationship to PAS-LTP suggests that the timing of sensory evoked potentials is relevant to the design and interpretation of PAS studies.

In examining EEG recordings during median nerve stimulation, a prominent peak in the frontal region, with average latency of approximately 20 ms, replicates a number of studies that have reported this P20 potential in the frontal cortex (T Allison et al., 1991; García Larrea et al., 1992; Valeriani et al., 2000; Yamada et al., 1984). This lends support to the hypothesis that the stimulus from the median nerve takes close to 25 ms to reach the frontal cortex, and provides evidence that the 25 ms interval used for PAS in the DLPFC is a good choice.
It was also found that the timing of the P20 SEP in the left frontal region was an important predictor of potentiation in healthy and schizophrenia participants of all ages, as participants whose P20 latency closely matched the 25 ms interstimulus interval used during PAS showed overall greater PAS-LTP. In a number of earlier PAS studies, SEP times in each participant have been used to select individualized PAS interstimulus intervals. This approach has been found to be effective in the motor cortex, where a negative potential occurring at 20 ms (N20) is the earliest identified potential (T Allison et al., 1991; García Larrea et al., 1992; Valeriani et al., 2000; Yamada et al., 1984). Research groups have used the N20 latency or N20+2ms as the interstimulus interval when performing PAS, with successful results (Jung & Ziemann, 2009; Müller-Dahlhaus et al., 2008; Ziemann et al., 2004). The results of this analysis suggest that this approach could have benefits over the use of a fixed interval for all participants, as it reduces the variability between individuals and maximizes PAS-LTP.

These results also have significance for PAS studies that compare groups of participants whose P20 latencies may differ. While an analysis of P20 times in people with schizophrenia found no evidence of abnormal nerve conduction in this population – nor has evidence of abnormal latency in schizophrenia been found in the literature - PAS researchers should take into consideration that SEP times might differ between subject groups. Arm length has been correlated with longer SEP latencies in response to median nerve stimulation. This is important for comparing PAS-LTP between sexes, as men tend to be taller than women and display longer SEP latencies (Truett Allison, Wood, & Goff, 1983). Increased SEP latency has also been observed in some forms of dementia (Abbruzzese et al., 1984). Of particular relevance to this
study is the effect of aging on nerve conduction velocity. In multiple studies, age has been associated with a slowing of conduction velocity in the median nerve and spinal cord and resulting increase in SEP latency (Truett Allison et al., 1983; Dorfman & Bosley, 1979; Stetson, Albers, Silverstein, & Wolfe, 1992), which could contribute to PAS-LTP results.

Whereas PAS-LTP was found to be significantly affected by P20 time, potentiation of theta-gamma coupling was not. This suggests that different mechanisms are employed in potentiating the two measures. The mechanism for PAS-LTP is understood to be similar to that of LTP, given that is dependent on stimulus timing and NMDA receptor activation. The mechanism for potentiation of theta-gamma coupling by PAS is not well understood, and could be a direction for future research.

