Alcohol Effects on Human Neurophysiology

by

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Abstract

Background: Alcohol exerts its behavioural and neurophysiological effects by acting on multiple neurotransmitter systems in the brain, including the excitatory glutamatergic and inhibitory GABAergic neurotransmitter systems. Alcohol's actions on these neurotransmitter systems may, in turn, have important implications for alcohol's effect on neuroplasticity.

Objectives: First, to evaluate the effect of alcohol intoxication on paired associative stimulation (PAS)-induced neuroplasticity in the motor cortex up to a day following PAS. Second, to evaluate the effect of alcohol intoxication on PAS-induced neuroplasticity in the dorsolateral prefrontal cortex (DLPFC). Third, to evaluate the effect of alcohol intoxication on N100 amplitude to transcranial magnetic stimulation (TMS) stimulation of the DLPFC, as an index of $GABA_B$ receptor mediated neurotransmission.

Hypotheses: First, it was hypothesized that alcohol intoxication would impair PAS-induced neuroplasticity in the motor cortex and that this impairment would also be evident the day following PAS. Second, it was hypothesized that alcohol intoxication would impair

PAS-induced neuroplasticity and the potentiation of theta-gamma coupling in the DLPFC. Third, it was hypothesized that alcohol intoxication would produce a decrease in the N100 amplitude to TMS stimulation of the DLPFC.

Results: The first study found that alcohol intoxication impaired PAS-induced neuroplasticity in the motor cortex. Potentiation levels were no longer different between the alcohol and placebo conditions the day following PAS administration. The second study found that alcohol intoxication impairs PAS-induced neuroplasticity in the DLPFC. The third study found that alcohol intoxication produces a decrease in N100 amplitude to TMS stimulation of the DLPFC.

Conclusions: Alcohol intoxication impairs neuroplasticity in the motor cortex and DLPFC. The disruption of neuroplasticity by alcohol, may be explained, in part, by alcohol's impairment of $GABA_B$ receptor mediated neurotransmission. These findings may provide a potential mechanism for alcohol's motor and cognitive impairments.

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List of Symbols and Abbreviations

α	Alpha
ß	Beta
δ	Delta
μ	Mu
θ	Theta
γ	Gamma
ACC	Anterior Cingulate Cortex
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid
APB	Abductor Pollicis Brevis
APV	(2R)-amino-5-phosphonovaleric acid
AUD	Alcohol Use Disorder
BAC	Blood Alcohol Concentration
BNST	Bed Nucleus of the Stria Terminalis
Ca^{2+}	Calcium Ions
CAMKII	Calcium/Calmodulin-Dependent Kinase II
cAMP	Cyclic Adenosine Monophosphate
CEA	Cortical Evoked Activity
CRF	Corticotrophin Releasing Factor
CRHR1	Corticotrophin Releasing Hormone Receptor 1
CSP	Cortical Silent Period

CYP2E1	Cytochrome P450 2E1
DALYs	Disability-Adjusted Life Years
DLPFC	Dorsolateral Prefrontal Cortex
EEG	Electroencephalography
EMG	Electromyography
EPSP	Excitatory Post-Synaptic Potential
ERP	Event-Related Potential
FAEE	Fatty Acid Ethyl Ester
GABA	Gamma Aminobutyric Acid
GluR	Glutamate Receptor
GMFA	Global Mean Field Amplitude
HAD	High Alcohol Drinking
ICA	Independent Component Analysis
ICF	Intracortical Facilitation
IPSP	Inhibitory Post-Synaptic Potentials
ISI	Inter-stimulus Interval
LICI	Long-Interval Cortical Inhibition
LTD	Long-Term Depression
LTP	Long-Term Potentiation
MEOS	Microsomal Ethanol Oxidizing System
MEP	Motor Evoked Potential
mGLUR	Metabotropic Glutamate Receptor

mM	Millimolar
mRNA	Messenger Ribonucleic Acid
MST	Magnetic Seizure Therapy
NAc	Nucleus Accumbens
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NMDA	N-methyl-D-aspartic acid
OFC	Orbitofrontal Cortex
p(EPSP)	Population Excitatory Post Synaptic Potentials
PAS	Paired Associative Stimulation
PFC	Prefrontal Cortex
PKA	Protein Kinase A
PNS	Peripheral Nerve Stimulation
RMT	Resting Motor Threshold
rTMS	Repititve Transcranial Magnetic Stimulation
SICI	Short-Interval Cortical Inhibition
SSRIs	Selective Serotonin Reuptake Inhibitors
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
TEP	TMS-Evoked Potentials
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation
VTA	Ventral Tegmental Area

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Chapter 1

Introduction

1 Background

1.1 History of Alcohol Use

The word alcohol can be used to describe a wide series of compounds (Koob & Le Moal, 2006). The form of alcohol used as a beverage is ethanol. For the purpose of this thesis, all references to alcohol will mean ethanol. It is not clear when humans first began producing alcohol beverages. Beer containers from 8000BCE (the late Stone Age period) suggest that humans have been fermenting alcohol for at least 10000 years. It is thought that the earliest alcohol beverages may have been made by fermenting berries or honey (Patrick, 1970). Alcohol beverages have been use by many cultures throughout all of recorded history (*Social and Cultural Aspects of Drinking: A report to the European Commission*, 2008).

1.2 Behavioural and Physiological Effects of Alcohol

The behavioural and physiological effects of alcohol vary with blood alcohol concentration (BAC). Blood alcohol concentrations are expressed using a number of units, grams of alcohol per one hundred milliliters of blood (%BAC), grams of alcohol per one liter of blood (%w/v), grams of alcohol per one kilogram of blood (%w/w) and millimoles of alcohol per liter of blood (mM). With BAC's of 0.01%-0.05%, alcohol produces changes in personality and mood including increased talkativeness, mild increases in

feelings of euphoria, decrease in inhibitions, decrease in anxiety and increased confidence. A BAC of 0.08% (the level at which one is deemed legally impaired in Canada), produce significant disinhibition, impaired motor function, impaired cognition and impaired judgement. A BAC of 0.15% is associated with major motor impairment and possible blackouts. A BAC of 0.30% is associated with a stuporous state approaching coma while 0.50% is the lethal dose in 50% of the population (LD50) (Koob & Le Moal, 2006).

1.3 Alcohol Pharmacokinetics

The distribution of alcohol from the blood into tissues and fluids is relative to their water content (Cedarbaum, 2012). Alcohol has a low lipid solubility but easily passes through biological membranes by passive diffusion (Cedarbaum, 2012; Koob & Le Moal, 2006). Higher alcohol concentrations produce a higher concentration and greater absorption of alcohol. Alcohol does not bind to plasma proteins (Cedarbaum, 2012). Alcohol has a low lipid:water partition coefficient (Ingolfsson & Andersen). This means that for any given weight, an individual with a higher percentage body fat will reach higher blood alcohol concentrations for an equal volume of alcohol (Cedarbaum, 2012). Given that women commonly have a higher percentage body fat, they will have a higher blood alcohol concentration when given the same dose of alcohol in grams per kilogram. This discrepancy is avoided by calculating dose in grams per kg of body water (Watson, Watson, & Batt, 1980). Women also tend to have lower first pass metabolism of alcohol by the stomach, which can also lead to higher levels blood alcohol levels in women (Cole-Harding & Wilson, 1987; Frezza et al., 1990).

Twenty percent of absorption of alcohol occurs in the stomach (Koob & Le Moal, 2006). Food in the stomach reduces the rate of gastric emptying of alcohol and thereby reduces the blood alcohol level achieved. Metabolism of alcohol in the stomach occurs through gastric alcohol dehydrogenase (Pestalozzi, Buhler, von Wartburg, & Hess, 1983) as well as the microsomal ethanol oxidizing system (MEOS), involving cytochrome P450 2E1 (CYP2E1) (Lieber & DeCarli, 1968) and catalase (Handler & Thurman, 1990). The level of contribution of the stomach to first pass metabolism is controversial (Zakhari, 2006).

The metabolism of alcohol by the liver occurs through three enzymatic pathways (Nagy, 2004; Riveros-Rosas, Julian-Sanchez, & Pina, 1997). The first pathway occurs through the rate-limiting enzyme alcohol dehydrogenase which is responsible for the oxidation of alcohol to acetaldehyde (Koob & Le Moal, 2006). Acetaldehyde dehydrogenase is then responsible for the breakdown of acetaldehyde into acetic acid, water and carbon dioxide (Koob & Le Moal, 2006). Genetic variation in acetaldehyde dehydrogenase is responsible for the differences in alcohol elimination in human populations (i.e., the inactivation of acetaldehyde dehydrogenase 2 is responsible for the flush reaction to alcohol in Asian populations) (Koob & Le Moal, 2006).

The second pathway for alcohol metabolism is through the microsomal ethanol oxidizing system (MEOS) by cytochrome P450 2E1 (CYP2E1) (Koob & Le Moal, 2006). This pathway accounts for a large portion of metabolic tolerance of alcohol (Lieber, 1997) via the induction of CYP2E1. Furthermore, this pathway is responsible for the production of toxic alcohol metabolites in the liver of individuals with alcohol use disorders (AUDs) (Lieber, 1997). The third pathway responsible for the metabolism of alcohol is the non-

oxidative metabolism of alcohol by fatty acid ethyl ester (FAEE) synthase (Koob & Le Moal, 2006; Lange & Voas, 2001). FAEEs have been implicated in the toxic effects on organs by alcohol (Hamamoto, Yamada, & Hirayama, 1990; Lange & Voas, 2001).

1.4 Alcohol Pharmacodynamics

Alcohol acts on multiple neurotransmitter systems including gamma-aminobutyric acid (GABA), glutamate, dopamine, serotonin and opioid peptides to exert its effects ((Nevo & Hamon, 1995). Below is a review of the existing literature on the effects of acute alcohol consumption on each of these neurotransmitter systems.

1.4.1 Acute Effects of Alcohol on GABAergic Neurotransmission

GABA is the major inhibitory neurotransmitter. Alcohol's effect on the GABA receptor complex appears to be modulated allosterically. That is, binding of alcohol to the GABA receptor complex results in the opening of the chloride channel and hyperpolarization of the cell or potentiation of the hyperpolarization produced by GABA (Deisz, 1999a). The effect of alcohol at the pharmacological level can be antagonized with the $GABA_A$ antagonists. For example, doses as low as 1-3 mM alter GABA-gated measures of current (Deisz, 1999b), thereby demonstrating its high potency, *in vitro*. Similarly, administration of $GABA_A$ antagonist picrotoxin, block the anticonflict effect induced by alcohol in animal models (Stefan, Wycislo, & Classen, 2004). Administration of $GABA_A$ antagonists has also been shown to reverse the motor-impairing effects of alcohol (Finn, Justus,

Mazas, & Steinmetz, 1999; Maurage et al., 2012) and alcohol-induced sedation (Finn et al., 1999) in animal models. In contrast, $GABA_A$ agonists have been shown to potentiate a number of alcohol-induced effects, such as the sedative-effects (Finn et al., 1999) and the aerial righting reflex (Grattan-Miscio & Vogel-Sprott, 2005; Schweizer & Vogel-Sprott, 2008). In addition to $GABA_A$, the effects of alcohol appear to also be modulated by $GABA_B$ receptor activity. For example, administration of the $GABA_B$ agonist baclofen decreases self-administration in nondependent rats (Saults, Cowan, Sher, & Moreno, 2007) and also reduces the alcohol deprivation effect in alcohol-preferring rats (Boha et al., 2009). The effects of alcohol on $GABA_B$ receptor mediated neurotransmission are however, less well understood than its effects on $GABA_A$.

1.4.2 Acute Effects of Alcohol on Glutamatergic Neurotransmission

Glutamate is the major excitatory neurotransmitter. Glutamate acts on several receptor subtypes to exert its effects, including N-methyl-D-aspartic acid (NMDA) receptor. Findings from multiple studies demonstrate that alcohol inhibits NMDA receptors. For example, in rat hippocampal slices, Lovinger et al., 1990 demonstrated that increasing concentrations of alcohol produces increased inhibition of NMDA receptor-mediated population excitatory post synaptic potentials (EPSPs) at alcohol concentrations between 1 and 50 mM (Brown, Davies, & Randall, 2007). Findings from early experiments demonstrated that inhibition of NMDA receptors by alcohol is non-competitive and reversible. Single-cell recording studies in rodents demonstrate that alcohol decreases the

probability of NMDA channel opening, with a corresponding decrease in the mean open time of NMDA channels (Leung & Shen, 2007; Sohal, Zhang, Yizhar, & Deisseroth, 2009). MK801, a selective and potent NMDA receptor antagonist produces event-related potentials and effects identical to alcohol on electroencephalogram (EEG) (Oscar-Berman, 1990). Together, these findings suggest that acute alcohol consumption results in a suppression of glutamatergic neurotransmission (Brown et al., 2007; Leung & Shen, 2007; Sohal et al., 2009).

1.4.3 Acute Effects of Alcohol on Dopaminergic Neurotransmission

Dopamine is the primary neurotransmitter of the mesolimbic system, the neurocircuitry involved in mediating the brain's response to reward. The mesolimbic system projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (Purves et al., 2012). A dose dependent increase in firing the rate of dopaminergic neurons in the VTA is seen with low doses of alcohol (Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985). Acute consumption of alcohol, as well as anticipation of alcohol consumption results in increased release of dopamine into the NAc (Weiss, Lorang, Bloom, & Koob, 1993). In one study, injection of low doses of alcohol into the blood (~2-4mg/kg of body weight) stimulated dopamine release in the NAc (Lyness & Smith, 1992). Similarly, intraperitoneal injection of alcohol at doses of 1g/kg and 2g/kg in rats resulted in significantly higher levels of dopamine in the NAc (Yoshimoto, McBride, Lumeng, & Li, 1992). Dose-dependent increases in extracellular dopamine levels in the amygdala have

also been reported following intraperitoneal injection of alcohol in rats (Yoshimoto et al., 2000). Oral consumption of alcohol by rats also produces an increase in dopamine release in the NAc (Weiss, Lorang, Bloom, & Koob, 1995). Injection of dopamine antagonists directly into the NAc blocks alcohol self-administration by rats (Hodge, Samson, & Chappelle, 1997; Rassnick, Pulvirenti, & Koob, 1992). Interestingly, lesioning of the mesolimbic dopamine system does not completely block self-administration of alcohol by rat. This finding suggests that while the mesolimbic dopamine system plays an important role alcohol reinforcement, it is not essential for the reinforcement (Rassnick, Stinus, & Koob, 1993).

1.4.4 Acute Effects of Alcohol on Serotoninergic Neurotransmission

In animal studies, acute consumption of alcohol produces an increase of brain serotonin levels (LeMarquand, Pihl, & Benkelfat, 1994b). Such an increase in serotonin levels may be due alcohol producing an increase in serotonin release or a delay in serotonin reuptake from synapses (Lovinger, 1997). In humans, acute alcohol consumption produces a decrease in blood serotonin levels and an increase in the uptake of serotonin by the platelets, suggesting that alcohol decreases serotonergic neurotransmission (LeMarquand, Pihl, & Benkelfat, 1994a). Similarly, acute consumption of alcohol results in an decrease in serotonin metabolites in urine in humans (Aizenstein & Korf, 1979). However, alcohol results in the potentiation of 5-HT3 (serotonin receptor) cation currents from 5-HT3 receptors transiently expressed in human embryonic kidney 293 cells (Lovinger & Zhou, 1994), suggesting that it increases serotonergic neurotransmission. Administration of

selective serotonin reuptake inhibitors (SSRIs), which prolong the action of serotonin by blocking the reuptake of the neurotransmitter from the synaptic cleft, results in a decrease of voluntary consumption of alcohol by rats in preference paradigms (Daoust et al., 1985). Alcohol preferring P rats, high alcohol drinking (HAD) rats and alcohol preferring Fawn-Hooded rat lines have low serotonin levels (McBride & Li, 1998). The administration of serotonin antagonists to alcohol preferring P rats suppresses daily voluntary alcohol intake (Rodd-Henricks et al., 2000). Together, these findings suggest that serotonin plays an important role in mediating the drive to consume alcohol, with lower levels of serotonin being associated with an increased drive to consume alcohol. However, the use of selective serotonin reuptake inhibitors have not demonstrated great promise as a treatment for AUDs (Marcinkiewicz, Lowery-Gionta & Kash, 2016). It has been hypothesized that targeting specific 5-HT receptors may allow for better treatment outcomes (Marcinkiewicz, Lowery-Gionta & Kash, 2016).

1.4.5 Acute Effects of Alcohol on Opioid Neurotransmission

Multiple lines of evidence suggest that endogenous opioids play an important role in mediating the rewarding effects of alcohol. Acute alcohol consumption in rats causes an increase in brain enkephalin and ß-endorphin (Schulz, Wuster, Duka, & Herz, 1980). Naltrexone, an opioid receptor antagonist, reduces alcohol-reinforced operant responding. This suppression is associated with an impairment of the alcohol-induced release of dopamine in the NAc (Gonzales & Weiss, 1998). Knockout of the μ-opioid receptor in mice prevents alcohol self-administration (Roberts et al., 2000). Alcohol preferring rats

demonstrate decreased activity of the endogenous opioid system in the mesolimbic dopaminergic system (Nylander, Hyytia, Forsander, & Terenius, 1994). In humans, elevated levels of ß-endorphins are correlated with an increased risk for developing alcohol use disorders (AUDs) (Gianoulakis, Krishnan, & Thavundayil, 1996). Together, these findings suggest that the endogenous opioid system plays an important role in mediating the rewarding effects of alcohol.

1.5 Alcohol Use Disorders

1.5.1 Epidemiology and Cost to Society

AUD's are a major health and social problem worldwide (Rehm et al., 2007). According to the Global Burden of Disease Study in 2010, AUDs account for 9.6% of disability-adjusted life years (DALYs) (Whiteford et al., 2013). Alcohol attributable deaths account for 3-8% of all global deaths and alcohol accounts for 4-6% of global disability-adjusted life-years lost (Rehm et al., 2009). Alcohol contributes to increased rates of mortality and morbidity through increased occurrence of diseases such as liver cirrhosis, cancers, cardio-vascular disorders, alcoholic encephalopathy and increased rates of motor vehicle accidents, violence and homicides (Rehm & Gmel, 2003; Rehm, Gmel, Sempos, & Trevisan, 2003; Rossow, Pernanen, & Rehm, 2001; Tunstall-Pedoe et al., 1999).

1.5.2 Binge Drinking

Binge drinking is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as "…a pattern of drinking that brings BAC levels to 17.4 mM (0.08% BAC). This typically occurs after 4 drinks for women and 5 drinks for men—in about 2 hours." (http://www.niaaa.nih.gov/). Binge drinking is considered an initial step to developing AUDs (Canolty & Knight, 2010; Li, Hewitt, & Grant, 2007; Lisman & Idiart, 1995).

1.5.3 Neurochemistry of Alcohol Use Disorders

1.5.3.1 GABA in Alcohol Use Disorders

While acute alcohol consumption appears to facilitate GABAergic inhibitory neurotransmission (Deisz, 1999b; Stefan et al., 2004), chronic alcohol consumption results in counter-adaptive changes in neurotransmission that result in a suppression of GABAergic neurotransmission (Enoch, 2008). A decrease in $GABA_A$ receptor density has been observed following chronic alcohol consumption (Golovko, Golovko, Leontieva, & Zefirov, 2002). Chronic alcohol exposure also results in a decrease in α_1-subunits, which are believed to result in $GABA_A$ receptors that are less responsive to alcohol (Criswell et al., 1995). Reduction in GABAergic neurotransmission contributes to the symptoms of alcohol withdrawal. For this reason, $GABA_A$ agonists, such as the benzodiazepine diazepam, are used to treat the symptoms of alcohol withdrawal acutely (Sellers & Romach, 1991). Additionally, in clinical studies, baclofen, a $GABA_B$ agonist has been

reported to reduce alcohol craving and withdrawal (Bartos, Vida, & Jonas, 2007; Traub, Bibbig, LeBeau, Cunningham, & Whittington, 2005; Wang & Buzsaki, 1996).

1.5.3.2 Glutamate in Alcohol Use Disorders

Following chronic alcohol consumption, counter adaptive changes result in an increase in glutamatergic neurotransmission (Gass & Olive, 2008). Findings from molecular studies demonstrate that chronic alcohol consumption results in an increase in mRNA and levels of proteins required to build certain NMDA receptor subunits (Follesa & Ticku, 1995). Studies from neuronal cultures of the cerebellum and cortex have revealed that following prolonged exposure to ethanol, NMDA receptor activity is increased in the absence of alcohol (Ahern, Lustig, & Greenberg, 1994; Iorio, Reinlib, Tabakoff, & Hoffman, 1992). Additionally, following chronic ethanol exposure to hippocampal neurons grown in culture, an increased number of NMDA receptors are found at the synapse (Carpenter-Hyland, Woodward, & Chandler, 2004). Similarly, findings from animal studies have demonstrated that prolonged exposure to alcohol results in increased production of NMDA receptor subunits in brain regions including the hippocampus, amygdala and cerebral cortex (Floyd, Jung, & McCool, 2003; Kalluri, Mehta, & Ticku, 1998; Snell et al., 1996). Increased NMDA receptors are also observed in post-mortem tissue of alcohol dependent individuals (Freund & Anderson, 1996). Changes in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have been reported following chronic alcohol exposure. Increased levels of GluR1 and GluR2/3 subunits are seen in some brain regions and in neuronal cultures following prolonged alcohol exposure.

Additionally, higher levels of AMPA receptor mediated Ca^{2+} flow into the neurons have been reported following chronic alcohol exposure (Chandler, Norwood, & Sutton, 1999; Dettmer et al., 2003).

Together, these findings suggest that chronic alcohol consumption results in an increase in glutamatergic neurotransmission in the absence of alcohol. Increased NMDA receptor activity and glutamatergic neurotransmission in the absence of alcohol can cause seizures in alcohol dependent individuals. Additionally, NMDA receptor mediated hyperexcitability produces an increase in excitotoxic cell death. Chronic alcohol's effect on NMDA receptor mediated activity may also have important implications for NMDA-receptor mediated neuroplasticity. Acamprosate, a drug that acts on glutamatergic neurotransmission is a commonly used as a pharmacotherapy for AUDs.

1.5.3.3 Dopamine in Alcohol Use Disorders

In animal models of alcohol dependence, a decrease of dopamine release in the NAc is observed (Weiss et al., 1996). In these models, dopamine release in the NAc is restored following consumption of alcohol (Weiss et al., 1996). In abstinent alcohol dependent individuals, reduced synthesis of dopamine and a reduced quantity of dopamine D2/3 receptors has been reported (Heinz et al., 2005; Martinez et al., 2005; Volkow et al., 1996; Volkow et al., 2002; Volkow et al., 2007). A negative correlation between dopamine levels in alcohol dependent and levels of craving and relapse has been reported (Heinz et al., 2005). It has been hypothesized that the decrease in dopamine release in the

mesolimbic dopamine system during withdrawal may be related to the negative affect observed during this state. The consumption of alcohol during withdrawal is likely reinforced by the restoration of dopamine levels in the mesolimbic dopamine system (Hui & Gang, 2014).

1.5.4 Pharmacotherapies for Alcohol Use Disorders

A number of pharmacotherapies are currently used for the treatment of alcohol use disorders. Acamprosate, disulfiram, and naltrexone have been approved for the treatment of AUDs by the U.S. Food and Drug Administration. Naltrexone is an opioid receptor antagonist (Littleton & Zieglgansberger, 2003). Naltrexone has the highest affinity for the μ-opioid receptor, resulting in its selectivity for this receptor (Littleton & Zieglgansberger, 2003). Naltrexone binds first to the μ-opioid receptor, then to the δ-opioid receptor and lastly to κ-opioid receptor, in a dose-dependent manner (Takemori, Ho, Naeseth, & Portoghese, 1988; Takemori & Portoghese, 1992). Given that alcohol is believed to exert part of its reinforcing effects by producing a release of endogenous opioids, inhibition of the activation of opioid receptors by naltrexone can thereby reduce the positively rewarding effects of alcohol and its cues (Littleton & Zieglgansberger, 2003). Although effects are modest, naltrexone reduces rates of alcohol consumption and increases abstinence rates (Winslow, Onysko, & Hebert, 2016).

Acamprosate is thought to exert its actions by acting on glutamatergic neurotransmission. Findings from a number of studies suggest that it inhibits glutamatergic neurotransmission

(Carpenter & Dickenson, 2001; Harris et al., 2002; Mayer et al., 2002). While the exact mechanisms underlying acamprosate's therapeutic effects are still unclear, it believed that acamprosate may inhibit the hyperglutamatergic state during acute abstinence by indirect antagonism of NMDA receptor mediated neurotransmission (Rammes et al., 2001) or by inhibition of metabotropic glutamate receptors (Harris et al., 2002). It has also been argued that the active moiety of acamprosate is calcium (Spanagel, Venegeliene, et al., 2014). While findings are not consistent, acamprosate has been shown to produce a reduction in alcohol consumption and increases abstinence rates in some studies (Winslow et al., 2016).

Another drug used to treat AUDs, although less commonly used in recent years, is disulfiram. Administration of disulfiram prior to drinking produces adverse effects that make alcohol consumption unpleasant (Vallari & Pietruszko, 1982). The adverse symptoms produced by alcohol consumption while taking disulfiram include increased heart rate, nausea, vomiting, throbbing headache, confusion, flushing of the face and neck, and blurred vision. These negative effects are produced by alcohol inhibiting aldehyde dehydrogenase, the enzyme responsible for the metabolism of acetaldehyde (produced by the conversion of alcohol into acetaldehyde) into acetic acid, water and carbon dioxide (Reed, Kalant, Gibbins, Kapur, & Rankin, 1976). The inhibition of aldehyde dehydrogenase by disulfiram results in an increase of acetaldehyde levels in the blood following alcohol consumption (Reed et al., 1976). Poor compliance is an issue with disulfiram given that it does not decrease the craving and the nature of the aversive effects patients experience if they consume alcohol while taking disulfiram (Fuller et al., 1986).