In two studies performed in older people with schizophrenia, the effects of age, schizophrenia and antipsychotic treatment were investigated. At the outset of the study, it was hypothesized that PAS-LTP would be impaired in schizophrenia and would decrease with increasing age in both the healthy and schizophrenia groups. As expected, PAS-LTP was significantly lower in the schizophrenia participants in all age groups, however it did not decrease with increasing age. The finding that P20 times do not differ between the schizophrenia and control groups indicated that peripheral nerve transmission is not likely to contribute to this difference. More likely, the impairment in PAS-LTP is caused by abnormalities in the central nervous system. These might include altered glutamatergic and dopaminergic signaling, along with disrupted NMDA receptor function (Ben-Shachar & Laifenfeld, 2004; Olney, Newcomer, & Farber, 1999).
Contrary to the initial hypothesis, PAS-LTP did not decrease in older participants. PAS studies evaluating neuroplasticity in the motor cortex have all found that PAS-LTP decreases with increasing age (Tecchio et al., 2008; Fathi et al., 2010; Müller-Dahlhaus et al., 2008). One possible reason that it was not observed to differ here could be that aging has a heterogeneous effect on neuroplasticity in different cortical regions, with the motor cortex being more strongly affected, or affected at an earlier age. This is supported by another PAS study that found that PAS-LTP is in fact increased in the somatosensory cortex in older people, indicating that aging does not cause global loss of neuroplasticity (Litvak et al., 2007). FMRI research reporting increased activation of the prefrontal cortex in older people performing cognitive tasks is another indicator that the excitability of this region might be increased as a mechanism to compensate for declining neural function associated with aging (Cabeza, 2002; Grady, 2012). A multitude of studies report that older people show greater activation in the prefrontal cortex during memory tasks, as well as a larger activated area compared to younger participants. In some cases there is an association between better task performance in the older individuals who showed increased activation compared to those who show lower activation. This relationship is seen only in older participants (Grady, 2012). If this is interpreted as a compensatory mechanism, this adaptation is in itself a form of neuroplasticity. Furthermore, it is indicative of increased excitability in the prefrontal cortex of older people. This increase in excitability may translate to increased cortical evoked activity evoked by TMS and potentiated by PAS.

Another explanation for the lack of observed age effect might be the increased latency of the P20 potential with age, which would bring the average P20 time of the older group closer to the 25
ms latency that was shown to result in higher PAS-LTP. While there was no significant increase in P20 latency observed in the sample studied here, a larger study reports a slowing in median nerve conduction of 1.3 m/s per decade (Stetson et al., 1992). Assuming an average distance that the stimulus travels along the median nerve is 60 cm, a 30-year old person with a P20 latency of 21 ms (the average time found in this analysis) would have a latency of approximately 23 ms at age 60. Based on the analysis of PAS-LTP with respect to P20 time this latency difference may be sufficient to mask the effects of age on PAS-LTP.

The three studies performed in the motor cortex in older people which found impaired PAS-LTP differed in their choice of PAS interstimulus intervals (Tecchio et al., 2008; Fathi et al., 2010; Müller-Dahlhaus et al., 2008). The earliest observed SEP in the motor cortex when the contralateral median nerve is stimulated is a negative deflection at 20 ms, therefore certain research groups use this SEP time to guide the selection of PAS interstimulus interval in each participant. The earliest study performed in the the motor cortex of older people used a fixed 25 ms interval for each participant, and found that PAS-LTP was impaired in older women but not older men (Tecchio et al, 2008) The second found each participant's N20 time and used individual interstimulus intervals of N20+2 ms, and found impaired PAS-LTP in older people of both sexes (Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008). The third study used a fixed interstimulus interval of 25 ms and also found that PAS-LTP was impaired in older people of both sexes (Fathi et al., 2010). As all of these studies found impairments in PAS-LTP in the motor cortex, regardless of the interstimulus interval used. It is therefore less likely that SEP timing is responsible for the absence of age effect observed in the DLPFC, and more likely that it
is instead the result of a difference in the effect of age on neuroplasticity in different cortical regions.