Disulfiram is also an inhibitor of dopamine β-hydroxylase, an enzyme that converts dopamine to norepinephrine (Goldstein, Anagnoste, Lauber, & McKeregham, 1964).

Other pharmacotherapies, not yet approved by the US Food and Drug Administration for the treatment of AUDs, that have shown some effectiveness for the treatment of AUDs include some anticonvulsants and antidepressants. Topiramate is an anticovulsant drug that has shown some effectiveness for the treatment of AUDs. Topiramate blocks state-dependent sodium channels (Kawasaki, Tancredi, D'Arcangelo, & Avoli, 1998; Shank, Gardocki, Streeter, & Maryanoff, 2000), potentiates GABA activity (White, Brown, Woodhead, Skeen, & Wolf, 2000), antagonizes glutamatergic AMPA/kainate receptors and inhibits voltage-gated L-type calcium channels (Zhang, Velumian, Jones, & Carlen, 2000). Topiratmate has been shown to produce fewer heavy drinking days and longer time to relapse (Pani, Trogu, Pacini, & Maremmani, 2014). Other anticonvulsants such as gabapentin (Furieri & Nakamura-Palacios, 2007; Mason et al., 2014) and pregablin (Martinotti et al., 2010) have also shown effectiveness for the treatment of AUDs. Antidepressants, such as sertraline have shown to be helpful in treating AUDs in patients with comorbid mental health disorders. However, they are not effective for the treatment of AUDs in patients without comorbid illnesses (Torrens, Fonseca, Mateu, & Farre, 2005).

The use of transcranial magnetic stimulation (TMS) with electromyography (EMG) and EEG holds value as a diagnostic tool to study the effects of alcohol and alcohol use disorders on human neurophysiology. Furthermore, TMS with EMG and EEG can be used to index neurophysiological changes following treatment for AUDS. Below is a review of the literature on the use of TMS with EMG and EEG.

1.6 Transcranial Magnetic Stimulation

TMS is a non-invasive technique, introduced in 1985 by Barker et al., that is used to stimulate a given region of the brain (Barker, Jalinous, & Freeston, 1985). Barker et al., 1985 demonstrated that administration of a TMS pulse over the motor cortex produces a response in the muscles that are innervated by the motor cortical area that is stimulated. These motor evoked potentials can be recorded via surface EMG electrodes. TMS uses a transducing coil connected to a high-voltage, high-current discharge system (Jalinous, 1991). A TMS pulse is produced by an electrical current discharged through the coil, which in turn, produces a magnetic field perpendicular to the coil (Groppa, Oliviero, et al., 2012). When the coil is placed on the head tangentially and a TMS pulse is administered, secondary eddy currents are induced in the intracranial tissue. The electrical field induced in the brain is perpendicular to the magnetic fields and travels in the opposite direction of electrical current in the coil (Barker et al., 1985). The action potentials that fire in response to TMS stimulation propagate transynaptically to other neurons. This results in the spread of neuronal activation to connected cortical and subcortical regions (Groppa, Muthuraman, et al., 2012).

1.6.1 Transcranial Magnetic Stimulation with Electromyography

The first investigations of TMS by Barker involved the examination of the effect of TMS to the motor cortex (Barker et al., 1985). Barker et al. demonstrated that single pulse TMS

to the motor cortex could produce a motor evoked potential (MEP) in the periphery, such as in the hand or leg muscles, based on the region of the motor cortex that is stimulated. These MEPS are recorded using EMG recordings placed on the target muscles. Various parameters of the MEPs, including the amplitude, latency and duration are used to investigate the activation threshold, integrity of corticospinal pathways, excitability and corticortical connectivity (Farzan, Barr, Fitzgerald, & Daskalakis, 2012). Furthermore the use of paired pulse TMS to the motor cortex allows for the investigation of various cortical excitatory and inhibitory processes.

1.6.1.1 Motor Threshold

The motor threshold is a measure of cortico-spinal excitability. Resting motor threshold (RMT) is commonly described as the minimum intensity required to elicit an MEP of at least 50μV in the target muscle in a minimum 5 out of 10 trials (Rossini et al., 1994). Administration of the NMDA antagonist has been demonstrated to produce a reduction in the RMT (Di Lazzaro et al., 2003) while drugs that block voltage-gated sodium channels increase the RMT (Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001; Chen, Samii, Canos, Wassermann, & Hallett, 1997; Mavroudakis, Caroyer, Brunko, & Zegers de Beyl, 1994). The use of other drugs to modulate other neurotransmitter systems, including GABA, dopamine, norepinephrine, serotonin, or acetylcholine has no effect on the RMT (Ziemann, 2004). Given these findings, it is thought that RMT is associated with the functional status of voltage-gated sodium channels (Boroojerdi et al., 2001; Chen et al., 1997; Mavroudakis et al., 1994).

1.6.1.2 Motor Evoked Potential

The MEP size is another measure of corticospinal excitability. The MEP size can be measured indexed using the MEP peak-to-peak amplitude or the area under the curve. MEP size can also be indexed using an input-output curve, which is calculated by applying TMS pulses to the motor cortex at progressively higher intensities until the MEP amplitude reaches a plateau (Abarbanel, Lemberg, Yaroslavski, Grisaru, & Belmaker, 1996; Pitcher & Miles, 2002).

1.6.2 Transcranial Magnetic Stimulation with Electroencephalography

The recording of brain waves from the surface of the scalp in humans was first conducted in the 1920's by Hans Berger who named the technique electroencephalography (EEG) (Swartz & Goldensohn, 1998). EEG allows for the measurement of neuronal electrical activity non-invasively (Siebner et al., 2009). Both spontaneous and event-related potentials over the complete surface of the brain can be measured with EEG (Siebner et al., 2009).

The combination of TMS with EEG in more recent years has allowed for the use of TMS to probe activity in various superficial brain regions outside of the motor cortex (Ilmoniemi & Kicic, 2010) as well as the study as the examination of oscillatory brain activity across brain regions (Frantseva et al., 2014). The neurophysiological activity induced by a TMS pulse can be indexed by the topographical mapping of TMS-evoked potentials (TEPs) (Rogasch et al., 2014). Unlike the measurement of MEPs using EMG,

the combination of TMS and EEG allows for the direct measurement of cortical activity without the contribution confounding activity such as spinal cortical excitability (Chung, Rogasch, Hoy, & Fitzgerald, 2015).

1.6.2.1 TMS-Evoked Potentials

TMS stimulation of the cortex results in the TEPs on the EEG that are highly reproducible over time (Casarotto et al., 2010; Lioumis, Kicic, Savolainen, Makela, & Kahkonen, 2009). TEPs are sensitive to TMS parameters such as site of stimulation (Casarotto et al., 2010; Fitzgerald et al., 2008; Komssi et al., 2002), coil angle (Bonato, Miniussi, & Rossini, 2006; Casarotto et al., 2010) and stimulation intensity (Casarotto et al., 2010; Kahkonen, Komssi, Wilenius, & Ilmoniemi, 2005; Komssi, Kahkonen, & Ilmoniemi, 2004). TMS to the motor cortex produces a series of peaks and troughs that lasts for up to 300ms. These peaks are defined as N15, P30, N45, P55, N100, P180 and N280, with N representing negative deflections and P representing positive peaks (Komssi & Kahkonen, 2006). In general, the amplitude of these peaks and troughs convey the excitability of the cortical networks (Esser et al., 2006; Massimini et al., 2005).

Early TEPs are thought to represent the interplay of excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs). Findings from Premoli et al., 2014 suggest that the N45 is mediated by $GABA_A$ receptor mediated neurotransmission (Premoli, Castellanos, et al., 2014). Similarly, findings from a number of previous studies suggest that the N100 represents $GABA_B$ receptor mediated neurotransmission (Bonnard,

Spieser, Meziane, de Graaf, & Pailhous, 2009; Bruckmann et al., 2012; Farzan et al., 2013; Kicic, Lioumis, Ilmoniemi, & Nikulin, 2008; Premoli, Rivolta, et al., 2014; Rogasch, Daskalakis, & Fitzgerald, 2013; Spieser, Meziane, & Bonnard, 2010) and the P60 represents NMDA-receptor mediate neurotransmission (Cash et al., 2016).

The negative peak that occurs ~100ms following the TMS pulse is the largest component of the EEG (Nikouline, Ruohonen, & Ilmoniemi, 1999; Paus, Sipila, & Strafella, 2001). This component is believed to represent inhibitory processes, particularly $GABA_B$ receptor mediated inhibition (Nikulin, Kicic, Kahkonen, & Ilmoniemi, 2003; Rogasch et al., 2013; Rogasch & Fitzgerald, 2013). The N100 is modulated during motor tasks (Bonnard et al., 2009; Bruckmann et al., 2012; Kicic et al., 2008; Spieser et al., 2010). Similarly, motor measures of long-interval cortical inhibition (LICI) and the cortical silent period (CSP), thought to represent $GABA_B$ receptor mediated neurotransmission are correlated with N100 amplitude (Farzan et al., 2013; Rogasch et al., 2013). Administration of baclofen, a $GABA_B$ agonist increases the amplitude of the N100 (Premoli, Rivolta, et al., 2014).

1.6.2.2 TMS-Evoked Oscillations

TMS stimulation to the cortex also produces cortical oscillations in distinct frequency bands, which can be examined by pairing TMS with EEG. These cortical oscillations are a product of the synchronous firing of the underlying populations of neurons. This synchronous firing of neurons in a rhythmic manner allows for communication between

neuronal networks and the processing of information (Ward, 2003). Oscillations occur across frequencies from 0.05Hz -500Hz.

The frequencies are traditionally grouped into bands corresponding the physiological characteristics of the given band. The bands include δ (0-4Hz), θ (4-8Hz), α (8-12Hz), β (12-30Hz) and γ (30-70Hz) (Basar, Basar-Eroglu, Guntekin, & Yener, 2013). δ waves can occur either in the cortex or the thalamus (Maquet et al., 1997). They are commonly observed during sleep (Rasch & Born, 2013). δ waves also play an important role in motivational drive (Knyazev, 2007, 2012) and stimulate the release of hormones including prolactin and growth hormone stimulating hormone (Brandenberger, 2003). θ waves play an important role in various forms of learning and memory (Berry & Thompson, 1978; Liebe, Hoerzer, Logothetis, & Rainer, 2012; Macrides, Eichenbaum, & Forbes, 1982; Mitchell, Rawlins, Steward, & Olton, 1982; Mizumori, Barnes, & McNaughton, 1990; Rutishauser, Ross, Mamelak, & Schuman, 2010; Winson, 1978) and are also important for synaptic plasticity (Greenstein, Pavlides, & Winson, 1988; J. M. Hyman, Wyble, Goyal, Rossi, & Hasselmo, 2003; Larson, Wong, & Lynch, 1986). θ oscillations are observed during REM sleep, but not during the deeper stages of sleep (Vanderwolf, 1969; Winson, 1974). α waves were originally thought to originate primarily from the occipital cortex during a state of restful relaxation with eyes closed (Basar, Schurmann, Basar-Eroglu, & Karakas, 1997; Pfurtscheller, Stancak, & Neuper, 1996). More recent evidence suggests that α waves represent functional inhibition through event-related synchronization (Jensen & Mazaheri, 2010; Klimesch, 2012; Klimesch, Sauseng, & Hanslmayr, 2007). β waves are associated with active concentration during a wakeful state (Baumeister, Barthel, Geiss, &

Weiss, 2008; Neuper & Pfurtscheller, 2001). Over the motor cortex, β waves occur during muscle contractions in isotonic movements (Baker, 2007). β waves become suppressed before and during changes in movements (Baker, 2007). γ waves are associated with a number of behaviours including attention (Jensen, Kaiser, & Lachaux, 2007), working memory (Howard et al., 2003) and visual perception (Beauchamp, Sun, Baum, Tolias, & Yoshor, 2012).

1.7 Cortical Inhibition

1.7.1 Role of Interneurons in Cortical Inhibition

Cortical inhibition is the process by which the activity of other neurons is modulated by interneurons in the cortex. Interneurons are neurons that only act locally (Purves et al., 2012). Inhibitory interneurons use GABA as their principal neurotransmitter while excitatory interneurons use glutamate as their primary neurotransmitter. Inhibitory interneurons selectively attenuate the activity of neurons they synapse onto primarily by hyperpolarizing primary cells through GABA (for review, see (Freund & Kali, 2008)).

1.7.2 Types of Interneurons

Inhibitory interneurons are found in three different conformations in the cortex: basket cells, chandelier cells and double bouquet cells. Basket cells are the most common type of interneurons, found in layers III-IV of the cortex and synapse onto pyramidal cells in an axo-somatic conformation (Benes & Berretta, 2001). Chandelier cells are found in layers II-III of the cortex and synapse onto pyramidal cells in an axo-axonal conformation.

Double bouquet cells found in layers II-III of the cortex and synapse onto the apical and basal dendrites of pyramidal cells and onto adjacent interneurons (Somogyi & Cowey, 1981; Somogyi, Cowey, Halasz, & Freund, 1981).

1.7.3 Mechanisms Underlying Cortical Inhibition

When inhibitory interneurons synapse onto pyramidal cells, their activity is suppressed. Excitatory interneurons can further suppress output from pyramidal cells by synapsing on inhibitory interneurons, increasing activity of these neurons by activating NMDA receptors located on the inhibitory interneurons (Daskalakis, Fitzgerald, & Christensen, 2007). In this manner, the regulation of cortical output by pyramidal cells by interneurons is the mechanism underlying cortical inhibition.

1.7.4 Assessment of Cortical Inhibition and Excitability Using TMS

Transcranial magnetic can be used to measure cortical excitability and various cortical inhibitory processes. Cortical excitability can be measured using intracortical facilitation (ICF) (Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999) while cortical inhibition can be measured using long-interval cortical inhibition (LICI) (Nakamura et al., 1997; Werhahn et al., 1999), short-interval cortical inhibition (SICI) (Nakamura et al., 1997; Werhahn et al., 1999) and the cortical silent period (CSP) (Inghilleri et al., 1993). Intracortical facilitation involves intracortical excitatory transmission and is thought to be mediated by NMDA-receptors (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997; Reis et al., 2006). Both LICI and SICI involve

cortical inhibitory processes. A wide range of findings suggest that LICI involves $GABA_B$ receptor-mediated inhibitory processes (Chen, Kelly, & Wu, 1999; McDonnell, Orekhov, & Ziemann, 2006; Nakamura et al., 1997; Valls-Sole, Pascual-Leone, Wassermann, & Hallett, 1992) while SICI involves $GABA_A$ receptor-mediated inhibition. The cortical silent period (CSP) is another TMS measure that is thought to reflect inhibitory neural mechanisms. The early portion of the CSP is controlled by spinal mechanisms (Fuhr, Agostino, & Hallett, 1991; Ziemann, Netz, Szelenyi, & Homberg, 1993), while the late long-lasting portion is believed to be controlled by $GABA_B$ (Sanger, Garg, & Chen, 2001).

1.7.5 Effects of Acute Alcohol Intoxication on Cortical Excitability

Findings from a number of TMS studies suggest that alcohol enhances inhibitory transmission and suppresses excitatory transmission. For example, acute ethanol intake results in prolongation of the CSP (Conte et al., 2008; Ziemann, Lonnecker, & Paulus, 1995). Using a paired stimulation paradigm, Ziemann et al., 1995 found that alcohol dose-dependently enhances intracortical inhibition (evidenced through an increase in SICI) and reduces ICF. Similarly, Conte et al. 2008 found that alcohol consumption increased the activity of cortical inhibitory circuits, as evidenced through the increase in CSP during repetitive TMS (rTMS). There is a large background of evidence indicating that the CSP and cortical inhibition are largely controlled by GABAergic inhibitory interneurons (Inghilleri, Berardelli, Cruccu, & Manfredi, 1993; Pierantozzi et al., 2004; Werhahn et al., 1999). The studies by Ziemann et al., 1995 and Conte et al., 2008 provide further evidence

that alcohol enhances GABAergic transmission. Similarly, the findings from Ziemann et al., 1995, demonstrating that alcohol decreases ICF, support previous findings that alcohol suppresses NMDA receptor function (Vengeliene, Bilbao, Molander, & Spanagel, 2008).

1.7.6 Effects of Chronic Alcohol Intoxication on Cortical Excitability

While findings from a number of studies suggest that chronic alcohol use leads to counter-adaptive decreases in GABA function and increases in glutamatergic function, there have only been a limited of TMS studies to examine the changes in cortical excitability/inhibition following chronic alcohol abuse. One study using TMS to examine changes in cortical excitability in 13 subjects in alcohol withdrawal, 12 chronic alcoholics and 15 age-matched control subjects found that in subjects who were in withdrawal, ICF was increased compared to alcohol dependent individuals and healthy controls. While patients in withdrawal tended to have a reduced SICI, there was no significant difference in SICI and CSP duration between patients experiencing alcohol withdrawal and healthy controls (Nardone et al., 2010). However, the authors noted that the lack of significant difference observed in SICI in subjects in withdrawal may have been due to small sample sizes.

A study by Conte et al., 2008 examined whether 5Hz rTMS to the motor cortex produces differences in measures of cortical excitability (MEP amplitude) and inhibition (CSP and SICI. The group found that the length of the CSP did not differ significantly between chronic alcohol abusers (with negative breath alcohol) and healthy individuals (prior to

alcohol intake). However, they found that chronic alcohol abuse abolished the rTMS-induced facilitation of the MEP amplitude observed in healthy subjects. As this elimination of rTMS-induced facilitation was seen in the absence of differences in SICI between chronic alcohol abusers and healthy subjects, it was speculated that this difference was not likely caused by GABAergic hyperfunction. However, as the SICI was only evaluated in 4 chronic abusers and 4 healthy subjects (before ethanol intake), the authors highlighted the lack of differences in SICI may be due to the small sample size or a ceiling effect. The apparent depression of glutamatergic transmission seen in this study is difficult to reconcile with findings from animal and human studies indicating that chronic alcohol abuse results in an increase in glutamatergic activity (Darstein, Landwehrmeyer, & Feuerstein, 2000; Follesa & Ticku, 1996; G. Freund & Anderson, 1996; Gulya, Grant, Valverius, Hoffman, & Tabakoff, 1991; Hu, Follesa, & Ticku, 1996; Hu & Ticku, 1995; Michaelis, Michaelis, Freed, & Foye, 1993). It is important to note that facilitation of the MEP induced by rTMS is indicatory of mechanisms underlying short-term cortical plasticity, which are likely not the same as the mechanisms underlying ICF.

Naim-Feil et al. examined cortical inhibition in the motor and frontal cortex of 12 alcohol dependent subjects post-detoxification and 14 healthy controls in a TMS study using EEG (Naim-Feil et al., 2016). In this study, alcohol dependent individuals demonstrated decreased LICI, suggesting reduced $GABA_B$ in the frontal cortex compared to healthy controls (Naim-Feil et al., 2016). Examination of motor cortex excitability revealed no differences in SICI, ICF or CSP between alcohol dependent individuals and healthy controls. However, alcohol dependent individuals demonstrated decreased RMT, AMT

and 1mV threshold, suggesting altered cortical excitability in the motor cortex compared to healthy controls. The authors noted that there was greater variability in SICI amongst alcohol dependent individuals compared to healthy controls (Naim-Feil et al., 2016).

1.8 Neuroplasticity

Neuroplasticity broadly describes the change in synaptic strength or efficacy in response to experience or use (McCool, 2011). In 1894, Ramon y Cajal hypothesized that information was stored in the brain through modification in synaptic connections between neurons (Cajal, 1894). This theory gained further attention following the publication of D.O. Hebb's landmark book "The Organization of Behaviour" (Hebb, 1949). In this book, Hebb postulated that "repeated stimulation of specific receptors will lead slowly to the formation of an 'assembly' of association-area cells which can act as briefly as a closed system after stimulation has ceased; this prolongs the time during which the structural changes of learning occur (Hebb, 1949). This hypothesis gained support in from the findings of Bliss & Lomo in 1973 demonstrating long-term potentiation (LTP) of glutamatergic synapses in the hippocampus (Bliss & Lomo, 1973). The phenomenon of LTP has been studied extensively since this original finding.

1.8.1 Long-Term Potentiation

LTP is a form of neuroplasticity involves stable and long-lasting enhancement of synaptic efficacy (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). LTP results from the synchronous activity at pre- and post-synaptic elements. It was first documented by Bliss & Lomo, 1973 in glutamatergic synapses between the medial perforant path fibres from the entorhinal cortext and granule cells in the dentate gyrus of the hippocampus in of an anesthetized rabbit (Bliss & Lomo, 1973). Later, associative LTP was induced in various neocortical areas including the somatosensory cortex (Bindman, Murphy, & Pockett, 1988), the auditory cortex (Cruikshank & Weinberger, 1996) and the visual cortex (Fregnac, Burke, Smith, & Friedlander, 1994; Hirsch & Gilbert, 1993). A number of *in vivo* and *in vitro* studies have induced associative LTP in the primary motor cortex (Baranyi & Feher, 1981a, 1981b, 1981c, 1981d; Baranyi & Szente, 1987; Baranyi, Szente, & Woody, 1991; Hess & Donoghue, 1994; Iriki, Pavlides, Keller, & Asanuma, 1989, 1991)

The NMDA receptor plays an important role in the induction of LTP at most glutamatergic synapses in the CNS. While not all forms of synaptic plasticity are not dependent on the NMDA receptor, NMDA receptor dependent LTP is the most well studied form of LTP. This form of LTP has been extensively studies between the pyramidal neurons of the CA3 and CA1 regions of the hippocampus (for review, see (Luscher & Malenka, 2012)). The evaluation of NMDA receptor activity thus allows for the detection of coincident pre- and post-synaptic activity as these receptors are positioned

post-synaptically and bind glutamate that is released into the synaptic cleft after the arrival of an action potential at the presynaptic terminal. However, the binding of glutamate to the NMDA receptor alone does not result in the opening of the receptors intrinsic cation channel because the channel is blocked by a magnesium ion when the channel is at near-resting membrane potentials (Nowak, Bregestovski, Ascher, Herbet, & Prochiantz, 1984). Opening of the NMDA receptor's intrinsic cation channel occurs after the post-synaptic cell is sufficiently depolarized, resulting in the magnesium ion being expelled from the cation channel, which allows an influx of sodium and calcium ions into the cell. The influx of calcium into the cell activates calcium-sensitive signaling mechanisms such as calcium/calmodulin-dependent kinase II (CAMKII) or the cyclic adenosine monophosphate (cAMP)-dependent pathways. These molecules initiate LTP expression. These molecules can act locally by phosphorylating receptors, and altering the intrinsic properties of their ion channels or by altering gene expression by signaling to the cell nucleus through transcription factors (Alberini, Ghirardi, Huang, Nguyen, & Kandel, 1995; Goelet, Castellucci, Schacher, & Kandel, 1986). Activation of CAMKII also initiates a biochemical cascade that results in the trafficking of

The role of GABAergic neurotransmission has been demonstrated by a number of studies. Activation of $GABA_A$ receptors by the benzodiaepine midazolam produces inhibits LTP induction in rat hippocampal brain slices (Evans & Viola-McCabe, 1996). Administration of the benzodiazepine lorazepam inhibits the potentiation of MEPs seen following practice of a motor task. These findings suggest that $GABA_A$ activation disrupts practice-dependent LTP (Butefisch et al., 2000; Ziemann, Muellbacher, Hallett, & Cohen, 2001) .

Administration of the benzodiazepine diazepam, which also results in activation of the $GABA_A$ receptor has also been reported to produce a trend towards a decrease in PAS-induced neuroplasticity, although the findings from this study were not statistically significant (Heidegger, Krakow, & Ziemann, 2010). Similarly, activation of $GABA_A$ receptors by administration of SICI to the motor cortex simultaneously during PAS blocks PAS-induced neuroplasticity (Elahi, Gunraj, & Chen, 2012). Together, this findings suggest that $GABA_A$ receptors play an important role in LTP.

1.8.2 Properties of Long-Term Potentiation

The key properties of LTP are cooperativity, associativity, input specificity and persistence (Malenka, 2003a, 2003b). Cooperativity refers to the requirement that the simultaneous activation of a given number of presynaptic fibres is required to induce LTP. In other words, a number of presynaptic fibres must "cooperate" to produce LTP. Cooperativity is required to sufficiently depolarize the postsynaptic cell for calcium and sodium ions in through the NMDA receptor channel. Associativity refers to the ability of LTP to be elicited at synapses that are activated by sub-threshold stimuli if there is a concurrent LTP-inducing stimulus on the same cell at a different set of synapses. Associativity occurs when the cell is depolarized by the LTP-inducing stimulus, which then travels through the dendritic tree to the NMDA receptors on the synapses that were concurrently activated by the sub-threshold stimulus. Associativity allows two pieces of information being transmitted at different sets of afferents synapsing onto the same post-synaptic cell concurrently to become associated. The property of input-specificity refers to

the phenomenon that if LTP is elicited at one synapse on a post-synaptic cell, others synapses that were remained inactivated during the induction protocol will not have LTP elicited. Lastly, the property of persistence refers to the fact that LTP persists for minutes to days following induction (Malenka, 2003a, 2003b).

1.8.3 Effects of Acute Alcohol on Neuroplasticity

Animal studies have shown that alcohol attenuates LTP in the hippocampus. Morrisett & Swartzwelder, 1993 examined the effect of alcohol on LTP and NMDA-mediated synaptic currents as well as $GABA_A$ and $GABA_B$-mediated synaptic currents from hippocampal slice (Morrisett & Swartzwelder, 1993). Alcohol was found to completely antagonize LTP induced by 5Hz theta-like stimulation patterns to the perforant path-dentate gyrus. NMDA-receptor mediated synaptic currents were inhibited by alcohol while there was no potentiation of $GABA_A$ currents and no attenuation of $GABA_B$-mediated fading of $GABA_A$ synaptic currents. These findings suggest that attenuation of LTP by alcohol is primarily due to the action of alcohol at the NMDA-receptor complex.

A series of experiments conducted by Schummers et al. using rat hippocampal sections suggest that alcohol's inhibition of LTP can be accounted for by alcohol's direct inhibition of NMDA receptors as well as its indirect inhibition of NMDA receptors, possibly by potentiating $GABA_A$ receptor-mediated neurotransmission (Schummers, Bentz, & Browning, 1997; Schummers & Browning, 2001). The concentrations of alcohol required for the inhibition of LTP are unclear. While most studies conducted in the rat

hippocampus suggest that high concentrations of alcohol (i.e., 50 mM) are required for the inhibition of LTP, there are reports suggesting that concentrations as low 5mM can inhibit LTP (Blitzer, Gil, & Landau, 1990). A study examining the effect of alcohol on NMDA receptor dependent LTP in the dorsomedial striatum using rat brain slices found that in this brain region, concentration as low as 2mM can decrease LTP and 10mM can completely block it (Yin, Park, Adermark, & Lovinger, 2007).

The effect of a single dose of alcohol on the synaptic strength of dopamine neurons 24 hours later has been demonstrated to be strain-dependent in mice (Wanat et al., 2009). The single exposure of alcohol reduced the AMPA receptor to NMDA receptor ratio in the VTA of the DBA strain of mice (alcohol non-preferring mice), while having no effect on LTP as indicated by the AMPA receptor to NMDA receptor ratio in C57BL/6 (alcohol preferring mice) (Wanat et al., 2009). The lack of effect of alcohol on AMPA to NMDA receptor ratio in C57BL/6 mice seen by Wanat et al., 2009 are discordant with the increase in AMPA to NMDA receptor ratio seen 24 hours following a single dose of alcohol in C57BL/6 seen by Ungless et al., 2001. These differences in findings have been speculated to be attributable to the different doses used in these studies (Wanat et al., 2009).

A single *in vivo* alcohol exposure also produces potentiation of GABAergic synapses in dopamine neurons in the VTA (Melis, Camarini, Ungless, & Bonci, 2002) . Dopaminergic activity with the VTA has been suggested to be important for the maintenance of alcohol consummatory behaviour (Kaczmarek & Kiefer, 2000; Ng & George, 1994; Rassnick et al., 1993). During acute withdrawal from chronic alcohol consumption, a decrease in the

activity of dopamine neurons in the mesolimbic dopamine system is observed while consumption of alcohol restores dopamine levels in the NAc (Weiss et al., 1996). Potentiation of the GABAergic synapses in the VTA following alcohol consumption, could at least partially explain decreased dopaminergic activity during alcohol withdrawal (Melis et al., 2002). This potentiation is protein kinase A (PKA) dependent (Melis et al., 2002). The potentiation of GABAergic synapses in the VTA may play a key role in the synaptic changes that contribute to the development of AUD (Melis et al., 2002).

The bed nucleus of the stria terminalis (BNST) plays an important role in the interaction between stress and drug abuse (Aston-Jones, Delfs, Druhan, & Zhu, 1999; Erb & Stewart, 1999). The BNST receives input from a number of brain centers in regards to stress and sends information to stress and reward pathways. It has been hypothesized that synaptic plasticity within the BNST could be involved with reinstatement of intake of a substance of abuse by stress (Weitlauf, Egli, Grueter, & Winder, 2004). Alcohol blocks the early portion of LTP at glutamatergic synapses in the dorsolateral BNST in a $GABA_A$ receptor dependent manner (Weitlauf et al., 2004). Alcohol has also been shown to dose dependently impair NMDA-receptor dependent LTP in the dorso-medial striatum, as measured using field potential recordings from rat brain slices (Yin et al., 2007). At the highest concentration of alcohol used, 50mM, alcohol promoted LTD in the dorso-medial striatum. The dorso-medial striatum plays an important role in goal directed actions. As such, impairment of neuroplasticity in this region by alcohol may also contribute to the neurophysiological changes involved in the development of AUDs.

1.8.4 Effects of Chronic Alcohol on Neuroplasticity

A number of neuroplasticity-associated changes have been reported in studies examining chronic alcohol consumption in animal models. Chronic consumption of alcohol by C57BL/6J mice for three months (under continuous access) results in an increase in levels of Homer2 protein the NAc. The family of Homer proteins is associated with a number of cellular functions including activity-dependent remodeling. This increase in Homer2 proteins was observed even two months after withdrawal from alcohol consumption. The increase in Homer2 protein levels was associated with an increase in levels of mGLUR1 and NR2b levels, noted at 2 days and 2 weeks but not at 2 months after withdrawal (Szumlinski, Ary, Lominac, Klugmann, & Kippin, 2008). The important role of Homer2 isoforms in alcohol-related plasticity is further supported by the finding that inducing an increase in NAc Homer2b expression (through virus-mediated gene delivery to the neurons of the NAc) from C57BL/6J mice results in a rise in the appetitive and consummatory behavior associated with alcohol reward and the tolerance to alcohol's sedative effects. The increase in glutamate receptors along with Homer2B in the NAc following chronic alcohol consumption is consistent with the rebound increase in glutamate during withdrawal from alcohol. Findings from this study suggest that increase in mGluR–Homer2b–NMDA signaling is an important cellular process that underlies the neuroplastiticy involved in the development of alcohol dependence (Szumlinski et al., 2008).

Binge drinking of alcohol by C57BL/6J mice results in an increase in NMDA receptor mediated currents in the VTA, which may be mediated by corticotrophin releasing factor (CRF) (Sparta et al., 2013) . Interestingly, in humans, there is genetic association of human corticotrophin releasing hormone receptor 1 (CRHR1) and patterns of alcohol intake (Treutlein et al., 2006). Binge-drinking of alcohol by C57BL/6J mice in the drinking in the dark paradigm, where 6g/kg of alcohol are consumed in 4 hours results in a CRF-mediated increase in NMDA receptor currents in the VTA, as measured through ex vivo whole-cell recordings. CRF is involved in various stress responses and mediates stress-induced reinstatement of alcohol self-administration in rats (Le et al., 2000; Stewart, 2000). Chronic consumption of alcohol and alcohol dependence in animal models has also been reported to result in increased CRF function in the central amygdale (Funk, Li, & Le, 2006; Sparta et al., 2013; Valdez et al., 2002) .

Additionally, chronic alcohol consumption has also been reported to alter synaptic plasticity of the prefrontal cortex of mice. After chronic intermittent alcohol exposure, C57BL/6 mice show an increase in the NMDA/AMPA current ratio in layer V of the medial prefrontal cortex, both immediately and 1 week following the last alcohol exposure (Kroener et al., 2012) . These findings were confirmed by western blot analysis, revealing that there was an increase in NMDA NR1 and NRB subunits while having no change in AMPA GluR1 subunits. Examination of spike-timing dependent LTP in the slice preparation revealed that the alcohol exposure resulted in an aberrant form of enhanced NMDA receptor-mediated neuroplasticity. These changes were associated with a reduced

cognitive flexibility, as revealed by a medial prefrontal cortex dependent attentional set-shifting task (Kroener et al., 2012).

1.9 Neuromodulatory Brain Stimulation

A number of brain stimulation paradigms are used to induce and study neuroplasticity in the healthy and diseased brain. Additionally, these brain stimulation paradigms can be used to study the effect of psychoactive drugs on neuroplasticity. These brain stimulation paradigms are collectively defined as neuromodulatory brain stimulation paradigms. Examples of neuromodulatory brain stimulation paradigms include repetitive transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS), magnetic seizure therapy (MST) and paired associative stimulation (PAS). Below the literature on PAS, the form of neuromodulatory brain stimulation used in the studies comprising this thesis will be reviewed.

1.9.1 Paired Associative Stimulation

PAS is a widely used experimental paradigm for inducing associative LTP in the human cortex by synchronously and repetitively pairing a suprathreshold peripheral nerve stimulation (PNS) with a suprathreshold TMS over the motor cortex. Stefan et al., 2000 demonstrated that when TMS over the optimal site for stimulating the abductor pollicis brevis (APB) muscle proceeds a low-frequency median nerve stimulation by 25 ms, the amplitude of MEPs measured from the resting target muscle were increased (Stefan,

Kunesch, Cohen, Benecke, & Classen, 2000). The potentiation induced by PAS is considered to be associative or "Hebbian" in nature, as it produces concomitant and synchronous activation of the postsynaptic cell from presynaptic inputs that result in concomitant and synchronous postsynaptic cell depolarization (Buonomano & Merzenich, 1998; Hebb, 1949). This facilitation of MEPs through PAS is largely dependent on NMDA receptor activation. PAS was also found to prolong the cortical silent period, an index of $GABA_B$ inhibitory neurotransmission (Cantello, Gianelli, Civardi, & Mutani, 1992) .As the afferent signal from the median nerve at wrist level requires ~20ms reach the somatosensory cortex and 3ms to reach the motor cortex from the somatosensory cortex, investigators identified that 25ms interval would be ideal for the effects from both sources to reach the motor cortex simultaneously. Potentiation induced by this method evolves within 30 minutes and lasts for a minimum duration of 30min-60mins and is reversible.

The original PAS paradigm described by Stefan et al., 2000 used EMG combined with PAS to the index PAS-induced potentiation in the motor cortex. More recently, the combination of the PAS with EEG has allowed for the indexing of PAS-induced potentiation in the cortex directly through EEG. Rajji et al., 2013 demonstrated that PAS-induced potentiation in the dorsolateral prefrontal cortex (DLPFC) by pairing TMS to the DLPFC with suprathreshold PNS and interstimulus intervals of 25ms.

1.9.2 PAS and Cortical Inhibition

A number of studies have examined the effect of PAS on measures of cortical inhibition and excitability, including SICI, ICF, LICI and CSP.

1.9.2.1 Effect of PAS on Short-Interval Intracortical Inhibition

In a study conducted in healthy individuals and sedentary individuals, no significant difference in measures of SICI were observed before and after PAS, in both sedentary and physically active individuals (Cirillo, Lavender, Ridding, & Semmler, 2009). Similarly, Stefan et al., 2002 reported that PAS had no effect on SICI after correction of the increased efficacy of the test stimulus following PAS (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002). Interestingly, Stefan et al., 2002 reported a transient decrease in SICI during PAS (Stefan et al., 2002). Quartarone et al. 2003 examined the effect of PAS on SICI on healthy individuals and patients with writer's cramp. PAS had no effect on SICI in both healthy subjects and patients with writer's cramps (Quartarone et al., 2003). In a study examining the effect of time of day and cortisol levels on PAS, Sale et al. reported that SICI was not significantly affected by the time of day and that PAS did not have any significant effect on SICI (Sale, Ridding, & Nordstrom, 2008). Taken together, these findings suggest that PAS does not have an effect on subsequent measures of SICI.

1.9.2.2 Effect of PAS on Long-Interval Cortical Inhibition

Some previous studies have demonstrated that PAS produces a change in LICI. Paired associative stimulation (PAS) using low/moderate intensity TMS stimulation produces a

decrease in subsequent LICI (Meunier, Russmann, Shamim, Lamy, & Hallett, 2012; Russmann, Lamy, Shamim, Meunier, & Hallett, 2009). Meunier et al., 2012 found that TMS stimulation that produces an average MEP amplitude of 0.5mV paired with PNS at 25ms inter-stimulus intervals (ISI)s produced a decrease in LICI. Russman et al., 2009 observed a similar decrease in LICI with TMS stimulation at 0.5mV MEP during PAS, while PAS10 (TMS stimulation preceded by PNS at ISI of 10 ms) produced a transient increase in LICI (Russmann et al., 2009). Overall, these studies suggest that PAS with low intensity TMS at ISIs of 25 ms produces a decrease in LICI while PAS10 produces an increase in LICI.

1.9.2.3 Effect of PAS on Intracortical Facilitation

Finding previous studies suggest in general, that PAS has no effect on subsequent ICF. Administration of PAS had no effect on ICF in a study evaluating the effect of a number of plasticity inducing paradigms on cortical excitability and inhibition (Di Lazzaro et al., 2011). Similarly, Sale et al., 2007 examined the effect of a short PAS paradigm (132 pairs of stimuli administered at 0.2 Hz frequency for 11 minutes) and a long PAS paradigm (90 pairs of stimuli administered at 0.1 Hz frequency for 30 minutes) to the motor cortex. Neither PAS paradigm had a significant effect on subsequent ICF (Sale, Ridding, & Nordstrom, 2007). Schaburn et al., 2013 reported that PAS consisting of 90 pairs of stimuli administered at a frequency of 0.05Hz for 30 minutes produced no effect on ICF (Schabrun, Weise, Ridding, & Classen, 2013).

1.9.2.4 Effect of PAS on Cortical Silent Period

Numerous studies have reported that administration of PAS alters the CSP (Cirillo et al., 2009; De Beaumont, Tremblay, Poirier, Lassonde, & Theoret, 2012; Quartarone et al., 2003; Sale et al., 2007, 2008; Stefan et al., 2000; Stefan et al., 2004). In the first paper demonstrating the PAS-induced neuroplasticity can be induced in the human motor cortex, Stefan et al. reported that along with an increased in MEP amplitude, PAS produced an increase in the CSP duration (Stefan et al., 2000). Similarly, Quartarone et al., 2003 reported that motor PAS-induced an increase in the CSP in both healthy controls and patients with writer's cramps (Quartarone et al., 2003). In a study examining the effect of attention on the effects of motor PAS, Stefan et al, reported that in addition to mediating PAS-induced neuroplasticity, attention also mediated PAS's effect on CSP (Stefan et al., 2004). The PAS-induced increase of CSP was blocked when attention was diverted away from the hand receiving PNS stimulation by a cognitive task (Stefan et al., 2004). Sale et al., 2007 reported that both a short (132 paired stimuli at 0.2Hz) and long (90 stimuli at 0.05Hz) PAS protocol induced an increase in CSP duration following PAS (Sale et al., 2007). Cirillo et al., 2009 reported that PAS-increased CSP duration in both sedentary individuals and physically active individuals (Cirillo et al., 2009). Together these studies suggest PAS produces an increase in CSP duration.

1.9.3 Effects of Pharmacological Interventions on PAS

A number of previous studies have explored the effect of various drugs on PAS-induced neuroplasticity. Below is a review of the literature examining the effect of various drugs on PAS-induced neuroplasticity.

1.9.3.1 Dopamine Effects on PAS

A study examining the effect of various drugs on PAS-induced neuroplasticity found that the dopamine agonist cabergoline had no effect on PAS, while the dopamine antagonist haloperidol produced a decrease in PAS-induced neuroplasticity (Korchounov & Ziemann, 2011). However, another study found that sulpiride, a selective dopamine D2 antagonist, increases PAS-induced neuroplasticity (Nitsche et al., 2009); while the dopamine D2/D3 agonist ropinirole produces a decrease in PAS-induced neuroplasticity (Monte-Silva et al., 2009). Additionally, it has been reported that global activation of dopamine receptors (both the D1 and D2 receptors) through levodopa increases both the extent and duration of PAS-induced neuroplasticity (Kuo, Paulus, & Nitsche, 2008). Interestingly, this increase in PAS-induced plasticity by levodopa is not observed in the presence of D2 receptor antagonist sulpiride (Nitsche et al., 2009). Given these findings, it is believed that the balanced activation of both D1 and D2 receptors is required to produce an increase in PAS-induced neuroplasticity.

1.9.3.2 Acetylcholine Effects on PAS

A few studies have examined the effect of drugs that act on the cholinergic system on PAS-induced neuroplasticity. Kuo et al., 2007 reported that 3mg of rivastigmine, a brain selective cholinesterase inhibitor resulted in an increase in the magnitude and duration of PAS-induced neuroplasticity (Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007). These findings suggest that increased levels of acetylcholine lead to increase PAS-induced neuroplasticity. Administration of varenicline, a nicotinic acetylcholine receptor partial agonist, was seen to increase PAS-induced potentiation in patients with schizophrenia while decreasing PAS-induced potentiation in healthy individuals (Bridgman et al., 2016). Administration of tacrine, an acetylcholine agonist, had no effect on PAS-induced neuroplasticity (Korchounov & Ziemann, 2011). It has been hypothesized that the lack of effect of tacrine on PAS-induced neuroplasticity may be due to tacrine's autoinhibition of electrically evoked acetylcholine release (Korchounov & Ziemann, 2011). Biperidine, an acetylcholine antagonist, decreased PAS-induced neuroplasticity, suggesting that blocking cholinergic neurotransmission blocks PAS-induced neuroplasticity (Korchounov & Ziemann, 2011).

1.9.3.3 Glutamate Effects on PAS

A number of studies have demonstrated that blocking of NMDA receptor mediated neurotransmission by the NMDA receptor antagonist dextromethorphan results in a decrease in PAS-induced neurotransmission (Stefan et al., 2002; Weise, Mann, Rumpf, Hallermann, & Classen, 2016; Wolters et al., 2003). PAS-induced neuroplasticity is also

blocked by voltage-gated L-type calcium channel antagonist nimodipine, providing further evidence of the importance of NMDA receptor mediated neurotransmission in PAS-induced neuroplasticity (Weise et al., 2016; Wolters et al., 2003).

1.9.3.4 GABA Effects on PAS

A number of studies have examined the effect of drugs that act on GABA-mediated neurotransmission on PAS-induced neuroplasticity. Diazepam, a $GABA_A$ agonist inhibits PAS-induced LTP (Ilic, Petrovic, Grajic, & Ilic, 2012). Administration of the $GABA_B$ agonist baclofen decreases PAS-induced neuroplasticity in the motor cortex (McDonnell, Orekhov, and Ziemann (2007). Together, these findings suggest that both $GABA_A$ and $GABA_B$ receptor mediated neurotransmission play an important role in the LTP-like neuroplasticity induced by PAS.

1.9.4 Factors Influencing Neuroplasticity Induction by PAS

The induction of neuroplasticity by PAS is mediated by a number of factors including attention, age, cortisol levels and hormone levels. Attention has been demonstrated to be an important mediator of neuroplasticity. In a study examining varied levels of attention on PAS-induced plasticity, induction of plasticity by PAS was highest when attention was directed at the target hand (the hand receiving the PNS stimulation) (Stefan et al., 2004). When attention was completely directed away from the target hand by cognitive task being administered during PAS, the induction of plasticity was completely blocked (Stefan et al., 2004). PAS-induced neuroplasticity was partially blocked when the

subjects' vision was occluded so that they could only feel their hand but could not see it. Another factor mediating PAS-induced neuroplasticity is age. Potentiation induced by PAS has been reported to decrease with age in both males and females in the motor cortex (McEwen et al., 1991; Muller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Tecchio et al., 2008). A similar impairment with age has not been observed in the somatosensory cortex suggesting that there are disparities in the effect based on brain region (Pellicciari, Miniussi, Rossini, & De Gennaro, 2009).

Hormones have also been demonstrated to modulate the neuroplasticity produced by PAS. Increased cortisol levels in circulation are negatively associated with PAS-induced potentiation, suggesting that cortisol inhibits PAS-induced neuroplasticity (Sale et al., 2008). Along these lines, potentiation by PAS is higher in the evening, when endogenous levels of cortisol are low (Sale et al., 2008). In females, testosterone levels have been significantly correlated with PAS-induced neuroplasticity, while levels of insulin-like growth factor are correlated with PAS-induced neuroplasticity in males (Polimanti et al., 2016).

1.10 Cognition

1.10.1 Effect of Alcohol on Cognition

Alcohol intoxication is associated with a number of effects on cognition. In general, the acute effects of alcohol on cognition vary with BAC. Complex cognitive tasks (ie. tasks requiring divided attention) are impaired at BACs as low as 0.01% (Moskowitz & Fiorentino, 2000). At higher BACs, as seen following binge drinking (≥0.08%BAC), a

much wider range of cognitive impairments are seen, including a slowing of simple reaction time (Fillmore, Stockwell, Chikritzhs, Bostrom, & Kerr, 2007). Outside of the controlled laboratory tasks, alcohol intoxication is associated with a number of behavioral consequences associated with impaired cognitive control and decision-making, including increased sexual risk taking (Halpern-Felsher, Millstein, & Ellen, 1996), aggressive behaviour (Hull & Bond, 1986) and increased risk of motor vehicle accidents (Koelega, 1995).

Impairment of inhibitory control is one of the major cognitive effects of alcohol intoxication. Inhibitory control refers to the ability of inhibit a motor response. Impulsivity can be attributed to impairments in inhibitory control (Olmstead, Hellemans, & Paine, 2006). Multiple studies (using laboratory tasks such as the Stop Signal and Cue Go/No-Go tasks) have demonstrated that inhibitory control is impaired with moderate to high doses of alcohol (producing a BAC around 0.06%) impair inhibitory (Marczinski, Abroms, Van Selst, & Fillmore, 2005; Marczinski & Fillmore, 2005). Impairment in tasks requiring inhibitory control over attention have also been reported at similar doses of alcohol (Abroms & Fillmore, 2004; Abroms, Gottlob, & Fillmore, 2006).

1.10.2 Cognition in Binge Drinkers and AUDs

Binge drinkers and individuals with AUDs have been reported to have cognitive dysfunction across several domains, including working memory (for review, (Oscar-Berman, 1990)). Functional neuroimaging studies have reported altered brain activation in the prefrontal cortex of alcohol dependent individuals during working memory tasks

(Charlet et al.; Pfefferbaum et al., 2001). Furthermore, EEG studies have demonstrated reduced P300 (an event-related potential (ERP) that is believed to be related to the updating of context during information processing) amplitude during memory performance in alcohol dependent individuals (George, Potts, Kothman, Martin, & Mukundan, 2004). Reduced θ power and event-related synchronization in short-and long-term abstinent alcohol dependent individuals has also been observed. Functional neuroimaging studies have demonstrated that altered activation in the prefrontal cortex during working memory performance predicts relapse (Charlet et al., 2014), and clinical outcome (Wilcox et al., 2014). Binge drinkers and individuals with AUDs demonstrate elevated subjective craving for alcohol (Field, Schoenmakers, & Wiers, 2008) and an increased "attentional bias" for cues related to alcohol and increased impulsivity.

1.10.3 The Role of the Dorsolateral Prefrontal Cortex in Executive Function

The DLPFC is a brain region that plays a vital role in cognitive control and working memory (Anderson, Bunce, & Barbas, 2016; Fuster, 2001; MacDonald, Cohen, Stenger, & Carter, 2000). The circuitry of the DLPFC projects from the DLPFC to the dorsolateral head of the caudate nucleus. The DLPFC plays a key role in attention, planning, organizing, set shifting, working memory. Additionally, findings from neuroimaging involving cognitive inhibitory tasks have demonstrated that the DLPFC is involved in response inhibition (Blasi et al., 2006; Garavan, Ross, Murphy, Roche, & Stein, 2002).

Sustained neural firing in the DLPFC of monkeys has been demonstrated in delayed-response tasks during the retention interval, providing evidence that the DLPFC plays a role in the maintenance and manipulation of information on-line (Fuster & Alexander, 1971; Kubota & Niki, 1971). Ablation of the lateral prefrontal cortex (PFC) results in deficits including impairments in working memory. In neuroimaging studies, the DLPFC along with the anterior cingulate cortex (ACC) show activation during cognitive tasks where subjects most perform two tasks at once or remember increasingly long sequences of numbers (Cohen et al., 1997; D'Esposito et al., 1995).

The DLPFC is functionally connected to the orbitofrontal cortex (OFC), hippocampus and amygdala and is believed to play a crucial role in reward processing and guiding behaviours (for review, (Feil & Zangen, 2010)). Given its anatomical and functional connections, the DLPFC is well-positioned to guide goal-directed behaviours, including assimilating information regarding the potential outcomes of a given behaviour and selecting the most appropriate behaviour (Feil & Zangen, 2010).

1.11 Brain Stimulation as a Treatment for AUDs

In addition to measuring the changes in neurotransmission and neuroplasticity induced by alcohol consumption and dependence, brain stimulation techniques also hold promise as a treatment for alcohol dependence. Repetitive transcranial magnetic stimulation (rTMS), a form of neuromodulatory brain stimulation, targeting a number of brain regions including the dorsolateral prefrontal cortex (DLPFC), has shown particular potential as a treatment

for AUDs. Below is a review of the literature on the use of rTMS for the treatment of AUDs.