It was hypothesized that potentiation of theta-gamma coupling would be impaired in schizophrenia and would further decrease with increasing age. As was the case for potentiation of cortical evoked activity, potentiation of MI was decreased in schizophrenia but not by aging. However, pre-PAS and post-PAS MI did decrease with increasing age. Based on the results of the SEP analysis that found that P20 latency does not differ between schizophrenia and controls, and furthermore that potentiation of theta-gamma coupling is independent of age, this difference cannot be attributed to differences in nerve conduction time. A likely explanation for impairments in potentiation of theta-gamma coupling in schizophrenia is that parvalbumin-containing cells, found to be important for generating theta-gamma coupling, are reduced in multiple cortical areas in schizophrenia, including the prefrontal cortex (Wulff et al., 2009). The causes of decreased MI with increased age have not been investigated. Certain rat strains exhibit a reduction in parvalbumin-containing cells as they age, however these findings have not been replicated in humans (Bu, Sathyendra, Nagykery, & Geula, 2003; Krzywkowski, De Bilbao, Senut, & Lamour, 1995; Ouda, Druga, & Syka, 2008). The inhibitory action of fast-spiking \(\text{GABA}_A\) receptors has been found to be important for generating gamma oscillations and theta-gamma coupling (Wulff et al., 2009, Buzsaki & Wang, 2012). Therefore, a disruption of \(\text{GABA}_A\)ergic neurotransmission is another possible culprit for decreased coupling in older people. Animal evidence has not provided evidence that \(\text{GABA}_A\) receptor function is affected in aged mice or monkeys (Wenk, Walker, Price, & Cork, 1991). In humans, changes in the expression of
GABA\textsubscript{A} receptor subunits have been observed with increasing age, however changes in GABAergic transmission has not been extensively studied (Rissman, De Blas, & Armstrong, 2007). The causes of impaired theta-gamma coupling in older people are therefore unclear at this time.

It has been well-documented that cognitive ability and neuroplasticity are impaired in schizophrenia. Included are deficits in working memory (Heinrichs & Zakzanis, 1998, Nuechterlein et al., 2004, Frantseva et al., 2008, Daskalakis, Christensen, Fitzgerald, & Chen, 2008, Hasan et al., 2011). The results of the research presented here indicate that impaired PAS-LTP in the DLPFC may play a role in the cognitive deficits observed in this population. It is unclear, however, whether PAS-LTP in the DLPFC is in fact correlated with working memory performance. PAS studies performed in other brain regions have identified behavioural correlates of PAS-LTP. In the somatosensory cortex, PAS-LTP was correlated with performance on a tactile discrimination task (Litvak et al., 2007). Another study performed in the motor cortex of people with schizophrenia and controls found that PAS-LTP was correlated with performance on a motor learning task. However, this latter group indicated in discussion of their publication that this result was likely driven by differences between the control and schizophrenia participants, as the patient group had overall lower PAS-LTP and performance of the motor learning task (Frantseva et al., 2008). At this time, no research has been performed that relates PAS-LTP in the DLPFC with working memory performance. In general, neuroplasticity in the pathways between the prefrontal cortex and hippocampus has been suggested to play a central role in working memory and memory consolidation (Laroche, Davis, & Jay, 2000). Electrophysiology and gene
expression studies have shown that mechanisms for neuroplasticity are active during learning and memory tasks (Laroche, Davis, & Jay, 2000). It can therefore be expected that PAS-LTP in the DLPFC - as a measure of plasticity – would be related to working memory performance, however further study of this relationship is called for.

Behavioural research has found that working memory declines significantly beginning in the mid-fifties, and anatomical evidence shows that the prefrontal cortex is one of the first regions to experience loss of grey matter volume due to aging (Daigneault, Braun, Whitaker 1992, Raz et al 1997). However, the research presented in this thesis found that there was no effect of age on PAS-LTP in the DLPFC. It can be surmised from these results that declining neuroplasticity is not the only factor that could contribute to impaired cognitive skills in older people. Other cognitive abilities including processing speed and attention regulation have been implicated in declining cognition.(Jones et al 2006, Burke et al 2006). Abnormalities in these faculties would result in the observed decline in working memory without necessarily producing or resulting from abnormal neuroplasticity. In the prefrontal cortex, the compensatory mechanisms described earlier might in fact exist to diminish the deleterious effects of processing speed slowing and poor regulation of attention that occur in older people (Cabeza, 2002; Grady, 2012).

Theta-gamma coupling has been shown to have a strong association with cognition, in particular with memory, where it has been hypothesized to play a role in segmenting elements in a sequence and transmission of information between cortical regions (J. E. Lisman & Idiart, 1995). The results of this research indicate that potentiation of theta-gamma coupling by PAS is
impaired in schizophrenia, which may have implications for understanding cognitive impairments in this population. Impaired theta-gamma coupling in schizophrenia has been suggested to be a cause of certain schizophrenia symptoms. For example, deficits in segmenting and ordering information could potentially lead to the kinds of disordered thoughts that are seen in schizophrenia (J. Lisman & Buzsaki, 2008). Early results from a study of theta-gamma coupling in working memory indicate that on a high-load working memory task, higher coupling is related to better task performance in healthy people (Rajji et al., 2014). As with PAS-LTP, however, it is not well-understood whether there is an association between potentiation of theta-gamma coupling by PAS and cognitive performance. Further research into the correlation between task performance and coupling would provide insight into the results of this analysis.

Theta-gamma coupling decreased with increasing age in both the schizophrenia and healthy groups. The decline in coupling and potentiation of coupling agrees with cognitive research that shown reduced working memory performance in older people (Daigneault et al., 1992). Theta-gamma coupling may therefore be a more informative measure than PAS-LTP for understanding cognition in older people, as the latter shown no decline with increasing age. As discussed earlier, the absence of age effect on potentiation of cortical evoked activity in the DLPFC indicates that other cognitive abilities - for example processing speed and attention regulation – likely play a role in the cognitive decline associated with aging. Age-related impairments in theta-gamma coupling could be another cause of declining cognitive performance. The important role that theta-gamma coupling has been found to play in memory make it a particularly good
target for future research in understanding the neural basis for working memory impairments in older people.

Four participants received two sessions of PAS: one session at a high dose of antipsychotic and another approximately 6-8 weeks later after a gradual dose reduction of 40%, however due to the small number of participants results are inconclusive. It was hypothesized that antipsychotics impair neuroplasticity by blocking D2 receptors, and so PAS-LTP and would be higher after participants had had their medication dose reduced. The results from the 4 participants do not provide evidence for this hypothesis, as two experienced an increase in potentiation and the other experienced a decrease. Another hypothesis was that potentiation of theta-gamma coupling would be improved on the lower antipsychotic dose. This was not case, as three of the four participants experienced a decrease in coupling, with the fourth experiencing no change. These results were not significant, however, and additional subjects will need to be tested before conclusions can be drawn from this line of research.

There are limitations to this research that introduce difficulties in interpreting the results and suggest directions for additional study. One important limitation of the PAS studies, in particular with regards to interpretation of N-back data, is the sample size. The heterogeneous nature of participants' performance makes it impossible to draw conclusions about performance, neuroplasticity and theta-gamma coupling.
Another limitation of the study is suggested by the absence of a detectable effect of aging on PAS-LTP. Two participants in the healthy group and two in the schizophrenia group were over the age of 80, however many more were in their 50s and early 60s. If participants are indeed using a compensatory mechanism in the DLPFC to maintain neuroplasticity, it is possible that a decline might only be detected in very old participants.

Finally, due to constraints of participant recruitment and testing the PAS studies did not include a control group. It would have been preferable to perform a control PAS protocol using a 100ms interstimulus interval in a subset of participants as a basis for comparison.

This research provides evidence for impaired plasticity of cortical excitability and theta-gamma coupling, however further exploration is required to understand their cause and to connect these deficits to cognitive abilities.
8 Conclusions

The results of this research provide evidence for several of the initial hypotheses, including supporting the use of an interstimulus interval of approximately 25 ms for PAS and indicating that neuroplasticity is impaired in older people with schizophrenia. SEP analysis of median nerve stimulation replicated the finding of a positive potential at approximately 20 ms latency in the frontal cortex in both healthy controls and people with schizophrenia. PAS, which depends on the simultaneous arrival of peripheral and cortical stimuli to enhance excitability, was found to be predicted by how closely the P20 latency matched the 25 ms PAS interstimulus interval.