1.11.1 Repetitive Transcranial Magnetic Stimulation

Repetitive transcranial magnetic (rTMS) stimulation is a non-invasive brain stimulation paradigm that alters cortical excitability. High-frequency short trains of suprathreshold stimuli produce a progressive increase in MEP size that starts during stimulation and lasts for a few milliseconds after the stimulation ends, reflective of short-term plasticity (Berardelli et al., 1998; Gilio et al., 2007; Jennum, Winkel, & Fuglsang-Frederiksen, 1995; Lorenzano et al., 2002; Pascual-Leone, Valls-Sole, Wassermann, & Hallett, 1994; Ziemann et al., 2008). The mechanisms responsible for the MEP size increase are similar to the NMDA-dependent short-term potentiation seen in animal studies (Bliss & Lomo, 1973; Cooke & Bliss, 2006; Zucker, 1989). Additionally, rTMS has been demonstrated to produce changes in a number of neurotransmitter systems (Daskalakis et al., 2006; de Jesus et al., 2013; Di Lazzaro, Oliviero, Mazzone, et al., 2002; Di Lazzaro, Oliviero, Pilato, et al., 2002; Fitzgerald, Brown, Daskalakis, Chen, & Kulkarni, 2002; Modugno et al., 2003; Peinemann et al., 2000; Wu, Sommer, Tergau, & Paulus, 2000).

A number of studies have evaluated the efficacy of high-frequency rTMS for the treatment of alcohol dependence (for review, see (Barr et al.; Bellamoli et al., 2014)). Mishra et al., 2010 performed a single-blind, sham-controlled study in 45 alcohol dependent individuals to evaluate the effect 10 daily sessions of high frequency (10Hz) rTMS over the right

DLPFC on alcohol craving. 30 patients received active rTMS while 15 patients received sham rTMS. Active rTMS produced a significant reduction in alcohol craving (Mishra, Nizamie, Das, & Praharaj). This group also compared the efficacy of left versus right high frequency (10Hz) rTMS over the DLPFC and found that while there was a significant reduction in craving scores after 10 sessions of stimulation, there was no significant difference between left versus right stimulation (Mishra, Praharaj, Katshu, Sarkar, & Nizamie, 2015).

Hoppner et al., 2011 examined the effect of high frequency 20Hz rTMS over the left DLPFC on craving and mood in female detoxified alcohol dependent individuals. 10 subjects received rTMS for 10 consecutive working days, while 9 received sham stimulation. Authors noted no difference in between the two groups in terms of alcohol craving or mood (Hoppner, Broese, Wendler, Berger, & Thome, 2011). De Ridder et al., 2011 administered 1Hz rTMS for 3 weeks to the dorsal anterior cingulate using a double cone coil on an alcohol dependent individual. fMRI and resting state EEG were also conducted before rTMS and after both successful rTMS (no relapse) and unsuccessful rTMS (with relapse). EEG β activity and connectivity between the dorsal anterior cingulate cortex and posterior cingulate cortex was associated with craving, and these findings disappeared after successful rTMS. Findings suggest that rTMS to the anterior cingulate can temporarily suppress craving for alcohol in dependent individuals (De Ridder, Vanneste, Kovacs, Sunaert, & Dom, 2011). Herremans et al. 2012 conducted a prospective study of 31 hospitalized alcohol dependent individuals. Following detoxification, subjects were randomized to receive either a single active or sham high

frequency (20Hz) rTMS session over the right DLPFC. The single session did not have an effect on the level of craving for alcohol (Herremans et al., 2012).

Deep rTMS involves the use of an H-coil rather than the standard figure of eight coil to achieve deep brain stimulation. Ceccanti et al., 2015 used the H-coil to administer high frequency rTMS to the medial prefrontal cortex alcohol dependent individuals following detox (Ceccanti et al., 2015). Ten sessions of deep rTMS at 20Hz resulted in a decrease in alcohol craving, a decrease in mean number of alcoholic drinks/day and decrease of drinks on days of maximum alcohol intake. These differences were not observed in the group that received sham rTMS.

1.11.2. Effect of rTMS on Neurotransmitter Systems

The effect of rTMS on GABAergic, glutamatergic and dopaminergic neurotransmission has been investigated in a number of studies. Below is a review of the literature on the effect of rTMS on neurotransmission.

1.11.2.1Effect of rTMS on GABAergic Neurotransmission

High frequency rTMS to the motor cortex produces an increase in CSP (Daskalakis et al., 2006; de Jesus et al., 2013). The therapeutic effect of rTMS may be mediated through the normalization of cortical excitability and inhibition, which in turn normalizes aberrant neuroplasticity. The effect of rTMS on cortical excitability and inhibition may vary based on baseline cortical excitability and inhibition and by subject population. For example, at

both low and high frequency stimulations, rTMS reduced SICI in individuals with relatively higher baseline SICI while it increased SICI in individuals with lower baseline SICI (Daskalakis et al., 2006). It has been hypothesized that this phenomenon is due to homeostatic plasticity, a process by which the excitability of neurons and synaptic strength is dynamically adjusted in the direction required to promote stability of the neural network (Surmeier & Foehring, 2004; Turrigiano & Nelson, 2004). While the mechanisms underlying homeostatic plasticity are still unclear, it has been argued that they may be related to the interaction of $GABA_A$ and $GABA_B$ receptor mediated inhibitory mechanisms (Daskalakis et al., 2006). $GABA_B$ inhibitory mechanisms inhibit $GABA_A$ receptor mediated inhibitory mechanisms. Therefore, in individuals with elevated $GABA_A$ receptor mediated inhibitory mechanisms (i.e., high SICI), an increase in $GABA_B$ receptor mediated neurotransmission by rTMS would result in a significant decrease in SICI. However, in individuals with reduced $GABA_A$ receptor mediated activity, it is expected that $GABA_B$ mediated inhibition of $GABA_A$ would be intact and the potentiation of $GABA_B$ activity with rTMS would produce no significant or further change of SICI. In this case, rTMS potentiation of $GABA_A$ receptor mediated inhibition would predominate and SICI would increase. By regulating $GABA_A$ and $GABA_B$ receptor mediated inhibition, rTMS may help to normalize aberrant plasticity in alcohol dependent individuals (Table 1)

1.11.2.2 Effect of rTMS on NMDA Receptor-Mediated Glutamatergic Neurotransmission

The findings on the effect of rTMS on NMDA receptor mediated neurotransmission, as indexed through ICF, have been inconsistent. Review of the literature suggests that the effect of rTMS on ICF may vary with stimulation parameters and subject population. Some studies have shown no effect of low frequency rTMS to the motor cortex on ICF (Di Lazzaro, Oliviero, Mazzone, et al., 2002; Fitzgerald et al., 2002; Modugno et al., 2003; Peinemann et al., 2000; Wu et al., 2000). However, Brighina et al., 2005 reported that low frequency rTMS administered to the motor cortex produced a decrease in ICF in healthy individuals, while producing an increase in ICF in migraine patients (Brighina et al., 2005). Romero et al., 2002 also reported that low frequency rTMS to the motor cortex produces a decrease in ICF in the motor cortex (Romero, Anschel, Sparing, Gangitano, & Pascual-Leone, 2002). Wu et al., 2000 found that high frequency (15Hz) rTMS to the motor cortex produced an increase in ICF in healthy individuals (Wu et al., 2000). Similarly, Massie et al., 2013 reported that high frequency 10Hz functional rTMS to the motor cortex produced an increase in ICF in stroke survivors (Massie, Tracy, & Malcolm, 2013; Massie, Tracy, Paxton, & Malcolm, 2013). Given the inconsistent findings of the effect of rTMS on ICF, further studies exploring the effect of various stimulation intensities in the same subject group may give us a clearer understanding of the effect of rTMS on ICF. Furthermore, as studies using the same stimulation frequency have found opposing results in healthy individuals versus various patient populations, it is likely that the effect of rTMS on ICF is dependent on the baseline cortical excitability of the subject

population. Previous studies in alcohol dependent individuals in withdrawal suggest that cortical excitability is elevated in the population. In this population, rTMS, through mechanisms of homeostatic plasticity, could decrease ICF and thereby regulate neuroplasticity (Table 1).

1.11.2.3 Effect of rTMS on Dopaminergic Neurotransmission

Alcohol dependence and withdrawal is associated with decreased dopamine function (Martinez et al., 2005; Melis, Spiga, & Diana, 2005; Volkow et al., 2007). Findings from animal and human studies indicate that high frequency rTMS produces an increase in dopaminergic activity in brain regions including cortex, striatum and limbic system (Feil & Zangen). Administration of high frequency left DLPFC rTMS to healthy individuals results in dopamine release in the ipsilateral orbitofrontal cortex, cingulate cortex and striatum (Cho & Strafella, 2009). Furthermore, administration of high frequency rTMS to the frontal cortex of rats induces the release of dopamine in the mesolimbic and mesostriatal circuits (Kanno, Matsumoto, Togashi, Yoshioka, & Mano, 2004; Keck et al., 2002; Zangen & Hyodo, 2002). This effect was more pronounced in rats undergoing withdrawal from alcohol (Erhardt et al., 2004). The induction of dopamine release in these regions by rTMS has been argued to be a potential mechanism contributing to the therapeutic effects of rTMS for the treatment of alcohol dependence. Importantly, dopamine has also been implicated as an important modulator of neuroplasticity (for review, see (Jay, 2003). For example, D1 antagonists block LTP in the prefrontal cortex while D1 agonists facilitate LTP in this region (Gurden, Takita, & Jay, 2000). Therefore,

regulation of dopamine function by rTMS can also contribute to the regulation of aberrant neuroplasticity in alcohol dependence (Table 1).

Table 1 Summary of Effects of rTMS on Neurotransmission

	Changes Following rTMS
GABAergic	▪ High frequency rTMS produces an increase in CSP (Daskalakis et al., 2006; de Jesus et al., 2013) ▪ High and low frequency rTMS reduced SICI in individuals with higher baseline SICI while it increased SICI in individuals with a lower baseline SICI (Daskalakis et al., 2006)
Glutamatergic	▪ Inconsistent findings ▪ No effect of low frequency rTMS on ICF in the motor cortex (Di Lazzaro, Oliviero, Mazzone, et al., 2002; Fitzgerald et al., 2002; Modugno et al., 2003; Peinemann et al., 2000) ▪ Low frequency rTMS on the motor cortex decreases ICF in healthy individuals while increasing ICF in migraine patients (Brighina et al., 2005) ▪ High frequency rTMS to the motor cortex produced an increase in ICF in healthy individuals (Wu et al., 2000)
Dopaminergic	▪ High frequency left DLPFC rTMS to healthy individuals results in dopamine release in the ipsilateral orbitofrontal cortex, cingulate cortex and striatum (Cho & Strafella, 2009) ▪ High frequency rTMS to the frontal cortex of rats induces the release of dopamine into the mesolimbic and mesostriatal circuits. This effect was more pronounced in rats undergoing withdrawal from alcohol (Erhardt et al., 2004)

1.12 Overview of Rationale, Hypotheses and Objectives

1.12.1 Examining the Effect of Alcohol Intoxication on Neuroplasticity in the Motor Cortex

1.12.1.1 Rationale

Neuroplasticity is dependent, in part on, $GABA_A$ and NMDA receptor activities. Given that alcohol disrupts GABA and NMDA activity, alcohol administration may result in disruption of neuroplasticity (Morrisett & Swartzwelder, 1993; Schummers et al., 1997; Schummers & Browning, 2001; Yin et al., 2007). PAS is a TMS protocol that indexes LTP-like neuroplasticity *in vivo*. While evidence from studies using rat brain slice preparations suggests that alcohol blocks neuroplasticity (Blitzer et al., 1990; Morrisett & Swartzwelder, 1993; Schummers & Browning, 2001; Yin et al., 2007) only one study has examined the effect of acute alcohol administration on LTP-like neuroplasticity in humans using PAS. Lucke et al., examined the effect of two different low doses (BAC of <5mM and <20mM) of alcohol on PAS induced LTP-like neuroplasticity (Lucke et al., 2014b). The doses used in this study were below the quantities defining a binge episode. That is, the lowest dose was equivalent to approximately 1 standard drink for an average woman (weight 66kg) and 2 standard drinks for an average man (weight 83 kg); while the highest dose was equivalent to approximately 2 standard drinks for women and 3 standard drinks for men. This study found that both alcohol doses suppressed LTP-like neuroplasticity in the motor cortex up to 30 minutes following PAS. However, the effect of alcohol intoxication at higher doses (producing BACs equivalent to those seen after binge drinking) on neuroplasticity has not been evaluated yet.

1.12.1.2 Primary Objective

The primary objective of the current study was to evaluate the effects of alcohol intoxication episode on PAS-induced neuroplasticity in a sample of fifteen healthy alcohol drinkers through a within-subject randomized cross-over study design.

1.12.1.3 Primary Hypotheses

It was hypothesized that alcohol intoxication would impair PAS-induced neuroplasticity in the motor cortex of alcohol drinkers.

1.12.1.4 Secondary Objective

The secondary objective of the study was to compare the long-lasting effects of PAS-induced neuroplasticity one day following PAS administration with alcohol intoxication versus the placebo beverage.

1.12.1.5 Secondary Hypotheses

It was hypothesized that the impairment in PAS-induced neuroplasticity would also be evident the day following PAS administration with alcohol intoxication.

1.12.2 Examining the Effect of Alcohol Intoxication on Neuroplasticity in the Dorsolateral Prefrontal Cortex

1.12.2.1 Rationale

Findings from previous studies suggest that alcohol intoxication affects neurophysiology in the DLPFC (for review, see (Loheswaran et al., 2016)). The DLPFC is a brain region within the mesocortico-limbic pathway that is implicated in the pathophysiology of addiction (Park et al., 2007) The DLPFC plays an important role in reward processing to guide behaviour and mediates cognitive functioning including working memory (for review, see (Owen, McMillan, Laird, & Bullmore, 2005)). Given that neuroplasticity is the principle mechanism underlying learning and memory important in cognitive functioning (Rosenzweig, Breedlove, & Leiman, 2002; Weinberger, 2004), indexing the effect of consuming high doses of alcohol on DLPFC neuroplasticity may reveal a potential mechanism of cognitive dysfunction observed during alcohol intoxication.

Despite the important implications of neuroplasticity impairment by alcohol in the DLPFC, no study to date has examined the effect of alcohol intoxication on neuroplasticity in this region in humans *in vivo*. Our group has demonstrated that LTP-like neuroplasticity can be indexed from the DLPFC by combining PAS with electroencephalography (EEG) (Rajji et al., 2013). This potentiation of cortical evoked activity (CEA) by PAS was associated with an increase in coupling of cortical oscillations of θ phase and γ amplitude (θ-γ coupling) (Rajji et al., 2013), believed to be associated with the working memory function of the DLPFC (Lisman & Idiart, 1995). This novel

technique holds the promise of revealing the effect of alcohol on neuroplasticity in the DLPFC and allows for the indexing of the effect of alcohol θ-γ coupling.

1.12.2.2 Primary Objective

The primary objective of the study was to examine the effect of alcohol intoxication on PAS-induced neuroplasticity in the DLPFC in fifteen healthy alcohol drinkers using PAS with EEG.

1.12.2.3 Primary Hypothesis

It was hypothesized that alcohol intoxication would impair PAS-induced neuroplasticity in the DLPFC in healthy alcohol drinkers.

1.12.2.4 Secondary Objective

The secondary objective was to examine the effect of alcohol intoxication on PAS-induced potentiation of θ-γ coupling in the DLPFC.

1.12.2.5 Secondary Hypothesis

It was hypothesized that alcohol intoxication would impair the PAS-induced potentiation of θ-γ coupling in the DLPFC.

1.12.3 Examining the Effect of Acute Intoxication on N100 Amplitude

1.12.3.1 Rationale

The combination of TMS with EEG has allowed for the direct measurement of cortical excitability and inhibition from the cortex (Daskalakis et al., 2008; Fitzgerald et al., 2008) with high temporal precision. The negative peak that occurs ~100ms following the TMS pulse is the largest component of the EEG (Nikouline et al., 1999; Paus et al., 2001). This component is believed to represent inhibitory processes, particularly $GABA_B$ receptor mediated inhibition (Matsunaga, Akamatsu, Uozumi, Urasaki, & Tsuji, 2002; Nikulin et al., 2003; Rogasch et al., 2013). The N100 is modulated during motor tasks similar to cortical inhibition (Bonnard et al., 2009; Bruckmann et al., 2012; Kicic et al., 2008; Spieser et al., 2010). Similarly, motor measures of LICI and CSP, both thought to represent $GABA_B$ receptor mediated neurotransmission, are correlated with N100 amplitude. Administration of baclofen, a $GABA_B$ agonist increases the amplitude of the N100. Together, these findings provide strong evidence that the N100 represents $GABA_B$ receptor activities. A recent study reported that alcohol consumption abolished the N100 response to TMS applied to the motor cortex (Kahkonen & Wilenius, 2007). The authors speculated that this was due to alcohol altering the cortico-cortical connectivity of the motor cortex and/or an overall suppression of alcohol on the motor cortex.

Some of alcohol's impairing effects of alcohol are likely mediated, at least in part, to alcohol's impairing effect on neurotransmission the DLPFC, a brain region that plays a key role in executive and cognitive functioning. Along these lines, high frequency rTMS

to the DLPFC has shown promise as a treatment for alcohol use disorders (Mishra, Nizamie, Das, & Praharaj). However, no studies have examined the effect of alcohol intoxication on N100 response to TMS of the DLPFC. Interestingly, previous studies using PAS to induce associative neuroplasticity in the human motor cortex have reported that measure of CSP and LICI, indices of $GABA_B$ receptor mediated neurotransmission, are altered following PAS administration (Russmann et al., 2009; Stefan et al., 2000). These findings suggest that PAS may possibly counteract alcohol's effects, if any, on N100 amplitude.

1.12.3.2 Primary Objective

The primary objective of the study was to evaluate the effect of alcohol intoxication on the amplitude of the N100 response to TMS stimulation of the DLPFC.

1.12.3.3 Primary Hypothesis

It was hypothesized that alcohol intoxication would produce a reduction in the N100 response amplitude to TMS stimulation of the DLPFC.

1.12.3.4 Secondary Objective

The secondary objective of the study was to examine if DLPFC PAS could be used to counteract the effect of alcohol intoxication on the N100 amplitude in response to TMS stimulation of the DLPFC.

1.11.3.5 Secondary Hypothesis

It was hypothesized PAS administered to the DLPFC will counteract the effect of alcohol intoxication on the N100 amplitude in response to TMS stimulation of the DLPFC.

Chapter 2

Materials and Methods

2.1 Examining the Effect of Alcohol Intoxication on Neuroplasticity in the Motor Cortex

2.1.1 Study Design

The current study was a within-subject, randomized cross-over study design consisting of a total of 4 study visits following enrollment into the study. These visits consisted of one study visit where subjects received the alcohol beverage (with PAS), one study visit where subjects received the placebo beverage (with PAS) and a brief next day visit to each of the aforementioned visits (with no beverage or PAS) during which the Post Day 1 MEP measures were collected. The order of the alcohol and placebo study visits was randomized with a minimum one-month washout period between beverage testing visits.

2.1.2 Study Visits

At the start of all study visits, breath measures of BAC and carbon monoxide (CO) were obtained and urine drug screens were administered. All females of child bearing age were administered urine pregnancy tests at the beginning of the beverage visits. The resting motor threshold (RMT) and the intensity required to produce an average motor evoked potential (MEP) amplitude of 1mV ($1mV_{T1}$), was then obtained. During the beverage visits, subjects were given 15 min to consume the beverage to achieve a rapid increase in

BAC. Their BAC was obtained via breath measures every 15 minutes after beverage consumption and PAS administration began when BAC was ≥17.4mM (≥0.08%). 1mV intensity ($1mV_{T2}$) was obtained again following beverage consumption and a 3 minute baseline session of 20 single TMS pulses were administered at this intensity (SI_{1mVT2}.) This was followed by PAS administration for 30 min at SI_{1mVT2}). Potentiation was indexed by 3 min sessions of 20 single TMS pulses at SI_{1mVT2} at post 0 min, post 15 min, post 30 min and post 60 min and Post Day1 to examine the longitudinal effects of alcohol on LTP-like activity. Figure 1 summarizes the study design and study visit timeline.

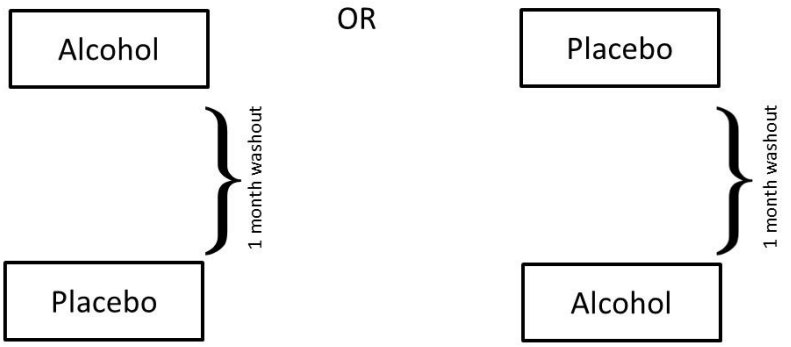


Figure 1. Study visits (alcohol/placebo) were randomized with a 1 month washout period between study visits. 20 TMS pulses were administered at 1mV stimulus intensity prior to beverage consumption (SI_{1mVT1}). Subjects were given 15 minutes to consume the beverage. BAC was obtained every 15 minutes following beverage consumption (except during PAS). Another 20 TMS pulses were administered following beverage consumption

and stimulus intensity was adjusted if necessary (SI_{1mVT2}). PAS was then administered, followed by 20 TMS pulses immediately following PAS ($SI_{1mV0min}$), post 15 min ($SI_{1mV15min}$), post 30 min ($SI_{1mV30min}$), post 60 min ($SI_{1mV60min}$) and the next day ($SI_{1mVDay1}$).

2.1.3 Subjects

Subjects were recruited through ads in local magazines and on-line, as well as ads posted at the University of Toronto and the Centre for Addiction of Mental Health. All subjects provided written informed consent prior for participation in the study. All experiments were in accordance with the Declaration of Helsinki and were approved by the research ethics board at the Centre for Addiction and Mental Health. Fifteen otherwise healthy alcohol drinkers (mean age 33.07, ± 7.42, 25-42 years of age, 11Males) participated in the study. Subjects had endorsed at least one heavy drinking episode (defined at 5 standard drinks for men and 4 standard drinks for women) ("NIAAA Guidelines," 2004), within the last month, as assessed using the Alcohol Timeline Follow-Back (Sobell, Sobell, Leo, & Cancilla, 1988). Subjects were between the age of 19 and 60 years of age and were of legal drinking age in Ontario, Canada. Subjects were non-smokers (had not smoked any cigarettes in the last three months) and did not meet DSM-IV criteria for any current drug abuse or dependence or any psychiatric disorders, as assessed using the Structured Clinical Interview for DSM Disorders IV (SCID, (First, Spitzer, Robert, Gibbon, & Williams, 2005). Subjects were excluded if they had a history of seizures, neurological disease or cognitive impairment (determined by a score of <24 on the Mini Mental State

Examination (Folstein, Folstein, & McHugh, 1975)) and none reported regular use of any therapeutic or recreational psychoactive drugs during the last three months. None of the female subjects were pregnant or breast feeding during their period of enrollment in the study. Table 1 includes the demographic information of the subjects.

2.1.4 Beverages

The alcohol beverage was made using 95% United States Pharamcopeia (USP) alcohol at a dose of 1.5g/l of body water. The alcohol beverage was mixed in a 1:5 ratio with orange juice and tonic water. The placebo beverage was made from an equivalent volume of orange juice and tonic water. Absolut Vodka (0.2mL of 40% alcohol) was added to both alcohol and placebo beverages immediately prior to administering the beverage to the subject to produce the odour of alcohol but is minimal enough to not produce any additional alcohol effects. Subjects were randomized to receive either alcohol or placebo beverage using a block randomization design generated by the pharmacy at Centre for Addiction and Mental Health. The beverages were prepared by the pharmacy and the investigators were blinded to the beverage type until after the first BAC measure was obtained 15 minutes following beverage consumption. Subjects were also blinded to the beverage type. Subjects were asked to guess which beverage they had received 30 minutes following beverage consumption. With the exception of one subject, all subjects correctly guessed the beverage type during both study visits.

2.1.5 BAC and CO Measurements

BAC was measured with an Alco-Sensor FST (DAVTECH Analytic Services, Canada) from breath samples at the beginning of all study visits and at 15 min intervals following beverage consumption (except during PAS administration). Alcohol on the breath is drawn onto a fuel cell with a porous disk and converted to acetic acid. Electrons released by this process generate current in proportion to the amount of alcohol oxidized. The current produced is then translated to BAC. A Micro+™ Smokerlyzer® CO monitor (Bedfont Scientific Ltd.) was used to obtain CO measures from breath samples at the beginning of all study visits.

2.1.6 TMS Stimulation

TMS pulses were administered to the left motor cortex using a 7 cm figure-of-eight coil, and two Magstim 200 stimulators (Magstim Company Ltd., UK) connected via a Bistim module and EMG data was collected using dedicated software (Cambridge Electronics Design, UK). The intensity of stimulation was determined based on the RMT from the left motor cortex. The RMT was determined according to the protocol outlined by Rossini et al., 1994 (Rossini et al., 1994). The RMT was defined as the minimum stimulus intensity that elicits a MEP of more than 50 mV in five of ten trials. The intensity of stimulation was then adjusted to produce a mean peak-to-peak MEP amplitude of 1 mV (Valls-Sole et al., 1992) in the left motor cortex. In stimulating the left motor cortex, the TMS coil was placed at the optimal position for eliciting MEPs from the right APB muscle. EMG was captured by placing two disposable disc electrodes over the right APB muscle in a tendon-

belly arrangement. The signal was amplified using a Model 2024F amplifier (Intronix Technologies Corporation, Bolton, Ontario Canada). The signal was filtered at band pass of 2 Hz to 2.5 kHz and digitized using the Micro 1401 (Cambridge Electronics Design, Cambridge UK).