PAS was used as a tool to study neuroplasticity in the DLPFC of people with schizophrenia throughout the lifespan. The hypothesis that plasticity is impaired in older people with schizophrenia was overall supported by the findings. PAS-LTP was significantly lower in people with schizophrenia. However, contrary to the hypothesis, it was not affected by age. Potentiation of theta-gamma coupling was also lower in people with schizophrenia and unaffected by age, however coupling both pre- and post-PAS did decline in older people.

Participants who participated in PAS also performed the N-back task for working memory. The goal of this was, initially, to examine the relationship between neuroplasticity and working memory performance. However, the low number of participants made this an impossibility. Examining the connection between PAS-LTP, theta-gamma coupling and cognition is a direction for future research.
Antipsychotics may also play a role in altering neuroplasticity and cognition in the dorsolateral prefrontal cortex. A small number of participants were assessed during two sessions: before and after undergoing antipsychotic dose reduction. Results of this line of research are inconclusive, and merit further investigation with a larger sample size.
9 Future Directions

The results of this research provide some insight into neuroplasticity throughout the lifespan in schizophrenia, however additional research would be of benefit in explaining some unexpected findings and understanding the mechanisms of altered neuroplasticity.

Firstly, plasticity of cortical evoked activity measured by PAS-LTP was found to be unaffected by age. Three previous PAS studies have found that PAS-LTP is impaired in the motor cortex of older people. It was therefore unexpected that no effect of age was observed in the DLPFC, additionally because working memory has been found to decline starting in the mid-fifties (Daigneault et al., 1992). There are different possible explanations for the observe absence of effect, which are detailed in the discussion. The increased SEP latency that previous studies have reported in older people might bring the average P20 SEP time closer to the 25 ms interstimulus interval used in PAS, which is associated with increased PAS-LTP. Another possibility is that compensatory mechanisms are engaged to preserve plasticity in the DLPFC, so that there is a heterogeneous effect of age on PAS-LTP in different cortical regions. In order to differentiate between these two hypotheses, a PAS experiment could be performed in the same demographic controlling for SEP time by using a protocol that selects individualized intervals for each participant based on their P20 latency. If it was found that PAS-LTP remained unaffected by age when the lengthening of P20 latency was accounted for, it would provide more convincing evidence that aging does not affect plasticity in all cortical areas to the same degree. This would introduce questions of which brain regions experience the greatest effects of age on PAS-LTP, and whether these age-related changes are reflected in cognitive decline in older people. As of
now, PAS studies have been performed in motor cortex, somatosensory cortex, DLPFC and posterior partietal cortex (Lucia, Lu, Bliem, & Ziemann, 2011; Pellicciari, Miniussi, Rossini, & De Gennaro, 2009; Rajji et al., 2013; Stefan et al., 2000). The results from P20 time analysis indicate that SEP times would be a good way to determine PAS interstimulus intervals in different brain regions in order to study PAS-LTP in various parts of the cortex.

Whereas PAS-LTP was found to be significantly affected by P20 latency, potentiation of theta-gamma coupling was not. This suggests that different mechanisms are employed in potentiating the two measures. Studies exploring mechanisms of potentiation and modulation of PAS-LTP have administered pharmaceutical agents to their participants to agonize or antagonize certain receptors (Korchounov & Ziemann, 2011; Monte-Silva et al., 2011; Stefan et al., 2000). Similar studies to examine the role of various neurotransmitters in potentiating theta-gamma coupling would provide some insight into its mechanism. A more thorough examination of the interstimulus interval that is effective for potentiating theta-gamma coupling could also further understanding of its mechanism. PAS-LTP has been shown to be sensitive to interstimulus interval in studies that have varied the interval from 25 ms to 5000 ms (Stefan et al., 2000). If potentiation of theta-gamma coupling is less sensitive to stimulus timing, as suggested by the results of SEP and PAS-LTP analysis, that would provide additional insight into the mechanism of potentiation of coupling.