2.1.7 Paired Associative Stimulation

PAS was conducted in accordance with the protocol originally described by Stefan et al., 2000 (Stefan et al., 2000). A series of 20 single TMS pulses at an intensity to produce a mean MEP amplitude of 1mV peak-to-peak were administered at a frequency of 0.1Hz to obtain the baseline MEP measurement (SI_{1mVT2}). During the PAS procedure, the right median nerve was stimulated at the wrist with standard bar electrodes (0.5 ms square wave constant current pulses), with the cathode positioned proximally at an intensity of 300% of the perceptual threshold through a peripheral nerve stimulator (Model SD9K, Grass Instruments, West Warick, Richmond, VA). This was followed by TMS over the left hemisphere at the optimal site for activating the APB muscle at SI_{1mVT2}. To derive LTP-like neuroplasticity, an interstimulus interval of 25 ms was used. In each experiment a total of 180 pairs of stimuli over 30 min were presented. Studies have shown that attention can modify neuroplasticity (Meintzschel & Ziemann, 2006; Stefan et al., 2004). During PAS, subjects were asked to attend to their hand and count the total number of stimulations delivered and reported the count randomly throughout the 30 minute session. The subjects were asked to report their final total count at the end of the 30 minute session. Following PAS, a train of 20 single pulses of TMS of 0.1 Hz were delivered at the

same intensity used for PAS and the 20 pulses administered before PAS (SI_{1mVT2}) at Post0 (immediately following PAS), Post15 (15 minutes following PAS), Post 30 (30 minutes following PAS) , Post 60 (60 minutes following PAS) and Post Day1 (the next day following PAS) to examine average MEP amplitude at each of these time points. The test parameters used for the PAS paradigm are shown in Table 2.

2.1.8 Statistics

Statistical analyses were performed using IBM SPSS Statistics (Version 22). Beverage effects were analyzed using a general linear model repeated measures (ANOVA) with the beverage (alcohol beverage and placebo beverage) and time (Post 0, Post 15, Post 30, Post 60, Post Day 1) as within-subject factors. Post-hoc analysis were conducted with paired t-tests and adjusted for multiple comparisons with Bonferroni correction. 1mV peak-to-peak intensities before and after ($1mV_{T1}$ and $1mV_{T2}$) alcohol and placebo were compared using paired t-tests.

2.2 Examining the Effect of Alcohol Intoxication on Neuroplasticity in the Dorsolateral Prefrontal Cortex

2.2.1 Study Design

The current study was a within-subject, randomized cross-over study design consisting of a total of two study visits following enrollment into the study. These visits consisted of one study visit where subjects received the alcohol beverage and one study visit where subjects received the placebo beverage. During both study visits, PAS was administered to

the DLPFC and EEG was collected. The order of the alcohol and placebo study visits was randomized with a minimum one-month washout period between beverage testing visits.

2.2.2 Study Visits

At the beginning of all study visits, breath measures of BAC and carbon monoxide were obtained and urine drug screens were administered. Females of child bearing age were administered urine pregnancy tests before study visits. The resting motor threshold (RMT) and the stimulus intensity required to produce an average motor evoked potential (MEP) amplitude of 1mV ($1mV_{T1}$) was obtained. The DLPFC was identified using the F5 electrode as the marker (Rusjan et al., 2010). To assess baseline cortical evoked activity (CEA), a train of 100 pulses at 0.1 Hz were delivered at stimulus intensity $1mV_{T1}$ pre PAS to the DLPFC (PrePAS) and EEG was collected. Subjects were then given 15 min to consume the beverage to achieve a rapid increase in BAC. Their BAC was obtained via breath measures every 15 minutes after beverage consumption and when BAC was ≥17.4mM (≥0.08%), the intensity required to produce an average motor evoked potential (MEP) amplitude of 1mV ($1mV_{T2}$) was reassessed and PAS was administered to the DLPFC. Immediately after the termination of PAS a train of 100 pulses of TMS of 0.1 Hz were delivered to the DLPFC at (Post 0), 15 minutes later (Post 15), 30 minutes post PAS (Post 30) and 60 minutes post PAS (Post 60) while EEG was collected to assess potentiation of CEA following PAS (Figure 2).

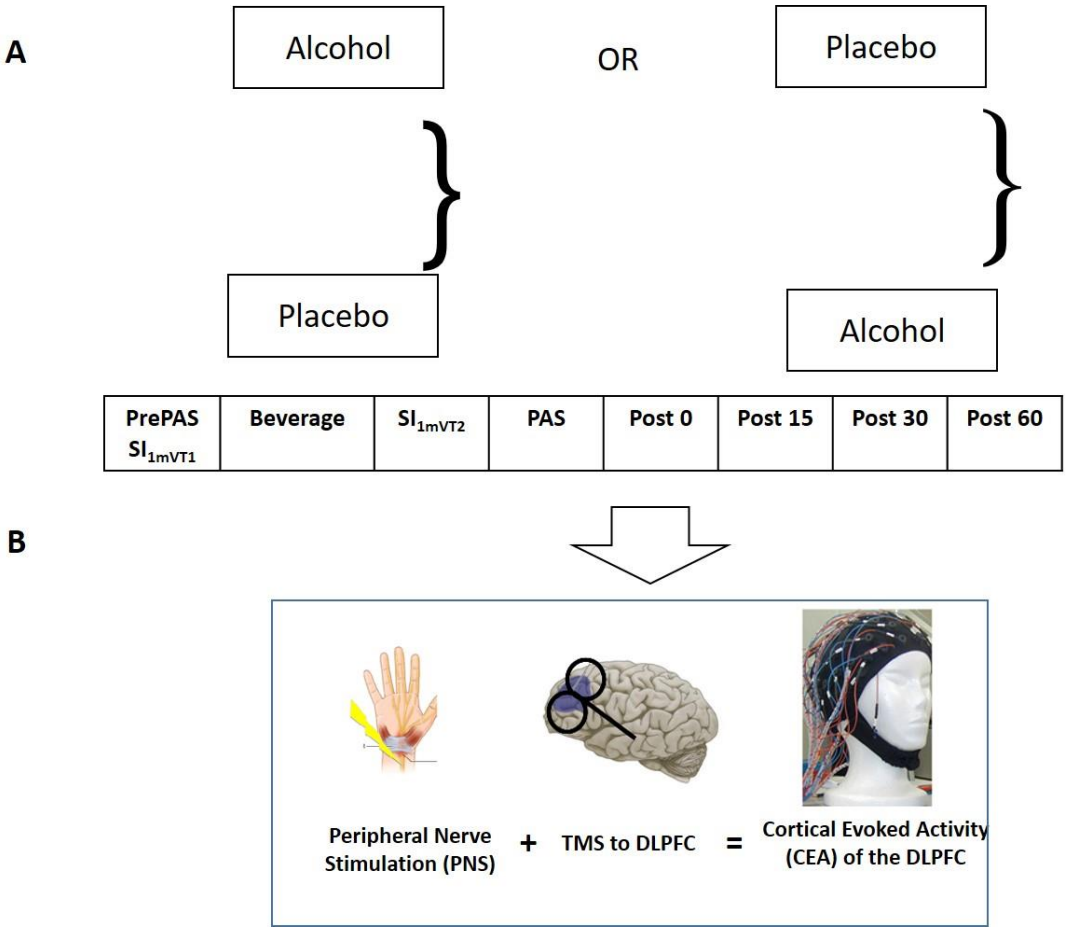


Figure 2 Study design. Study visits (alcohol/placebo) were randomized with a 1 month washout period between study visits. 100 TMS pulses were administered to the DLPFC at 1mV stimulus intensity prior to beverage consumption (SI1mVT1). Subjects were given 15 minutes to consume the beverage. BAC was obtained every 15 minutes following beverage consumption (except during PAS). Another 100 TMS pulses were administered following beverage consumption and stimulus intensity was adjusted if necessary (SI1mVT2). PAS was then administered using SI1mVT2, followed by 100 TMS pulses also at SI1mVT2 at Post 0 (immediately following PAS), Post 15 (15 minutes following PAS), Post 30 (30 minutes following PAS) and Post 60 (60 minutes following PAS). EEG was collected at all time points to measure CEA.

2.2.3 Subjects

Subjects were recruited through ads on-line and ads posted at the University of Toronto. All subjects provided written informed consent prior for participation in the study. All experiments were in accordance with the Declaration of Helsinki and were approved by the research ethics board at the Centre for Addiction and Mental Health. Fifteen healthy alcohol drinkers participated in the study (mean age 33.42, ± 7.52, 23-46 years of age, 10 Males). Subjects had endorsed at least one heavy drinking episode (defined at 5 standard drinks for men and 4 standard drinks for women) ("NIAAA Guidelines," 2004), within the last month, as assessed using the Alcohol Timeline Follow-Back (Sobell et al., 1988). Subjects were between the age of 19 and 60 years of age and were of legal drinking age in Ontario, Canada. Subjects were non-smokers (had not smoked any cigarettes in the last three months) and did not meet DSM-IV criteria for any current drug abuse or dependence or any psychiatric disorders. Subjects were excluded if they had a history of seizures, neurological disease or cognitive impairment (determined by a score of <24 on the Mini Mental State Examination (Folstein et al., 1975)) and none reported regular use of any therapeutic or recreational psychoactive drugs during the last three months.

2.2.4 Beverages

The alcohol beverage was made using 95% United States Pharmacopeia (USP) alcohol at a dose of 1.5g/l of body water. The alcohol beverage was mixed in a 1:5 ratio with orange juice and tonic water. The placebo beverage was made from an equivalent volume of orange juice and tonic water. Absolut Vodka (0.2mL of 40% alcohol) was added to both

alcohol and placebo beverages immediately prior to administering the beverage to the subject to produce the odour of alcohol but is minimal enough to not produce any additional alcohol effects. Subjects were randomized to receive either alcohol or placebo beverage. Investigators were blinded to the beverage type until after the first BAC measure was obtained 15 minutes following beverage consumption. Subjects were also blinded to the beverage type.

2.2.5 BAC and CO Measurements

BAC was measured with an Alco-Sensor FST (DAVTECH Analytic Services, Canada) from breath samples at the beginning of all study visits and at 15 min intervals following beverage consumption (except during PAS administration). A Micro+™ Smokerlyzer® CO monitor (Bedfont Scientific Ltd.) was used to obtain CO measures from breath samples at the beginning of all study visits.

2.2.6 TMS Stimulation

TMS pulses were administered to the left motor cortex to obtain the resting motor threshold and 1 mV intensity and to the left DLPFC (for PrePAS, PAS and PostPAS) using a 7 cm figure-of-eight coil, and two Magstim 200 stimulators (Magstim Company Ltd., UK) connected via a Bistim module and EMG data was collected using dedicated software (Cambridge Electronics Design, UK). The intensity of stimulation was determined based on the RMT from the left motor cortex. The RMT was determined according to the protocol outlined by Rossini et al., 1994 (Rossini et al., 1994). The RMT

was defined as the minimum stimulus intensity that elicits a MEP of more than 50 mV in five of ten trials. The intensity of stimulation was then adjusted to produce a mean peak-to-peak MEP amplitude of 1 mV (Valls-Sole et al., 1992) in the left motor cortex. In stimulating the left motor cortex, the TMS coil was placed at the optimal position for eliciting MEPs from the right APB muscle. EMG was captured by placing two disposable disc electrodes over the right APB muscle in a tendon-belly arrangement. The signal was amplified using a Model 2024F amplifier (Intronix Technologies Corporation, Bolton, Ontario Canada). The signal was filtered at band pass of 2 Hz to 2.5 kHz and digitized using the Micro 1401 (Cambridge Electronics Design, Cambridge UK).

2.2.7 CEA from the DLPFC

TMS was applied (using the 1mV intensity obtained from the left motor cortex) over the F5 electrode and CEA was measured using EEG. EEG signals were acquired through a 64-channel Synamps 2 EEG system. The impedence of all electrodes (Ag/AgCl ring electrodes) was lowered to ≤ 5 kΩ. All electrodes were referenced to an electrode positioned posterior to the Cz electrode. In addition, four electrodes were placed on the outer corner of each eye, as well as, above and below the left eye, to monitor the eye movement artefact. EEG signals were recorded using DC and a lowpass filter of 100 Hz at 20 kHz sampling rate, which has been shown to avoid saturation of amplifiers and minimize TMS related artefact (Daskalakis et al., 2008).

EEG data were down-sampled to 1000Hz and segmented from -1000ms to 2000ms relative to the onset of the TMS pulse. The data was then baseline corrected with respect to the pre-stimulus interval -500 ms to -110 ms. To avoid TMS artefacts, EEG data were then re-segmented from 25ms to 2000ms. Thereafter, the EEG data was digitally filtered by using a second order, Butterworth, zero-phase shift 1-55 Hz band pass filter (24dB/Oct). In order to apply the same objective and subjective criteria to de-noise data, the EEG recordings from all 5 sessions (PrePAS, Post0, Post 15, Post 30 and Post 60) were concatenated together. Initially, EEG data were visually inspected to eliminate trials and channels that were highly contaminated with noise (muscle activity, electrode artifacts). Then, an electrodes-by-trials matrix of ones was created and assigned a value of zero if an epoch had: (1) an amplitude larger than +/- 150 μV; (2) a power spectrum that violated the 1/f power law; or (3) a standard deviation 3 times larger than the average of all trials. Additionally, electrodes were rejected if their corresponding row had more than 60% of columns (trials) coded as zeros and epochs were removed if their corresponding column had more than 20% of rows (electrodes) coded as zeros. Lastly, an independent component analysis (ICA) (EEGLAB toolbox; Infomax algorithm) was performed to remove eyeblink traces, muscle artifacts, and other noise from the EEG data and data was re-referenced to the average for further analysis.

To assess potentiation of CEA by PAS, first, the TMS evoked potential (TEP) for each session was calculated by averaging the response over all epochs. Next, using the Hilbert transformation, the area between 50-275 ms under the instantaneous amplitude of TEP

was determined. This area indicates the overall power of the TEP. The first interval (i.e., 50 ms) was chosen because it represents the earliest artifact-free data and the second interval (i.e., 275 ms) was chosen to cover the activity of $GABA_B$ receptors (Deisz, 1999a, 1999b). To quantify the PAS-induced potentiation on each session, we calculated the ratio of TEP power at each time post after over pre- PAS responses. As the post-PAS timing of maximum potentiation of CEA could vary among participants, we selected the maximum CEA ratio for each participant after PAS. To assess PAS-induced potentiation in the DLPFC, the 4 left frontal electrodes encompassing the DLPFC (F1, F3, F5 and F7) were used.

2.2.8 Paired Associative Stimulation

PAS was administered to the DLPFC in the accordance with the protocol first described by Rajji et al., 2013. Briefly, PAS administration consisted of 180 TMS stimuli delivered over the F5 electrode at a frequency of 0.1Hz. The TMS stimuli over the DLPFC were preceded by peripheral nerve stimulation (PNS) delivered to the right median nerve by 25 ms. Electrical median nerve stimulation was delivered at 300% of the sensory threshold. The sensory threshold was identified as the minimum detectable PNS stimulus. As described by Rajji et al., 2013, given that the post timing for maximum potentiation varies among participants, the maximum ratio for CEA for each participant following PAS was selected. One outlier (with CEA values 3 standard deviations above the mean during the placebo visit) was removed from CEA calculations.

2.2.9 θ-γ Coupling

The analysis of coupling of θ-phase and γ-amplitude was performed on the time averaged response of the TMS evoked potential of each participant using Matlab. The averaged signal was first filtered into separate θ [4-7]Hz and γ [30-50]Hz waveforms with a zero-phase shift filter and then a Hilbert transform was applied to separate the phase and amplitude of the signal. The γ amplitudes were sorted into six bins (i.e., _180° to _120°, _120° to _60°, _60° to 0°, 0°–60°, 60°–120°, 120°–180°) using the phase information of the θ wave and were then averaged. Given that the angle values correspond to the cosine reference, the peak of the waveform falls at zero degrees. An entropy based modulation index (MI) (Tort et al., 2010) was used to quantify coupling: *MI=[log(N)-H(P)]/log(N)]*

Where N is the number of phase bins, log(N) represents the 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:

$H(P) = -\sum_{j=1}^{N} P(j) \log [P(j)]$

The relative amplitude distribution P for each participant was calculated by dividing the amplitude of each phase by the sum of all amplitudes across bins. The maximum entropy for a relative amplitude distribution happens when the amplitude is 1/N, which occurs when the distribution is uniform. Given that an increase in coupling represents an increase of order, higher coupling translates to lower entropy H(P). This in turn produces a high MI value. The choice of the post-PAS time-point for each participant was based on the maximum time of potentiation.

2.2.10 Statistics

Statistical analyses were performed using IBM SPSS Statistics (Version 22). Beverage effects on potentiation and theta-gamma coupling were analyzed using a general linear model repeated measures (ANOVA) with beverage (alcohol versus placebo) as the within-subjects factor. For evaluation of mean potentiation, potentiation from all Post-PAS time-points were averaged (Post 0, Post 15, Post 30 and Post60). For evaluation of maximum potentiation, the time point with the maximal potentiation index was selected. Post-hoc analyses were conducted using paired t-tests. Mean 1mV peak-to-peak intensities (TMS test stimulus intensity) before and after beverage were compared using paired t-tests.

2.3 Examining the Effect of Alcohol Intoxication on N100 Amplitude

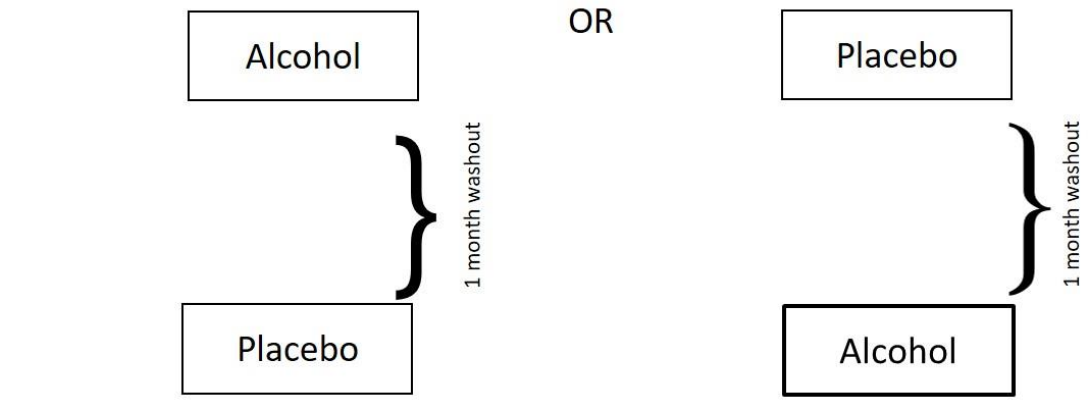
2.3.1 Study Design

The study was a within-subject, randomized cross-over design. Subjects who met eligibility criteria were enrolled into the study. Following enrollment, subjects attended two study visits. Subjects were required to drink an alcohol beverage during one study visit and a placebo beverage during the other visit. 100 TMS was administered to the left DLPFC during study visits before (PreBev) and after beverage (PostBev) consumption and EEG was collected. PAS was then administered to the left DLPFC. Following the 30 minute PAS session, 100 TMS pulses were then administered to the left DLPFC at Post 0, Post 15, Post 30 and Post 60 to measure the effect of PAS on N100. There was a one-

month washout period during both study visits and the order of the study visits was randomized.

2.3.2 Study Visits

Upon arrival of the subject to each study visit, breath measures of carbon monoxide (CO) and BAC were obtained. Urine pregnancy tests were administered to all females of child bearing age prior to study commencement. The study visit was only commenced if urine pregnancy tests and BAC measures were negative. The resting motor threshold (RMT) and the stimulus intensity required to produce an average motor evoked potential (MEP) amplitude of 1mV (1mVT1) was obtained. The DLPFC was identified using the F5 electrode as the marker (Rusjan, Barr et al. 2010). Assessment of baseline TEPs was performed by applying a train of 100 pulses at 0.1Hz at stimulus intensity 1mVT1 (PreBev). In order to achieve a rapid increase in BAC, subjects were given 15 minutes to consume the beverage. BAC was obtained via breath measures every 15 minutes after beverage consumption. The stimulus intensity necessary to produce an average MEP of 1mV (1mVT2) was reassessed when each subjects BAC reached ≥17.4mM (≥0.08%). Another train of 100 pulses at 0.1H was administered to the left DLPFC at stimulus intensity 1mVT2 (PostBev). PAS was then administered to the left DLPFC at stimulus intensity 1mVT2. Following PAS, 100 pulses were administered to the left DLPFC at Post 0, Post 15, Post 30 and Post 60. EEG was collected throughout all testing sessions (Figure 3).



PreBev 100 TMS Stimuli ($1mVT_1$)	Beverage	PostBev 100 TMS Stimuli ($1mVT_2$)	PAS (SI_{1mVT2})	Post0 100 TMS Stimuli ($1mVT_2$)	Post15 100 TMS Stimuli ($1mVT_2$)	Post15 100 TMS Stimuli ($1mVT_2$)	Post30 100 TMS Stimuli ($1mVT_2$)	Post60 100 TMS Stimuli ($1mVT_2$)

Figure 3 Study visits (alcohol/placebo) were randomized with a 1 month washout period between study visits. 100 TMS pulses were administered to the DLPFC at 1mV stimulus intensity prior to beverage consumption (SI_{1mVT1}). Subjects were given 15 minutes to consume the beverage. Another 100 TMS pulses were administered following beverage consumption and stimulus intensity was adjusted if necessary (SI_{1mVT2}). PAS was administered at SI_{1mVT2} to the DLPFC for 30 minutes. This was followed by 100 TMS pulses to the DLPFC at SI_{1mVT2} at Post0, Post15, Post30 and Post60.

2.3.3 Subjects

Subject recruitment was done through ads posted at the University of Toronto as well as online ads (Craigslist, Kijiji, etc). Informed consent was provided by all subjects prior to participation in the study and all experiments were conducted in accordance with the Declaration of Helsinki. The study was approved by the research ethics board at the Centre for Addiction and Mental Health. Fifteen healthy alcohol drinkers (mean age 33.42, ± 7.52, 23-46 years of age, 10 Males) who had endorsed at least on heavy drinking episode (defined at 5 standard drinks for men and 4 standard drinks for women) ("NIAAA Guidelines," 2004), within the last month, as assessed using the Alcohol Timeline Follow-Back (Sobell et al., 1988), participated in the study. Subjects were non-smokers (had not smoked any cigarettes in the last three months) and were between the age of 19 to 60 years of age. Subjects did not meet did not meet DSM-IV criteria for any current drug abuse or dependence or any psychiatric disorders. Subjects were excluded if they had a history of seizures, neurological disease or cognitive impairment (determined by a score of <24 on the Mini Mental State Examination (Folstein et al., 1975). None of the subjects reported regular use of any therapeutic or recreational psychoactive drugs during the last three months.

2.3.4 Beverages

Beverages consisted of either an alcohol beverage made with 95% United States Pharmacopia (USP) alcohol or a placebo beverage of equal volume made of orange juice and tonic water. The alcohol beverage was mixed in a 1:5 ratio with orange juice and

tonic water. Both the placebo and alcohol beverages had 0.2mL of Absolute Vodka (0.2mL of 40% alcohol) added to immediately before beverage administration to the subjects. This very small amount of alcohol was added to the beverage to produce the odor of alcohol without producing any of alcohol's effects. Subjects were randomized to receive either alcohol or placebo beverage first and received the other beverage in the subsequent visit. Subjects were blinded to the type of beverage they received. Investigators were also blinded to the type of beverage until after the first BAC measure was obtained 15 minutes following beverage consumption.

2.3.5 BAC and CO Measurements

An Alco-Sensor FST (DAVTECH Analytic Services, Canada) was used to obtain a breath sample at the beginning of all study visits and at 15 min intervals following beverage consumption. CO measures were obtained at the beginning of all study visits using Micro+™ Smokerlyzer® CO monitor (Bedfont Scientific Ltd.)

2.3.6 TMS Stimulation

The RMT and 1mV intensity were obtained by applying TMS pulses over the left motor cortex using a 7 cm figure-of-eight coil and two Magstim 200 stimulators (Magstim Company Ltd, UK) connected via a Bistim module. The site of stimulation in the left motor cortex was determined by identifying the optimal position for eliciting MEPs from the right APB muscle. Two disposable disc electrodes over the right APB muscle in a tendon-belly arrangement were used to capture EMG. RMT was evaluated using the

protocol outlined by Rossini et al., 1994 (Rossini, Barker et al. 1994). The RMT was defined as the minimum stimulus intensity that elicits a MEP of more than 50 μV in five of ten trials. The intensity of stimulation was determined based on the intensity required to produce a mean peak-to-peak MEP amplitude on 1mV. Dedicated software (Cambridge Electronic Design, UK) was used to collect electromyography data. Once the 1mv intensity was determined, TMS pulses were applied to the left DLPFC for the test paradigms. The signal was amplified using a Model 2024F amplifier (Intronix Technologies Corporation, Bolton, Ontario Canada) and filtered at band pass of 2 Hz to 2.5 kHz and digitized using the Micro 1401 (Cambridge Electronics Design, Cambridge UK). TMS stimuli were administered over the F5 electrode at 1mV intensity at 0.1Hz during all test paradigms and PAS (PreBev, PostBev, PAS, Post0, Post15, Post30 and Post 60).

2.3.7 EEG Data Collection and Analysis

A 64-channel Synamps 2 EEG system was used to acquire EEG data. The electrode positioned posterior to the Cz electrode was used as the reference electrode. The impedence of each of the electrodes (Ag/AgCl ring electrodes) was lowered to <5kΩ. To monitor eye movement artefacts, four electrodes were placed on the outer corner of each eye, as well as, above and below the left eye, to monitor the eye movement artefact. In order to minimize TMS related artefacts and avoid saturation of the amplifiers, EEG signals were recorded using DC and a low pass filter of 100 Hz at 20 kHz sampling rate (Daskalakis et al., 2008).

EEG data was down-sampled to 1000Hz. The data was segmented was -1000ms to 2000ms relative to the TMS pulse. Baseline correction was performed with respect to the pre-stimulus interval. To eliminate EEG artifacts, the EEG data was re-segmented from 25ms to 2000ms. The data was then digitally filtered using a second order Butterworth zero-phase shift 1-55Hz band pass filter (24dB/Oct). The recordings from both sessions (PreBev, PostBev, Post 0, Post 15, Post 30, Post 60) were concatenated to apply the same criteria to both recordings to remove noise from the data.

Initially, EEG data were visually inspected to eliminate trials and channels that were highly contaminated with noise (muscle activity, electrode artifacts). Then, an electrodes-by-trials matrix of ones was created and assigned a value of zero if an epoch had: (1) an amplitude larger than +/- 150 μV; (2) a power spectrum that violated the 1/f power law; or (3) a standard deviation 3 times larger than the average of all trials. Additionally, electrodes were rejected if their corresponding row had more than 60% of columns (trials) coded as zeros and epochs were removed if their corresponding column had more than 20% of rows (electrodes) coded as zeros. Lastly, an independent component analysis (ICA) (EEGLAB toolbox; Infomax algorithm) was performed to remove eyeblink traces, muscle artifacts, and other noise from the EEG data and data was re-referenced to the average for further analysis.

The TEP was calculated by averaging the response over all epochs for each of the sessions. The Hilbert transformation was then used to determine the area under the instantaneous amplitude between 50-275ms. The first interval (i.e., 50 ms) was chosen

because it represents the earliest artifact-free data and the second interval (i.e., 275 ms) was chosen to cover the activity of $GABA_B$ receptors.

2.3.8 N100 Analysis

The amplitude of the N100 was calculated by measuring the amplitude under the curve of the global mean field amplitude (GMFA) at 100ms±20ms. The GMFA is used to index global field activity and is measured by calculating the root mean squared value of the CEA across electrodes (Lehmann & Skrandies, 1980). The GMFA was used to calculate in order to capture the N100 regardless of which electrodes it may occur over.

Chapter 3

Results

3.1 The Effect of Alcohol Intoxication on Neuroplasticity in the Motor Cortex

3.1.1 Subject Demographic

Eleven males and four females participated in the study. Subjects reported having three heavy drinking episodes on average in the last month. Subjects reported speaking an average of 2 languages and had an average of 16 years of education. They reported consuming an average of 30 standard drinks in the last month. Subject demographics are presented in Table 1.

Table 1 Subject Demographics

	Mean ± 1 Standard Deviation (SD)
Age	33.07 ± 7.42
Sex	11 males; 4 female
Average # of years of education	16.33 ± 2.35
Average # of languages spoken	2±1
Mean MMSE score	29.53 ± 0.64
Average # of heavy drinking episodes/month	3±2
Average # of standard drinks/month	30±20

3.1.2 1mV Intensity (% stimulator output)

Paired t-tests were conducted to examine if the alcohol or placebo beverage had an effect on 1mV peak-to-peak intensity between T1 and T2. There was no significant difference between $1mV_{T1}$ and $1mV_{T2}$ for alcohol (t=-1.567; df=14; *p*=0.139) or placebo (t=-2.093; df=14; *p*=0.055) beverage (Table 2).

Table 2 Experimental characteristics for PAS

*The time points T1 and T2 refers to pre and post beverage respectively (refer to Figure 1)

*Values are in Mean ± 1 Standard Deviation (SD)

	Alcohol T1	**Alcohol T2**	**Placebo T1**	**Placebo T2**
Resting motor threshold (% stimulator output)	48±9	--	48±9	--
1mV Intensity (% stimulator output)	60±13	63±15	60±11	61±12
Peripheral nerve sensory threshold (mA)	--	2.1±0.66	--	2.1±0.72
Mean number of sensory stimuli detected (total)/180	--	168±19	--	172±16

3.1.3 Breath Alcohol Concentration

The mean peak blood alcohol concentration (BAC) was 24mM± 3mM (range 21.3mM-33.1mM). BAC was always at 0mM at $SI_{1mVT1\ and}$ peaked during $SI_{1mVT2.}$ BAC remained

above 17.4mM (the legal and binge drinking intoxication level) throughout the PAS administration during the alcohol beverage days. BAC for all participants had returned to 0mM during the Post Day1 (Table 3).

Table 3 Blood Alcohol Concentration. Values are in Means ± 1 Standard Deviation (SD)

	Pre-PAS	**PAS**	**Post 0**	**Post 15**	**Post 30**	**Post 60**	**Post Day 1**
BAC (mM)	24.4±3.1	22.5±3.6	22.1±3.3	22.4±3.4	21.3±4.6	18.9±4.1	0

3.1.4 Attention

Paired t-tests revealed no significant difference in number of sensory stimuli detected during PAS in the alcohol and placebo conditions, suggesting that attention levels were not significantly different between the two conditions (t=0.771; df=14; *p*=0.454; Table 2).

3.1.5 The Effect of Alcohol Intoxication on PAS

The repeated measures ANOVA revealed a significant main effect of beverage (F=6.513; df=1,27; *p*=0.017), reflective of reduced LTP-like neuroplasticity with alcohol compared to placebo. There was no significant main effect of time overall (F=0.169; df=4,108; *p*=0.954), which may be due to the variance between participants in the time for PAS-induced neuroplasticity to peak. However, there was a significant effect of time in the one-way ANOVA of pre and post MEP amplitudes for the placebo beverage (F=3.096;

df=5,60; p=.015), reflective of MEP potentiation in the placebo condition. Post-hoc analyses revealed using paired t-tests revealed a significant effect at Post 30 (t= -3.746; df=14; p=0.002) and Post 60 (t= -3.683; df=14; p=0.002) following correction for multiple comparisons. A significant beverage by time interaction (F=2.906; df=4,108; *p*=0.025) was also found. Post-hoc analyses using paired t-tests revealed a significant effect at Post 30 (t=-3.5475; df=14; p=0.003) and Post 60 (t=-4.852; df=14; p=0.000) following correction for multiple comparisons. The effect sizes for these findings were Cohen's *d*=-1.290 and *d*=-1.460, respectively. No significant difference was observed between alcohol and placebo beverage on Post Day 1 (*Figure 1).*

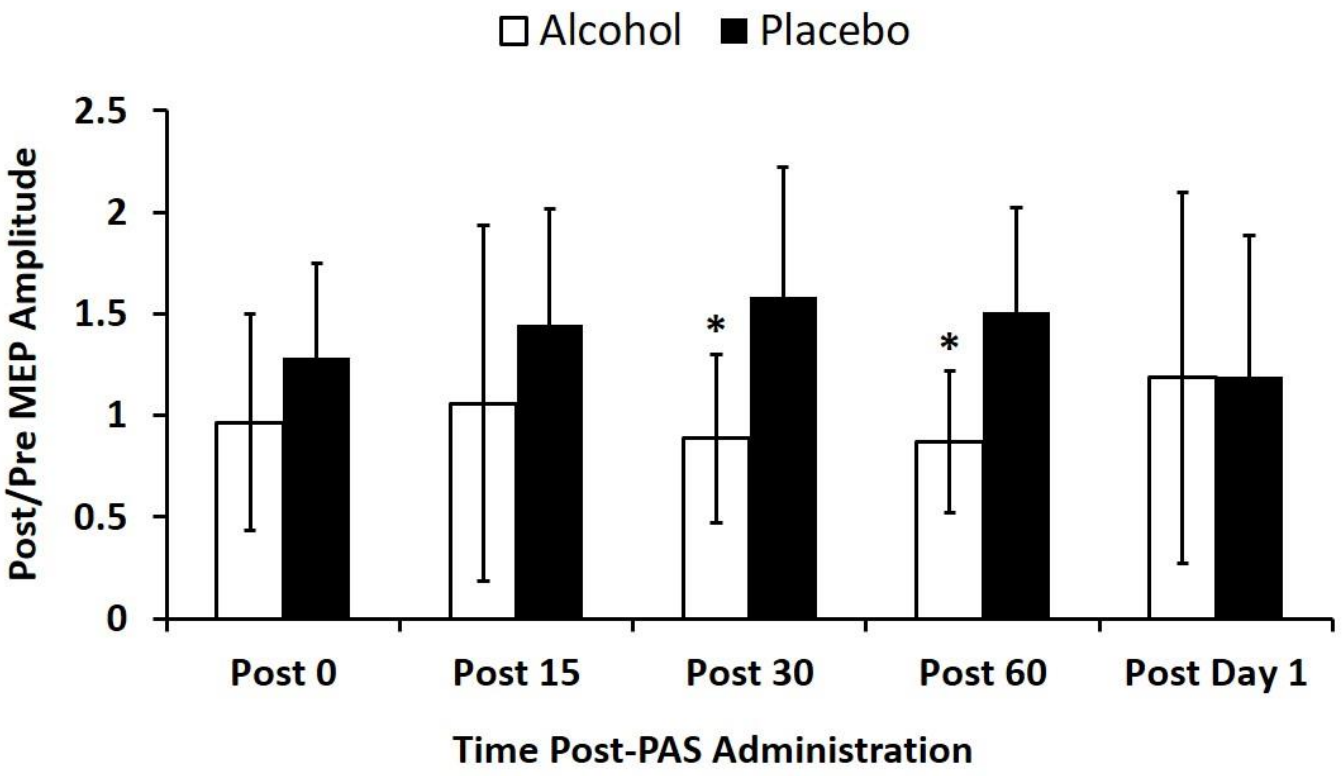


Figure 1 Mean ratio of 20 TMS pulses post-PAS at post 0 min ($SI_{1mV0min}$), post 15 min ($SI_{1mV15min}$), post 30 min ($SI_{1mV30min}$), post 60 min ($SI_{1mV60min}$) and the next day ($SI_{1mVDay1}$) to 20 TMS pulses pre-PAS for the alcohol (white bar) and placebo (black bar) conditions (n=15). Error bars represent the standard deviations. Alcohol significantly impaired PAS-induced neuroplasticity, particularly at 30 minutes and 60 minutes following PAS compared to placebo. There was no effect of alcohol on PAS the next day.

3.2 The Effect of Alcohol Intoxication on Neuroplasticity in the Dorsolateral Prefrontal Cortex

3.2.1 Subject Demographics

Ten males and five females participated in the study. Subjects reported having four heavy drinking episodes on average in the last month. Subjects reported speaking an average of 2 languages and had an average of 16 years of education. They reported consuming an average of 44 standard drinks in the last month. Subject demographics are presented in Table 4.

Table 4 Subject Demographics

	Mean ± 1 Standard Deviation (SD)
Age	32.60±7.79
Sex	10 males; 5 females
Average # of years of education	16.13±1.92
Average # of languages spoken	2±1
Mean MMSE score	29.33±0.98
Average # of heavy drinking episodes/month	4.53±4.91
Average # of standard drinks/month	44.10±30.27

3.2.2 1mV Intensity (% stimulator output)

Paired t-tests were conducted to examine if the alcohol or placebo beverage had an effect on 1mV peak-to-peak intensity between T1 and T2. There was no significant difference between $1mV_{T1}$ and $1mV_{T2}$ for placebo (t=-1.740; df=14; p=0.104) or alcohol (t=-0.893;

df=14; *p*=0.387) beverage (Table 5), suggesting that neither alcohol nor placebo beverage has an effect on corticospinal excitability at baseline.

Table 5 Experimental characteristics for PAS

	Alcohol T1	**Alcohol T2**	**Placebo T1**	**Placebo T2**
Resting motor threshold (% stimulator output)	58±8	--	59±8	--
1mV Intensity (% stimulator output)	71±12	72±14	71±11	71±11
Peripheral nerve sensory threshold (mA)	--	2.6±0.92	--	2.2±0.83
Mean number of sensory stimuli detected (total)/180	--	174±11	--	169±19

3.2.3 Breath Alcohol Concentration

The mean peak blood alcohol concentration (BAC) was 23.6 mM± 4.1 mM (range 18.5 mM-34.2 mM). BAC was always at 0 mM at $SI_{1mVT1\ and}$ peaked during T2. BAC remained above 17.4mM during PAS (the legal and binge drinking intoxication level), with the exception of two subjects who were just below this level during PAS administration (Table 6).

Table 6 Blood alcohol concentration. Values are in Means ± 1 Standard Deviation (SD)

	Before PAS	**PAS**	**Post 0**	**Post 15**	**Post 30**	**Post 60**
BAC (mM)	21.3±4.6	19.7±3.9	17.2±2.0	16.6±2.7	15.4±2.6	14.1±3.0

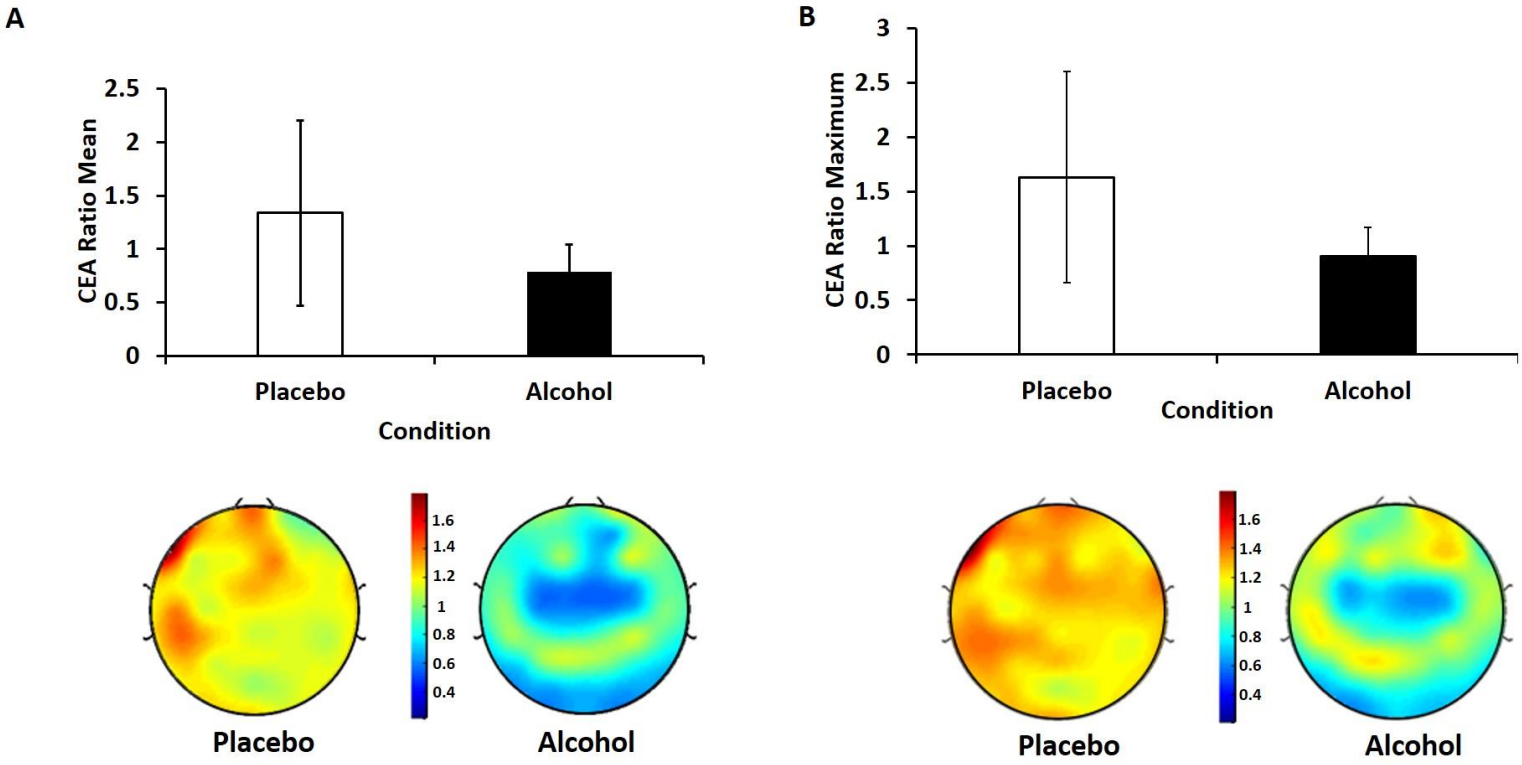
3.2.4 Attention

Attention has been shown to affect neuroplasticity (Stefan et al., 2004). Paired t-tests revealed no significant difference in number of sensory stimuli detected during PAS in the alcohol and placebo conditions, suggesting that attention levels were not significantly different between the two conditions (t=-0.946;df=14; p=0.360).

3.2.5 Effect of Alcohol Intoxication on Neuroplasticity in the DLPFC

The repeated measures ANOVA revealed a main effect of beverage (F=6.034; df=1,13, p=0.029), reflective of decreased PAS-induced neuroplasticity in the DLPFC with alcohol, compared to placebo. The single alcohol drinking episode resulted in a significant impairment of the mean potentiation compared to placebo beverage (t=2.456, df=13, p=0.029) in the DLPFC (Figure 4). A single drinking episode also resulted in impaired peak PAS-induced neuroplasticity in the DLPFC compared to the placebo beverage, as indexed using the mean maximum CEA ratio in the DLPFC (t=-2.945, df=13, p=0.011;

Figure 2). Alcohol intoxication significantly impaired mean (t=-3.051, df=13, p=0.009) and maximum DLPFC PAS-induced neuroplasticity globally (t=-3.260, df=13, p=0.006). A one sample t-test confirmed that potentiation occurred under the placebo condition, as the mean maximum CEA ratio was significantly greater than 1 in the DLPFC (t=2.432,



df=13, p=0.30) and globally (t=2.325, df=13, p=0.037).

Figure 2a) Mean ratio of 100 TMS pulses to the DLPFC across all post-PAS timepoints (Post 0 min, Post 15 min, Post 30 min, Post 60 min) to 100 TMS pulses to the DLPFC pre-PAS for the placebo (white bar) and alcohol (black bar) conditions (n=14). Error bars represent the standard deviations. Alcohol significantly impaired mean PAS-induced neuroplasticity. *b)* Mean ratio of 100 TMS pulses to the DLPFC at time point of maximum

potentiation compared to 100 TMS pulses to the DLPFC pre-PAS for the alcohol (black bar) and placebo (white bar) conditions (n=14). Error bars represent the standard deviations. Alcohol significantly impaired maximal PAS-induced neuroplasticity. The panels on the bottom represents average topoplots of alcohol compared to placebo, with hotter colours representing greater CEA following PAS.

3.2.6 Alcohol's Effects on θ-γ Coupling

The repeated measures ANOVA revealed a significant effect of time (PrePAS vs max potentiation time Post-PAS), reflective of increased θ-γ coupling following PAS ($F=7.516$, $df=1,14$, $p=0.016$). Post-hoc analyses revealed that PAS to the DLPFC resulted in an increase in MI, indicating increased θ-γ coupling during the placebo visit ($t=2.954$, $df=14$, $p=0.010$). This significant increase following PAS was not observed during the alcohol visit ($t=1.486$, $df=14$, $p=0.159$; Figure 3).

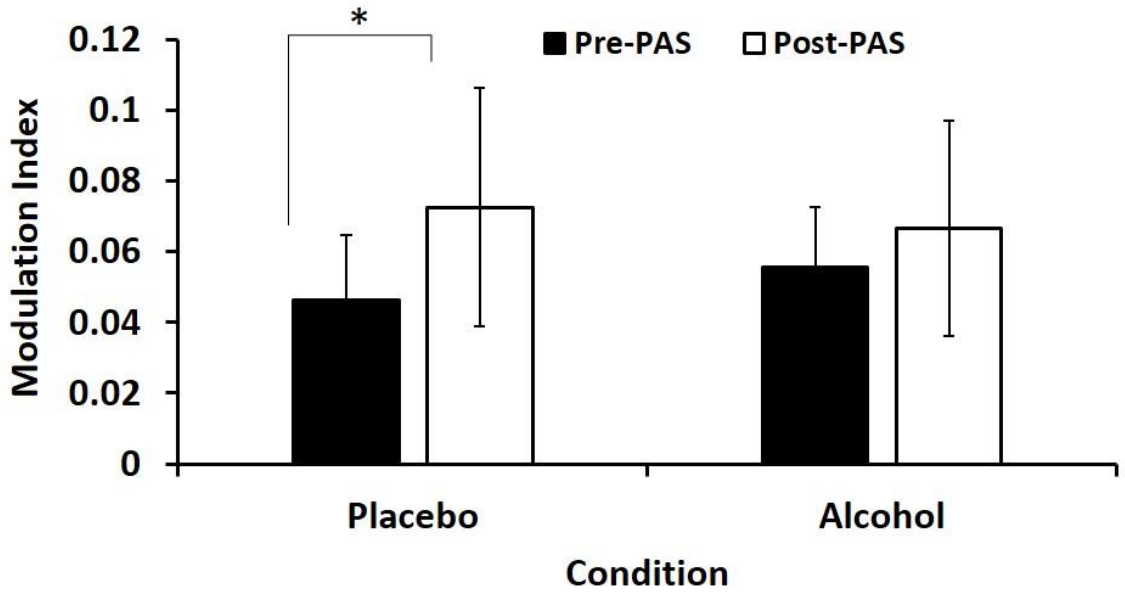


Figure 3 θ-γ coupling is indexed through the modulation index (MI). There was a significant increase in MI following PAS with placebo beverage. This significant increase in MI was not observed following PAS with the alcohol beverage. Error bars represent the standard deviations.

3.3 Effect of Alcohol Intoxication on N100 Amplitude

3.3.1 Subject Demographics

Ten males and five females participated in the study. Subjects reported having four heavy drinking episodes on average in the last month. Subjects reported speaking an average of 2 languages and had an average of 16 years of education. They reported consuming an average of 44 standard drinks in the last month. Subject demographics are presented in Table 7.

Table 7 *Subject Demographics*

	Mean ± 1 Standard Deviation (SD)
Age	32.60±7.79
Sex	10 males; 5 females
Average # of years of education	16.13±1.92
Average # of languages spoken	2±1
Mean MMSE score	29.33±0.98
Average # of heavy drinking episodes/month	4.53±4.91
Average # of standard drinks/month	44.10±30.27

3.3.2 1mV Intensity (% stimulator output)

Paired t-tests were conducted to examine if the alcohol or placebo beverage had an effect on 1mV peak-to-peak intensity between PreBev and PostBev. There was no significant difference between $1mV_{T1}$ and $1mV_{T2}$ for placebo (t=-1.740; df=14; p=0.104) or alcohol (t=-0.893; df=14; p=0.387) beverage, suggesting that neither alcohol nor placebo beverage has an effect on corticospinal excitability (Table 8).

Table 8 Experimental Characteristics of PAS

	Alcohol T1	Alcohol T2	Placebo T1	Placebo T2
Resting motor threshold (% stimulator output)	58±8	--	59±8	--
1mV Intensity (% stimulator output)	71±12	72±14	71±11	71±11
Peripheral nerve sensory threshold (mA)	--	2.6±0.92	--	2.2±0.83
Mean number of sensory stimuli detected (total)/180	--	174±11	--	169±19

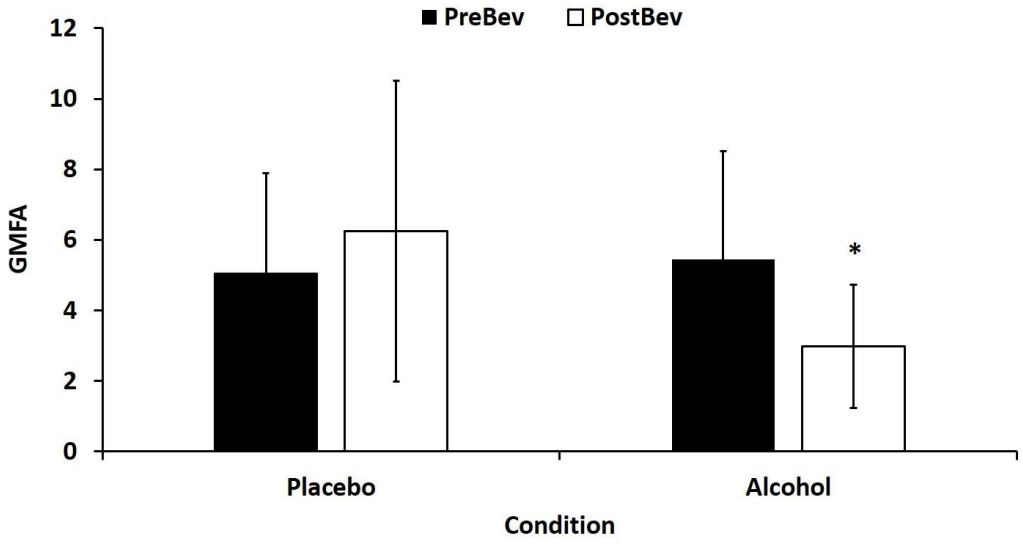
3.3.3 Breath Alcohol Concentration

The mean peak BAC was 23.6 mM± 4.1 mM (range 18.5 mM-34.2 mM). BAC was always at 0 mM at PreBev $_{and}$ peaked during PostBev. BAC remained above 17.4mM (the legal intoxication level) throughout PostBev.