One hypothesis that could not be explored with our sample size was whether PAS-LTP and theta-gamma coupling would relate to cognition. Due to behavioural variability and smaller effect sizes, such an analysis would require a larger sample size. Measures of cognitive ability
were collected as part of the PAS study, and with a larger sample an analysis of correlation between these cognitive measures – including performance on the N-back – and PAS-LTP and potentiation of theta-gamma coupling can be performed. Since cortical excitability and of theta-gamma coupling are likely potentiated through different mechanisms and seem to be affected differently by aging, they might also have different significance for cognition. For example, both baseline theta-gamma coupling and potentiation of coupling in the left frontal cortex decreased with increasing age. In this sense, these measure are more closely associated with age-related declines in working memory than PAS-LTP, which was not affected by age.

Further study with a larger sample size is also needed for understanding the effect of antipsychotic dose on neuroplasticity. Though previous researchers have not detected an effect of antipsychotic dose on PAS-LTP (Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Frantseva et al., 2008; Hasan et al., 2011), the benefit of a dose reduction protocol is the repeated-measures design that reduces variation in treatment effects, allowing for the effect of antipsychotics to be better isolated from other sources of variability.

The literature review presented earlier highlighted a dearth of studies of neuroplasticity and theta-gamma coupling in older people with schizophrenia. While this line of research has contributed in some ways to our understanding of neuroplasticity in schizophrenia throughout the lifespan, further study of the effect of aging on plasticity in the DLPFC, factors that affect theta-gamma coupling and the relationship between neuroplasticity and cognition will give a more complete picture.
References


Appendices

10 Appendix A: Inclusion and Exclusion Criteria for Paired Associative Stimulation

10.1 Inclusion Criteria for Patients

- Age 18 or above
- All races and ethnicities.
- Females and males.
- Meet DSM-IV TR criteria for a current diagnosis of schizophrenia or schizoaffective disorder.
- Clinically stable as operationalized by (1) either having not been hospitalized within 3 months or having been hospitalized for 3 months or more prior to assessment, and (2) having had no change in antipsychotic medication dosage within the 4 weeks prior to assessment.
- Willingness and ability to speak English
- Willingness to provide informed consent
- Corrected visual ability that enables reading of newspaper headlines and corrected hearing capacity that is adequate to respond to a raised conversational voice.

10.2 Exclusion Criteria for Patients

1. Meets criteria for a cognitive disorder secondary to a neurological or other medical disorder affecting the central nervous system (for example, multiple sclerosis, history of traumatic brain injury, stroke, untreated hypothyroidism).
2. Mini Mental Status Examination score of 17 and less because a subject with a very low MMSE score is unlikely to be able to compete the NP battery.

3. Diagnosis of bipolar disorder or current major depressive episode.

4. Meets diagnostic criteria for current alcohol or other drug dependence within 6 months of testing.

5. Electroconvulsive Therapy (ECT) within 6 months of testing.


7. Incompetency to consent

10.3 Inclusion Criteria for Healthy Controls

• Age 18 or above

• Willingness and ability to speak English

• Willingness to provide informed consent

• Corrected visual ability that enables reading of newspaper headlines and corrected hearing capacity that is adequate to respond to a raised conversational voice.

10.4 Exclusion Criteria for Controls

• DSM IV TR psychiatric diagnosis except for simple phobias or an adjustment disorder.

• Other neurological disorder affecting central nervous system.

• Psychotropic medication except for sedative /hypnotics at a stable dose for at least 4 weeks.
Family history of a primary psychotic disorder in a first-degree relative.

- Left handedness.