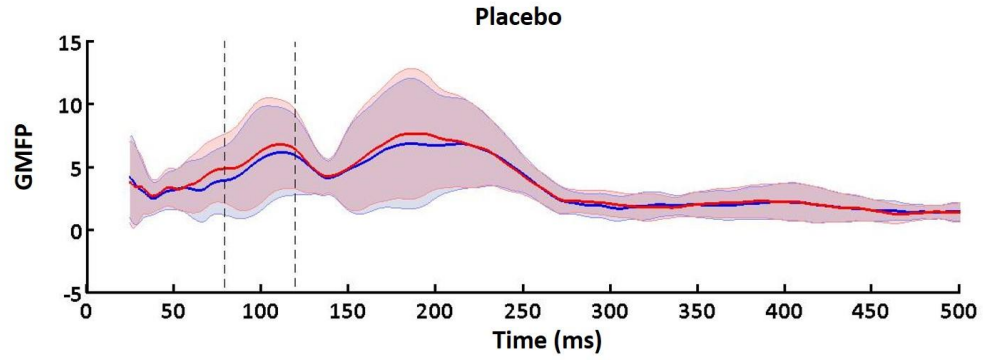
3.3.4 Effect of Alcohol Intoxication on N100 Amplitude

The repeated measures ANOVA revealed a beverage by time interaction ($F=26.60$; $df=1,14$; $p<0.001$). Post hoc analyses revealed that alcohol produced a significant decrease in N100 amplitude indicating reduced N100 amplitude at PostBev compared to PreBev ($t=4.316$, $df=14$, $p=0.001$). There was no significant time effect with the placebo beverage ($t=-1.856$, $df=14$, $p=0.085$; Figure 4).

A


PreBev
PostBev
GMFA
12
10
8
6
4
2
0
*
Placebo
Alcohol
Condition

B


Placebo
GMFP
15
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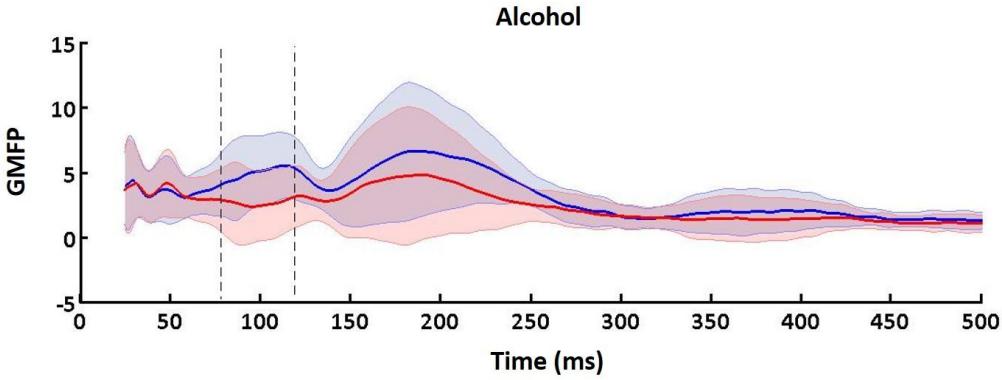
Time (ms)


Alcohol
GMFP
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-5
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150
200
250
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350
400
450
500
Time (ms)

Figure 4 N100 Amplitude. a) Average global mean field amplitude (GMFA) of 100 TMS pulses to the DLPFC before (PreBev) and after (PostBev) the placebo and alcohol beverages (n=15). Error bars represent the standard deviations. Alcohol significantly reduced the mean PAS-induced neuroplasticity. b) Average global mean field potential before (solid green line) and after (solid red line) for placebo and alcohol beverages. The standard deviations are marked with the corresponding dotted lines.

3.3.5 Effect of PAS on Alcohol's Impairment of N100 Amplitude

The repeated measures ANOVA comparing N100 before PAS (PreBev) to after PAS (Post 0, Post 15, Post 30, Post 60) revealed a significant effect of beverage ($F=8.677$; $df=1,13$; $p=0.011$) and a significant beverage by time interaction ($F=11.53$; $df=1,13$; $p<0.001$). Post hoc analyses revealed that there was a significant difference in N100 amplitude at Post0 ($t=4.212$; $df=14$; $p=0.001$), Post 15 ($t=4.182$; $df=14$, $p=0.001$), Post 30 ($t=4.371$; $df=14$; $p=0.001$) and Post 60 (4.138; $df=14$; $p=0.001$) compared to PrePAS in the alcohol condition. In contrast, there was no significant difference at Post 0 ($t=-1.271$; $df=14$; $p=0.224$), Post 15 ($t=-0.297$, $df=14$, $p=0.771$), Post 30 ($t=-0.729$; $df=14$; $p=0.478$) and Post 60 ($t=-0.360$; $df=14$, $p=0.724$) with the placebo condition (Figure 5).

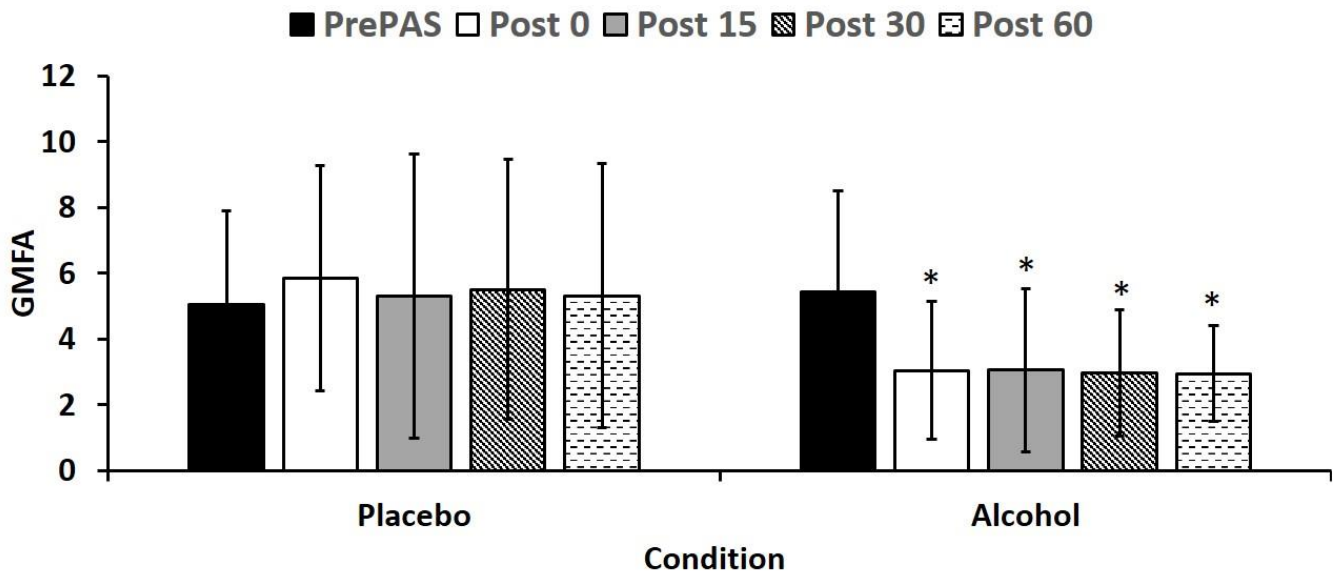

Figure 5 N100 Amplitude. *a)* Average global mean field amplitude (GMFA) of 100 TMS pulses to the DLPFC before (PrePAS) and after PAS (Post 0, Post 15, Post 30, Post 60) under the alcohol and placebo conditions (n=15). Error bars represent the standard deviations. PAS had no effect on N100 amplitude under the placebo condition. N100 remained reduced following PAS in the alcohol condition, suggesting that PAS did not counteract alcohol's impairing effects on the N100.

Chapter 4

Discussion

4.1 Summary of Thesis Findings

The findings described in this doctoral thesis demonstrated three main findings: 1) alcohol intoxication impairs PAS-induced neuroplasticity in the motor cortex; 2) alcohol intoxication impairs PAS-induced neuroplasticity in the dorsolateral prefrontal cortex; and 3) alcohol intoxication produces a decrease in the N100 amplitude, a marker of $GABA_B$ receptor mediated neurotransmission.

Study 1 examined the effect of acute alcohol intoxication (at a dose that constitutes a binge) on PAS-induced neuroplasticity in the motor cortex using PAS with EMG. The effects of alcohol intoxication were examined up to the day following PAS. An impairment of PAS-induced neuroplasticity in the motor cortex was observed up to 60 minutes following PAS. However, no effect of acute alcohol intoxication on PAS-induced neuroplasticity in the motor cortex was observed ~24 hours following PAS. The results of the study demonstrated that acute consumption of alcohol impairs neuroplasticity in the motor cortex. These findings provided a potential mechanism by which alcohol may produce its motor impairing effects on motor learning and memory. The results from Study 1 raised two important questions. Firstly, does acute consumption of alcohol also impair neuroplasticity in the DLPFC, a brain region that is known to play an important

role in cognition? Secondly, what neurophysiological mechanisms may underlie alcohol's impairing effects on neuroplasticity? While findings from previous studies have suggested that alcohol's impairment of NMDA receptor mediated neurotransmission and $GABA_A$ receptor mediated neurotransmission are linked to alcohol's impairing effects on neuroplasticity, less is known about alcohols effects on $GABA_B$ receptor mediated neurotransmission. Baclofen, a $GABA_B$ receptor agonist, has demonstrated some promise as a treatment for alcohol dependence (Saults, et al. 2007), suggesting that the $GABA_B$ receptor may mediate some of alcohol's effects. For this reason, we focused on the role of $GABA_B$ receptor mediated neurotransmission in Study 3. To answer these two questions we indexed neuroplasticity from the DLPFC with PAS-EEG in Study 2 and examined the effect of acute alcohol consumption on the N100 component associated with GABAB activity in Study 3.

In Study 2, the effect of acute alcohol consumption on PAS-induced neuroplasticity in the DLPFC was assessed. Additionally, the effect of acute alcohol consumption on θ-γ coupling, a measure associated with cognitive function, was examined. A previous study has demonstrated that neuroplasticity in the DLPFC can be indexed using PAS with EEG. Additionally, this study demonstrated that θ-γ coupling is increased following administration of PAS to the DLPFC (Rajji et al., 2013). It was hypothesized that acute alcohol consumption would impair neuroplasticity in the DLPFC. Additionally, it was hypothesized that the increase in θ-γ coupling seen following PAS would be attenuated following alcohol consumption. Findings from Study 2 demonstrated that alcohol consumption produces an impairment in neuroplasticity both in the DLPFC and globally

compared to the placebo beverage. Furthermore, while an increase in θ-γ coupling was observed following PAS with the placebo beverage, this potentiation of θ-γ coupling was not observed following PAS with the alcohol beverage. Results from this study demonstrated that alcohol impairs neuroplasticity in the DLPFC and that it may be acting on the same neuronal networks involved in cognitive function.

Lastly, the neurophysiological mechanisms that may underlie alcohol's impairment of neuroplasticity in the human cortex were explored. Results from multiple lines of investigation suggest that alcohol's impairment of neuroplasticity is mediated by alcohol's impairment of excitatory glutamatergic neurotransmission and inhibitory GABAergic neurotransmission (Morrisett & Swartzwelder, 1993; Schummers et al., 1997; Schummers & Browning, 2001; Ziemann et al., 1995). he N100 is the negative peak that occurs ~100ms following the TMS pulse and is the largest component in the EEG (Nikouline et al., 1999; Paus et al., 2001). Findings from numerous studies suggest that the N100 is a measure of $GABA_B$ receptor mediated inhibition (Nikulin et al., 2003; Rogasch et al., 2013; Rogasch & Fitzgerald, 2013). The aim of Study 3 was to examine the effect of acute alcohol consumption on the N100 amplitude in response to TMS stimulation of the DLPFC. It was hypothesized that acute alcohol consumption would produce a decrease in N100 amplitude. Findings from Study 3 demonstrated that acute alcohol consumption attenuated the N100 amplitude, suggesting that alcohol produces a decrease in $GABA_B$ receptor mediated neurotransmission. The N100 may serve as a marker of alcohol's effects on inhibitory neurotransmission.

4.1.1 Alcohol's Impairment of Neuroplasticity in the Motor Cortex

Alcohol intoxication was found to significantly impair LTP-like neuroplasticity in the motor cortex. Moreover, this effect was found to be significantly different at 30 and 60 minutes post PAS. These findings are consistent with Lucke et al who demonstrated reduced LTP-like neuroplasticity with two lower doses of alcohol (<5mM and <20mM) up to 30 minutes post PAS. However, this study did not test the effects of alcohol administration longitudinally. That is, Study 1 demonstrates the effect of alcohol intoxication on LTP-like neuroplasticity up to 60 minutes post PAS. Taken together, findings from Study 1 suggest that alcohol impairs PAS-induced neuroplasticity across a large dosage range (<5mM - >20mM). It is unknown, however, if consumption of high doses of alcohol results in a greater magnitude of impaired neuroplasticity. Future studies may wish to further evaluate the effect of different doses on the time course and extent of LTP-like neuroplasticity, which may be linked to conversion of social drinking to AUDs.

Alcohol intoxication did not have a residual effect on PAS-induced neuroplasticity in the motor cortex the day following PAS administration. This demonstrates that alcohol intoxication does not have lingering effects on MEP potentiation the next day. It is not yet clear how this relates to learning and memory the day following alcohol intoxication, as LTP is thought to be the cellular basis underlying these processes (Lisman, Lichtman, & Sanes, 2003; Malenka & Bear, 2004). Some previous studies have reported impairment in aspects of neurocognition the day following alcohol intoxication (Howland et al., 2010; Kim, Yoon, Lee, Choi, & Go, 2003; McKinney, Coyle, & Verster, 2012; Takala, Siro, &

Toivainen, 1958; Verster, van Duin, Volkerts, Schreuder, & Verbaten, 2003), while other reports have found no such effects (Howland et al., 2010; Lemon, Chesher, Fox, Greeley, & Nabke, 1993). It is possible that while MEP potentiation is no longer affected the next day, there may be other downstream changes (i.e., synaptic/structural) that can lead to impairments in learning and memory. This notion is supported by findings from Rajji et al., 2011 (Rajji et al., 2011). While PAS-induced neuroplasticity returns to baseline the day following PAS (Stefan et al., 2000), PAS-induced enhancement of motor learning was evident up to a week following PAS administration among healthy subjects. These findings suggest that PAS and motor learning may share common mechanisms but normalized potentiation is not synonymous with normalized performance, as there may be other downstream residual effects that are not captured by indexing PAS-induced neuroplasticity. Further studies examining PAS-induced neuroplasticity along with other measures of motor learning (such as the rotary pursuit task) are necessary to delineate if normalized PAS-induced neuroplasticity correlates with normalized learning and memory the day following alcohol intoxication. Furthermore, while PAS-induced potentiation is no longer affected the day following alcohol intoxication, it is possible that potential next-day effects on learning and memory may be mediated by metaplasticity. Metaplasticity is the process by which activity dependent alterations in neural function modulate subsequent neuroplasticity (Abraham & Bear, 1996a). Along these lines, impairment of neuroplasticity by alcohol intoxication may lead to changes in neuroplasticity induced the day after alcohol intoxication. Future studies may explore the effect of alcohol intoxication on LTP-like neuroplasticity induced by PAS conducted the next day to

examine the potential role in metaplasticity on the long-lasting effects of alcohol intoxication on learning and memory.

4.1.2 Alcohol's Impairment of Neuroplasticity in the DLPFC

Intoxication by alcohol impaired PAS-induced neuroplasticity in the left DLPFC and globally. Previously, it has been demonstrated that a single heavy drinking episode (Loheswaran et al., 2015) and even low doses of alcohol (Lucke et al., 2014a) impair PAS-induced neuroplasticity in the motor cortex. Using DLPFC PAS and EEG, findings from Study 2 demonstrate that this impairment of neuroplasticity by alcohol intoxication occurs in DLPFC. Interestingly, the impairment in neuroplasticity induced by alcohol was not just localized to the DLPFC. Rather, we also observed a global impairment of neuroplasticity. Global ERP deficits have been reported in binge drinkers compared to non-drinkers during basic and high level cognitive states (Maurage et al., 2012). Global aberrancies in neuronal responses may highlight neural inefficiencies in heavy drinkers (as subjects in the study reported at least one previous binge drinking episode in the last month) that contribute to a less localized induction of neuroplasticity in this population. Induction of less localized neuroplasticity in this population along with alcohol's widespread action on the brain (Nevo & Hamon, 1995) may explain the global impairment of neuroplasticity seen in the present study.

4.1.3 Alcohol's Impairment of θ-γ Coupling

An increase in θ-γ coupling in the DLPFC following PAS with placebo beverage was observed Study 2. These findings are consistent with Rajji et al., 2013 who demonstrated that PAS potentiated θ-γ coupling in the DLPFC among healthy subjects. Interestingly, this increase in θ-γ coupling following PAS was not observed with alcohol. θ-γ coupling is believed to be an index of DLPFC functioning, specifically working memory (Canolty & Knight, 2010; Lisman & Idiart, 1995). θ-γ coupling has been shown to increase during trials that required ordering information compared to trials that do not require ordering (Rajji et al., 2013). Previous studies have demonstrated that acute alcohol intoxication produces an impairment of working memory across several domains (Grattan-Miscio & Vogel-Sprott, 2005; Saults et al., 2007; Schweizer & Vogel-Sprott, 2008). Increased θ-γ coupling following PAS observed in Study 2 is speculated to be due to PAS activating the same neuronal networks in the DLPFC involved in working memory (Rajji et al., 2013). Therefore, alcohol's impairment of PAS induced potentiation of θ-γ coupling may be associated with alcohol's effects on neuroplasticity in neuronal networks involved in learning and memory.

4.1.4 Effect of Alcohol on N100 Response to TMS Stimulation of DLPFC

Findings from Study 3 demonstrated that alcohol produces a significant decrease in the N100 response to DLPFC TMS stimulation. These results are in accordance with a previous study that examined the effect of alcohol consumption (at a dose of 0.8kg/kg) on

motor cortex TMS stimulation induced N100 response (Kahkonen & Wilenius, 2007). The authors found that the N100 amplitude was reduced in ten electrodes with the most pronounced N100 component (based on visual assessment) following alcohol consumption. Study 3 demonstrated that alcohol also decreases the N100 amplitude in response to TMS of the DLPFC. This suggests that alcohol has a suppressive effect on the DLPFC similar to that previously observed in the motor cortex. Given that the N100 component is observed centrally (Hine, Thornton, Davis, & Debener, 2008; Kahkonen & Wilenius, 2007), these findings suggest that alcohol may interfere with connectivity between the DLPFC and other regions of the cortex. A disruption of connectivity between the DLPFC and other regions of the cortex may underlie the loss of inhibitory control from the frontal cortex observed during alcohol intoxication (Loeber & Duka, 2009a, 2009b).

The reduction of N100 amplitude by alcohol, suggesting a decrease in $GABA_B$ receptor neurotransmission, may be difficult to reconcile at first with the commonly accepted notion that alcohol produces an increase in GABAergic neurotransmission (Janak & Michael Gill, 2003; Koob, 2006; Liljequist & Engel, 1982; Sundstrom-Poromaa et al., 2002). However, while alcohol has been demonstrated to result in an increase in $GABA_A$ receptor mediated neurotransmission (Janak & Michael Gill, 2003; Koob, 2006; Liljequist & Engel, 1982; Sundstrom-Poromaa et al., 2002), its effect on $GABA_B$ receptor mediated neurotransmission is less well understood. Similar to the present study, Kahkonen & Wilenius (2007) reported a decrease in N100 amplitude following acute alcohol consumption (Kahkonen & Wilenius, 2007). Additionally, administration of $GABA_A$

agonists such as alprazolam and diazepam produce a decrease in N100 amplitude, similar to alcohol (Premoli, Castellanos, et al., 2014). It has been argued that the decrease in N100 amplitude produced by $GABA_A$ agonists may be due to inhibition of $GABA_B$ receptor mediated inhibitory post-synaptic potentials in the neocortex and hippocampal pyramidal neurons by $GABA_A$ receptor activation (Lopantsev & Schwartzkroin, 1999; Premoli, Castellanos, et al., 2014; Thomson & Destexhe, 1999). Given that alcohol is also agonistic at the $GABA_A$ receptor, a similar mechanism may underlie the reduction in N100 amplitude by alcohol. Alternatively, alcohol's antagonistic effect at NMDA receptors may contribute to a loss of activation of GABAergic neurons by alcohol and produce a reduction in $GABA_B$ receptor mediated neurotransmission (Giovannini, Mutolo, Bianchi, Michelassi, & Pepeu, 1994).

4.1.5 Effect of DLPFC PAS on N100 Response

The finding that the N100 amplitude was unaffected following PAS to the DLPFC in both conditions demonstrates that PAS cannot be used to counteract the effect of alcohol on the N100. No previous studies have reported the effect of PAS on N100 amplitude. Previous studies have reported that PAS produces a change in TMS measures of $GABA_B$ neurotransmission (Russmann et al., 2009; Stefan et al., 2000). Following motor PAS, Stefan et al., 2000 observed an *increase* in CSP duration, suggesting that PAS produced an increase in $GABA_B$ receptor mediated neurotransmission. In contrary, Russman et al., 2009 reported a *decrease* in LICI following motor PAS, suggesting that PAS produced a decrease in $GABA_B$ receptor mediated neurotransmission. These seemingly contradictory

findings may be attributable to differences in the PAS paradigms used in the two studies. During the PAS intervention, Stefan et al., 2000 used TMS stimulation intensities producing 1mV MEPS while Russman et al., 2009 used TMS intensities producing 0.5MmV MEPs. However, a number of studies have reported a dissociation between LICI and CSP findings (Benwell, Mastaglia, & Thickbroom, 2007; Hammond & Vallence, 2007; McDonnell et al., 2006).

There are a number of possibilities that may account for the PAS's lack of effect on N100. Firstly, in the current study, PAS was administered to the DLPFC, while in previous studies PAS was administered to the motor cortex. Thus, it is likely that differing neurocircuits were involved. Secondly, in the previous studies, LICI and CSP were indexed from the periphery through EMG while the present study involved direct measurement of N100 from the cortex. Future studies examining the effect of N100 amplitude following both motor and DLPFC PAS are required to confirm whether motor and DLPFC PAS have similar, if any, effect on N100 amplitude. PAS-induced potentiation may be reflected in an increase in other TEP components. For example, PAS may be associated with an increase in amplitude of the N45 or P60, TEP components implicated with $GABA_A$ and glutamatergic neurotransmission respectively (Cash et al., 2016; Premoli, Castellanos, et al., 2014). Future studies are required to identify the TEP components that are associated with DLPFC PAS-induced potentiation.

4.1.6 Neuroplasticity in the Motor Cortex: Implications for Motor Learning

Findings from Study 1 demonstrated that acute alcohol consumption produces an impairment of PAS-induced neuroplasticity in the motor cortex. Impairments in motor function and motor learning are commonly observed during alcohol intoxication. Difficulty in motor coordination is reported to increase with BAC (Tagawa et al., 2000). Similarly, a deterioration of performance on the rotary pursuit task has been reported at higher BAC levels (~0.05%BAC) (Reilly & Scott, 1993). The rotary pursuit task is a paradigm that measures motor learning and hand eye coordination. Rajji et al., 2011 reported that performance on the rotary pursuit task is improved 45 minutes and 1 week following PAS to the motor cortex compared to the control condition of control PAS (PAS-10) (Rajji et al., 2011). Similarly, learning of simple finger movements is improved following PAS to the motor cortex (Jung & Ziemann, 2009). The enhancement of performance in the motor learning following PAS suggests that PAS acts on neuroplasticity of the neuronal circuits recruited involved in motor learning. Demonstration of alcohol's impairment of PAS-induced neuroplasticity in the motor cortex in Study 1 provides a potential mechanism for alcohol's adverse effects on motor learning.

4.1.7 The Role of Neuroplasticity in Cognition

Findings from Study 2 demonstrated that acute alcohol consumption impairs neuroplasticity in the DLPFC. The DLPFC is a key brain region involved in cognition and executive function (Owen et al., 2005). Neuroplasticity in this region is likely to play an important role in cognitive functioning. This notion is supported by findings that cognitive function can be modulated by the administration of number of neuroplasticity-inducing non-invasive brain stimulation paradigms to the DLPFC, including tDCS, transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) (Brunoni & Vanderhasselt, 2014; Kuo & Nitsche, 2012; Kuo, Paulus, & Nitsche, 2014; Snowball et al., 2013). Rajji et al., 2013 reported that PAS to the DLPFC is associated with a potentiation of coupling of the θ and γ frequency bands (Rajji et al., 2013). θ-γ coupling has been demonstrated to increase with working memory load (Axmacher, Lenz, Haupt, Elger, & Fell, 2010; J. Y. Park, Jhung, Lee, & An, 2013). It has been hypothesized that specific information is represented in every γ oscillation that is coupled to a particular phase of the θ cycle. Additionally, the ordering of information is believed to be represented in the order of various γ oscillations (Canolty & Knight, 2010; Lisman & Idiart, 1995). The finding that PAS administration to the DLPFC produces an increase in θ-γ coupling suggests that PAS produces an activation of the neuronal networks involved in working memory (Rajji et al., 2013). Similar to the finding reported by Rajji et al., 2013, an increase in θ-γ coupling following PAS administration to the DLPFC was observed with placebo beverage in Study 2. However, this increase in θ-γ coupling was not observed with DLPFC PAS following acute alcohol consumption. It is well known

that consumption of alcohol is associated with deterioration in performance over a number of cognitive domains (Fillmore, 2007; Holloway, 1995; Moscowitz & Fiorentino, 2000) including working memory (Dry, Burns, Nettelbeck, Farquharson, & White, 2012; Gundersen, Gruner, Specht, & Hugdahl, 2008). The absence of an increase in θ-γ coupling following PAS during acute alcohol intoxication suggests that alcohol disrupts neuroplasticity and potentiation of the neuronal networks involved in working memory. Disruption of neuroplasticity and potentiation of θ-γ coupling provides a potential mechanism by which acute alcohol consumption impairs cognition.