### 11 Appendix B: Additional Inclusion/Exclusion Criteria for Dose Reduction

#### 11.1 Inclusion Criteria

- Age of 50 and older at time of scanning
- DSM-IV/SCID diagnosis of schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, or psychotic disorder NOS with onset before age 45
- Having been treated with either oral risperidone at a steady dose of $\geq 2$ mg/day or oral olanzapine $\geq 10$ mg/day for at least 12 months
- MMSE $\geq 18$
- PANSS score $\leq 3$ for delusions, unusual thought content, hallucinatory behaviour

#### 11.2 Exclusion Criteria

- Incapacity to provide consent to psychiatric treatment
- Participation in this study would result in exceeding the annual radiation dose limits (20 mSv) for human subjects participating in research studies.
- Substance abuse or dependence (within past six months)
- Positive urine drug screen
• Positive serum pregnancy test at screening or positive urine pregnancy test before PET scan

• Having taken more than one dose of antipsychotics other than risperidone or olanzapine within 12 months of screening

• History of treatment with long-acting (depot) neuroleptic antipsychotic medication, or Risperdal Consta, within past 12 months

• Addition of or change in dose of antidepressants, valproic acid, lithium, carbamazepine, or laotrigine for mental health reasons within 12 months of screening

• Metal implants or a pace-maker that would preclude the MRI scan

• History of head trauma resulting in loss of consciousness > 30 minutes that required medical attention

• Unstable physical illness or significant neurological disorder including a seizure disorder

• Size of head, neck, and body being unable to fit PET and MRI scanners

• Refusal to give consent to investigator to communicate with physician of record for the entire duration of the study

• Psychiatric concerns raised by the physician of record regarding participation in the study.

• History of psychiatric hospitalization within 12 months of screening

• History of suicidal gestures or attempts following previous attempts at antipsychotic dose reduction
Cognitive Testing

**Wechsler Test of Adult Reading (WTAR):** The WTAR is designed to assess pre-morbid level of intellectual functioning for individual’s age 16 to 89 years.

**Mini-Mental Status Exam (MMSE):** The MMSE is a well known and widely used brief mental status test of cognitive impairment in elderly individuals. The test consists of 13 questions administered directly to the subject, assessing orientation to place and time, learning and memory, construction ability, attention and calculation. The possible range of scores is 0-30, where 0 represents severe impairment and 30 represents no impairment.

**Executive Interview (EXIT):** This instrument was designed to assess executive cognitive function. There are 25 items, each having a possible score range of zero to a maximum of 2 points, with higher scores indicating greater executive dyscontrol.

**Repeatable Battery for the Assessment of Neuropsychological Status (RBANS):**

The RBANS is a brief, individually administered test that helps determine the neuropsychological status of adults ages 20-89 years who have neurological injury or disease such as dementia, head injury, or stroke. One can get a quick sampling of important cognitive areas using content and a format familiar to clinicians who use the Wechsler™ Scales. The overall battery length is less than 30 minutes, in order to maximize patient cooperation and to
minimize the effect of fatigue on performance. In addition, the RBANS has two parallel forms, ideal for measuring change in the client's neuropsychological status over time.

**N-Back Task:** The N-back task assesses the ability to maintain active information online. It is a continuous-recognition measure whereby letters are presented in sequence. At each letter presentation, the individual is asked to judge whether this letter matched the one presented N items back.

**Sternberg Task:** The Sternberg task assesses verbal working memory, the ability to maintain information such as a series of letters online over a brief period. This task involves the presentation of a series of letters ranging from 2-8 letters followed by a delay phase lasting several seconds in which memory of the letters must be retained. A single letter is then presented and participants must identify whether this letter was presented in the original series of letters.

**Semantic Priming Task:** Semantic memories refer to general knowledge such as knowledge of words and their meanings, knowledge of objects and their relationships and knowledge of the world. Semantic priming is an experimental paradigm used to test the storage and grouping of semantic memories. In this task, participants are presented with a priming word followed by a test word. Participants are required to indicate if the two words are related (cat-dog) or unrelated (cat-apple) and the reaction time is compared. Related words result in shorter reaction times compared to unrelated words.