4.1.8 The Effect of Alcohol on Cortical Inhibition

Findings from Study 3 demonstrated that acute alcohol consumption produces a reduction in $GABA_B$ receptor mediated neurotransmission. The effect of acute alcohol consumption $GABA_B$ receptor mediated neurotransmission is less clearly understood than the effects of alcohol on NMDA and $GABA_A$ receptor mediated neurotransmission. Ziemann et al., 1995 reported that acute consumption of alcohol produced an increase in CSP duration, suggesting an increase in $GABA_B$. This finding may at first be seemingly difficult to reconcile with our finding of decreased $GABA_B$ receptor mediated neurotransmission following acute alcohol consumption. However, it has been argued that CSP and LICI are not measures of the same processes (McDonnell et al., 2006). LICI is a measure of the *magnitude* of inhibition while CSP is a measure of the *duration* of inhibition (Tergau et al., 1999). It has been demonstrated that the early portion of the CSP is due to spinal mechanisms while the late portion is due to cortical inhibitory processes (Chen, Lozano, &

Ashby, 1999). Therefore, it is hard to distinguish whether the increase in the CSP duration produced by alcohol is due to alcohol's effects on cortical $GABA_B$ receptor mediated inhibitory processes, due to its effects on $GABA_B$ receptor mediated neurotransmission at the spinal level or both. Findings from Study 3 suggest that at the level of the cortex, alcohol decreases in $GABA_B$ receptor mediated neurotransmission. These findings are in accordance with findings from Kahkonen et al., demonstrating a decrease in N100 response (Kahkonen & Wilenius, 2007). Interestingly, other drugs that increase $GABA_A$ receptor mediated neurotransmission, such as benzodiazepines, have been shown to decrease the N100 amplitude (Premoli, Castellanos, et al., 2014). It has been proposed that the decrease in N100 amplitude by $GABA_A$ agonists is due to inhibition of $GABA_B$ receptor mediated post-synaptic potentials in the cortex by activation of $GABA_A$ (Lopantsev & Schwartzkroin, 1999; Premoli, Castellanos, et al., 2014; Thomson & Destexhe, 1999). The modulation of $GABA_B$ inhibitory post synaptic potentials by the voltage-dependent chloride influx through $GABA_A$ receptors has been demonstrated in hippocampal CA3 pyramidal cells and the neocortex (Lopantsev & Schwartzkroin, 1999; Premoli, Castellanos, et al., 2014; Thomson & Destexhe, 1999). A similar mechanism may account for the reduction in $GABA_B$ receptor mediated neurotransmission in the present study.

4.1.9 Functional Consequence of Alcohol on Neuroplasticity

Findings from Study 1 and Study 2 demonstrated that acute alcohol intoxication impairs neuroplasticity throughout the cortex. The mechanisms underlying alcohol's disruption of neuroplasticity are not yet completely understood. The contribution of NMDA, $GABA_A$ and $GABA_B$ receptor mediated neurotransmission to alcohol's effects on neuroplasticity are explored below.

The NMDA receptor is thought to play an essential role in LTP (Collingridge, Kehl, & McLennan, 1983). The NMDA antagonist (2R)-amino-5-phosphonovaleric acid (APV) blocks LTP in rat hippocampal and CA1 brain slices (Collingridge et al., 1983). Alcohol has been shown to inhibit NMDA-activated ion currents in voltage clamped sensory neurons (Lovinger, White, & Weight, 1989). However, it has been reported that alcohol's antagonistic effect at the NMDA receptor accounts for most, but not all, of alcohol's disruption of LTP (Schummers et al., 1997). Schummers et al., 1997 administered 5µM of ketamine to rat hippocampal sections. 5µM of ketamine produces the same level of inhibition of NMDA receptors as does 100mM of alcohol. However, while 100mM of alcohol completed abolished LTP induction, 5 µM of ketamine did not completely abolish LTP induction. Therefore, alcohol's antagonism of the NMDA receptor does not account for the complete abolishment of LTP by alcohol. It has been hypothesized that alcohol's impairment of LTP may be mediated by its effects on $GABA_A$ receptor mediated neurotransmission in addition to its direct antagonism of the NMDA receptor (Schummers et al., 1997). Further evidence in support of this theory was provided by an *in vitro*

experiment conducted in the CA1 region of the rat hippocampus. Alcohol's inhibition of the NMDA receptor is decreased when $GABA_A$ receptors are blocked by $GABA_A$ antagonist picrotoxin (Schummers & Browning, 2001). Given that the NMDA receptor plays an important role in LTP, alcohol's direct and indirect antagonism (via potentiation of $GABA_A$ receptor mediated neurotransmission) of the NMDA receptor likely contributes to alcohol's impairment of neuroplasticity.

Findings from brain stimulation studies also support the notion that alcohol's impairment of neuroplasticity is mediated by its antagonistic effect on NMDA receptor mediated neurotransmission and potentiating effect on $GABA_A$ receptor mediated neurotransmission (Lucke et al., 2014b; Schummers et al., 1997; Schummers & Browning, 2001; Ziemann et al., 1995). For example, Ziemann et al., 1995 found that alcohol consumption (producing a mean BAC of 13.9mM) resulted in an increase of TMS indices of $GABA_A$ and $GABA_B$ receptor mediated inhibition and a decrease in TMS indices of NMDA receptor mediated excitability among healthy subjects (Ziemann et al., 1995). It follows that Lucke et al., 2014 found that alcohol (<5mM and <20mM) produced an impairment in PAS-induced neuroplasticity, with no effect on saccadic peak velocity, a biomarker of α1-$GABA_A$ receptor mediated sedation (Lucke et al., 2014b). These findings suggest that alcohol does not exert its effect at this particular subunit and that the effects are likely mediated through other $GABA_A$ receptor subunits (i.e., α4β3δ and α6β3δ subunits) uniquely sensitive to alcohol (Sundstrom-Poromaa et al., 2002; Wallner, Hanchar, & Olsen, 2003). Producing an increase in GABAergic neurotransmission through pharmacological intervention (i.e., benzodiazepine administration) has been

shown to produce a decrease in both practice-dependent and PAS-induced LTP (Heidegger et al., 2010). Similarly, the increase in GABAergic neurotransmission by alcohol is likely to also contribute to alcohol's impairment of neurotransmission. Together, these findings suggest that both NMDA receptors and $GABA_A$ receptors are necessary for the complete reduction of neuroplasticity by alcohol.

Study 3 demonstrated that alcohol produces a reduction in the N100 in response to TMS stimulation of the DLPFC, suggesting that alcohol produces a decrease in $GABA_B$. Findings from a number of *in vitro* studies suggest that disinhibition by $GABA_B$ receptors is required for the induction of LTP. In a study using 5 Hz stimulation to induce neuroplasticity in hippocampal slices, the LTP-induction was associated with a decrease in $GABA_A$ receptor mediated inhibition. This decrease in $GABA_A$ inhibition was associated with an increase in NMDA-receptor mediated excitatory neurotransmission. Administration of a $GABA_B$ antagonist, 2-OH Saclofen, blocked the induction of LTP, along with blocking the reduction in $GABA_A$ inhibition and the increase in facilitation. Therefore, the reduced $GABA_B$ receptor mediated neurotransmission observed in Study 3 may represent an additional mechanism by which alcohol consumption impairs LTP.

4.1.10 Dysfunctional Neuroplasticity and the Pathophysiology of AUDs

Interestingly, it has been argued that aberrancies in neuroplasticity, related to modifications in the efficacy of glutamatergic neurotransmission, may be a key underlying

factor in alcohol dependence (Chandler, 2003; Jones & Bonci, 2005). There are multiple lines of evidence from animal and *in vitro* studies that suggest that neuroplasticity in the reward brain circuitry can be affected by drugs of abuse (for review, see (Kauer & Malenka, 2007). It is hypothesized that mechanisms of learning and memory play at least a partial role in the development of drug dependence (Guan & Ye, 2010). This has contributed to the notion that addiction may be a harmful, but powerful form of learning and memory (Hyman, Malenka, & Nestler, 2006). Specifically, the molecular and cellular mechanisms such a LTP that underlie associative memories in forebrain circuits that receive input from midbrain dopamine neurons are hypothesized to be major substrates of the compulsive drug use seen in drug dependence (Hyman et al., 2006). A key aspect of drug dependence is the transition from recreational drug use to compulsive drug taking. Neuroplasticity is thought to play a key role in this transition to dependence (Madsen, Brown, & Lawrence, 2012). Additionally, certain genes can produce aberrancies in neuroplasticity that predispose individuals to be susceptible to addiction (Hill, 2010). These genetic predispositions can contribute to an unfortunate cycle in which aberrant neuroplasticity makes individuals susceptible to AUDs, which then further exacerbates such aberrancies.

4.1.10.1 Neuroplasticity Following Chronic Alcohol Exposure in Animal Models

Findings from animal studies suggest that chronic alcohol consumption produces aberrant neuroplasticity in the prefrontal cortex as well as other cortical and subcortical regions

(Jeanes, Buske, & Morrisett, 2011; Kroener et al., 2012; Nimitvilai, Lopez, Mulholland, & Woodward, 2016). Chronic alcohol consumption produces an increase in NR2B subunit-containing NMDA receptors throughout the cortex (Carpenter-Hyland et al., 2004; Hendricson, Sibbald, & Morrisett, 2004). The NR2B containing NMDA receptors have been implicated in synaptic plasticity (Barria & Malinow, 2005; Tang et al., 1999). In C57BL/6 mice, chronic intermittent alcohol exposure results in an increase in the AMPA/NMDA current ratio in layer V of the medial prefrontal cortex (Kroener et al., 2012). This increase was observed both immediately and week following the last alcohol exposure (Kroener et al., 2012). These findings were confirmed by western blot analysis that revealed that there was an increase in NMDA NR1 and NRB subunits while having no change in AMPA GluR1 subunits. Examination of spike-timing dependent LTP in the slice preparation revealed that the chronic alcohol exposure resulted in an aberrant form of enhanced NMDAR-mediated plasticity. These changes were associated with a reduced cognitive flexibility as revealed by a medial prefrontal cortex dependent attentional set-shifting task (Kroener et al., 2012). Similarly, Jeanes et al., 2011 reported that elicitation of alcohol dependence in C57BL/6 mice by chronic intermittent alcohol exposure is associated with a switch from LTD to NMDA receptor-dependent LTP in the NAc shell (Jeanes et al., 2011). Nimitvilai et al., 2016 examined the effects of repeated cycles of chronic intermittent alcohol exposure on synaptic plasticity in the orbitofrontal cortex C57BL/6 mice. It was found that the repeated cycles of chronic intermittent alcohol exposure resulted in LTP of glutamatergic neurotransmission that was persistent following LTP-induction using a spike timing protocol. Following chronic alcohol exposure, action potential spiking was less sensitive to the inhibitor effects of acute alcohol exposure.

These changes were associated with an increase in the AMPA/NMDA receptor ratio (Nimitvilai et al., 2016). Taken together these findings suggest that chronic alcohol exposure in animal models produces an aberrant increase in glutamatergic neuroplasticity. However, the changes in neuroplasticity in alcohol dependent individuals have not yet been evaluated.

4.1.10.2 Hyperplasticity or Hypoplasticity in AUDs?

Aberrancies in neuroplasticity in either direction (i.e., hyperplasticity or hypoplasticity) could contribute to the impairments in behaviour and cognition as seen in alcohol dependence. Understanding the effect of alcohol on the direction of neuroplasticity in the human brain can help us understand how alcohol exposure can lead to dependence. Excessive neuroplasticity or hyperplasticity may be implicated in the transition to alcohol dependence. Enhanced NMDA receptor mediated neurotransmission in alcohol dependence, has been proposed to produce increases in synaptic strength that would contribute to metaplasticity (Clapp et al., 2008). Metaplasticity refers to the phenomena by which the system becomes sensitized to subsequent neuroplasticity processes (Abraham & Bear, 1996b). Through metaplasticity, increased NMDA-receptor mediated glutamatergic neurotransmission during alcohol withdrawal can lead to longer lasting changes in neuroplasticity. In addition to increased LTP, hyperplasticity in alcohol dependent patients may be caused by decreased long-term depression (LTD) in these individuals. A recent study has demonstrated that acute alcohol consumption enhances LTD (Fuhl, Muller-Dahlhaus, Lucke, Toennes, & Ziemann, 2015). Due to counteradaptive changes following

chronic alcohol consumption, alcohol dependent individuals are likely to have decreased LTD. An imbalance in the ratio of LTP to LTD in the brain reward circuitry would contribute to dysfunctional plasticity in this system.

4.1.10.3 Neuroplasticity in the DLPFC in AUDs

The disruption of neuroplasticity in the DLPFC is of particular interest for the development of AUDs. The DLPFC is involved in the addiction pathophysiology owing to its role in the brain reward system as part of the meso-cortico-limbic pathway (Park et al., 2007). The DLPFC is functionally connected to the orbitofrontal cortex, amygdala and hippocampus and plays a role in reward processing and guiding behaviours. It is believed that the DLPFC regulates the integration of goal-motivated behaviour by assimilating information regarding the potential negative and positive outcomes of selecting a behaviour. Therefore, aberrant functioning of the DLPFC can result in the selection of inappropriate behaviours despite their negative consequences, such as compulsive drug taking (Feil & Zangen, 2010). Additionally, alcohol intoxication impairs working memory (Boissoneault, Sklar, Prather, & Nixon, 2014) and patients with AUDs suffer from executive dysfunction across several domains including working memory (for review see (Oscar-Berman, 1990)), which is largely governed by the DLPFC (for review see (Owen et al., 2005)). Importantly, recent evidence suggests that working memory dysfunction may predict abstinence and clinical outcome in patients with AUDs (Charlet et al., 2013). Indirect evidence for impaired neuroplasticity in the DLPFC of alcohol dependent individuals comes from findings of neuromodulatory brain stimulation studies that have

demonstrated that modulation of neuroplasticity in the DLPFC through rTMS shows promise as a treatment of AUDs (De Ridder et al., 2011; Mishra et al., 2010). Identifying the existence, localization and direction of neuroplasticity impairment in the DLPFC of alcohol dependent individuals through PAS with EEG can help inform the future use of neuromodulatory brain stimulation for the treatment of individuals with AUDS.

4.1.11 Relevance of N100 to AUDs

The reduced N100 amplitude caused by alcohol suggests that an impaired N100 may also by affected following chronic alcohol abuse and in individuals who are alcohol dependent. Indeed, previous studies have reported a decrease in N100 amplitude during laboratory cognitive tasks in alcohol dependent individuals compared to healthy controls (Miyazato & Ogura, 1993; Ogura & Miyazato, 1991). Given these findings, the N100 may serve as a useful marker of alcohol dependence. Future studies are required to confirm whether a similar impairment is observed in TMS-evoked N100 response in alcohol dependent individuals and whether treatment for alcohol dependence is associated with regulation of the N100 response.

4.1.12 Proposed Mechanism of How Neuromodulatory Brain Stimulation Can Regulate Neuroplasticity to Treat AUDs

While therapeutic brain stimulation paradigms have shown potential as a treatment for alcohol dependence, the mechanisms by which they exert their effects are not clearly understood. In general, high frequency rTMS to the DLPFC has received a great deal of

attention as a promising treatment for alcohol dependence. rTMS has been shown to affect multiple neurotransmitter systems. It is possible that by regulating aberrancies that occur in these neurotransmitter systems in alcohol dependence, rTMS can regulate and normalize neuroplasticity in this population.

In general, alcohol dependence is characterized by hyperactive glutamatergic function and reduced GABAergic and dopaminergic function in the absence of alcohol. Each of these neurotransmitter systems is an important mediator of neuroplasticity and therefore, abnormalities in these systems are likely to contribute to aberrancies in neuroplasticity in the drug reward circuitry. Such aberrant neuroplasticity has been demonstrated to mediate increased cue reactivity to drug-associated cues and relapse (Gorelick, Zangen, & George, 2014). rTMS may exert its therapeutic effect by regulating the dysfunction in these neurotransmitter systems and thereby regulating aberrant neuroplasticity in the brain reward circuitry. Neuromodulatory brain stimulation has shown promise as a treatment for alcohol dependence. However, the mechanisms of action of these treatments are yet to be clearly understood. rTMS has been shown to act glutamatergic, GABAergic and dopaminergic neurotransmission and may exert its therapeutic effect by regulating the dysfunction in these neurotransmitter systems, thereby regulating aberrant neuroplasticity in the brain reward circuity. Gaining a better understanding of how neuroplasticity is altered in the DLPFC (using PAS with EEG) of alcohol dependent patients may help us understand how to optimize neuromodulatory brain stimulation parameters to treat dependence. Furthermore, there is likely individual variability in the extent of aberrancies in neurotransmission and neuroplasticity in alcohol dependent individuals. Using brain

stimulation to index neurotransmission and neuroplasticity in alcohol dependent individuals may allow neuromodulation parameters to be tailored to an individual's needs. Impairments in neuroplasticity detected using PAS with EEG can be modified and treated accordingly using rTMS (Figure 1).

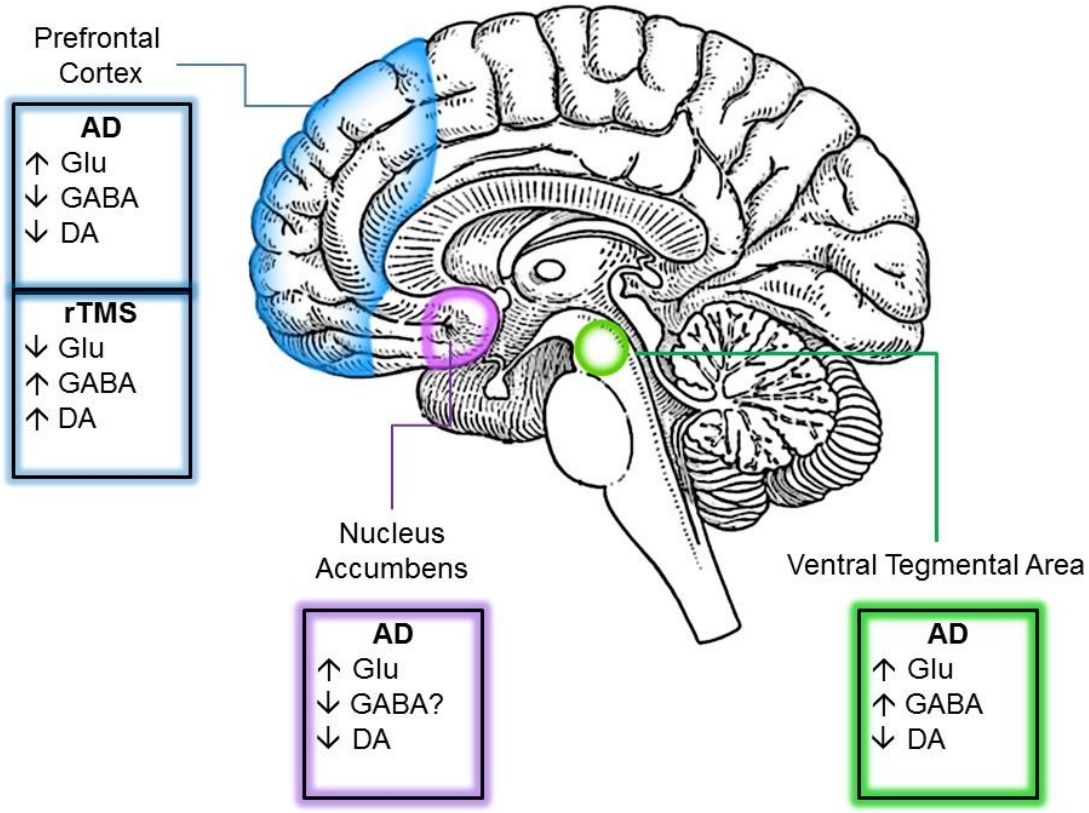

Figure 1 Simplified schematic of abnormalities in neurotransmission in AUD patients that would contribute to aberrant neuroplaticity and the proposed effect of high frequency rTMS to the dorsolateral prefrontal cortex on these neurotransmitter systems. Previous studies have demonstrated that alcohol dependent patients have increased glutamatergic activity in the brain reward circuitry (Freund & Anderson, 1996; Nam et al., 2012; Rao et

al., 2015). Due to counteradaptive changes following chronic alcohol exposure in AUD patients, GABAergic activity is generally reduced in the brains of these individuals (Clapp et al., 2008). Along these lines, GABAergic activity is decreased in the frontal cortex of AUD patients (Deckel et al., 1995; Gilman et al., 1996). However, GABAergic neurotransmission is likely to be increased in the ventral tegmental area (VTA) of patients, likely due to increased glutamatergic input into GABAergic neurons in this region. The increased GABAergic activity in the VTA leads to decreased activity of dopaminergic neurons in this region and decreased dopamine release into the nucleus accumbens (NAc). In AUD patients, dopaminergic activity is generally decreased throughout the brain rewards circuitry, including the prefrontal cortex (Narendran et al.). We propose that through mechanisms of homeostatic plasticity, high frequency rTMS would regulate the abnormalities in neurotransmission in the DLPFC, an important part of the brain reward circuitry. Normalization of neuroplasticity will reduce the maladaptive enhanced response to alcohol or cues associated with alcohol, in turn reducing relapse rates. (Glu=glutamatergic neurotransmission; GABA=GABAergic neurotransmission; DA=dopaminergic neurotransmission)

4.2 Limitations

There are a number of limitations to the studies that comprise this thesis work. For Study 1, it would have been informative to include a motor learning task (i.e., rotary pursuit) and/or a working memory task to examine how the impairment of LTP by alcohol relates to behavioural performance. These would be valuable additions to any future studies

replicating these findings. In regards to Study 1 and 2, PAS has been shown to be attention-dependent (Stefan et al., 2004). Therefore, one may argue that alcohol's impairment of neuroplasticity may be due to decreased attention during alcohol intoxication. However, subjects were instructed to count the number of stimuli to their wrist during the PAS paradigm and the mean count did not differ significantly between the two conditions, suggesting that impaired attention is not a contributing factor to alcohol's impairment of neuroplasticity. Another limitation of all studies were that BAC levels in the study were calculated from breath measures rather than directly from blood samples. However, taking regular blood samples throughout our beverage visits would have been unnecessarily disruptive and invasive, given that breath measures are shown to correlate highly with BAC (Peleg, 2010). For Study 2, it would have been informative to examine the effect of alcohol intoxication on working memory. This would have allowed us to determine if alcohol's impairment of neuroplasticity and θ-γ coupling is associated with working memory dysfunction. However, given that θ-γ coupling has been demonstrated to be related to working memory load, we can infer that alcohol is acting on the same neuronal networks involved in working memory to impair potentiation of θ-γ coupling. For Study 3, it would have been useful to include other TMS measures of cortical inhibition, such as LICI, to confirm alcohol's impairing effect of $GABA_B$ receptor mediated transmission. However, given that previous studies have confirmed that LICI is correlated to N100 amplitude, it can be inferred that a similar impairing effect would be observed on this measure.

4.3 Future Directions

4.3.1 Examining Neuroplasticity in Alcohol Use Disorders Using PAS

In the first two studies of this thesis work, it was demonstrated that acute alcohol consumption produces an impairment of neuroplasticity in the motor cortex and DLPFC. The next step in this line of investigation is to examine neuroplasticity in the motor cortex and DLPFC alcohol dependent individuals during acute withdrawal and following prolonged abstinence, compared to healthy controls. This work will help us gain a better understanding of whether there is an aberrant hyperplasticity or hypoplasticity in dependent individuals. Examining neuroplasticity in the motor cortex and DLPFC following prolonged abstinence will also help us gain a better understanding of whether neuroplasticity normalizes during abstinence and if so, when this normalization occurs. In particiular, identifying any abberancies in neuroplasticity in the DLPFC of alcohol dependent individuals is an important step to optimizing treatments to regulate neuroplasticity in this region.

4.3.2 Developing Neuromodulatory Treatments to Counteract Neuroplasticity Dysfunction in Alcohol Use Disorders

Following identification of the direction and magnitude of aberrancies of neuroplasticity in alcohol dependent individuals, the next step will be to explore how interventional neurodmodulatory brain stimulation can be used to counteract these aberrancies. While high frequency rTMS has shown promise as a treatment to reduce craving and relapse

rates in alcohol dependent individuals, findings have been inconsistent. This is possibly due to the inconsistent stimulation parameters used in previous studies. Knowledge of the existing aberrancies in neuroplasticity may be used to optimize stimulation parameters and identify the best stimulation protocols and parameters for the treatment of alcohol dependence. In addition to identifying the optimal parameters for rTMS, the effective use of multiple PAS sessions to counteract the neuroplasticity aberrancies in alcohol dependence can be explored as a treatment for alcohol dependence. Finally, the possibility of using PAS to identify aberrancies in neurplasticity on an individual basis can be used to taylor treatment for alcohol dependenct individuals should be explored.

4.3.3 Exploring N100 as a Marker of Inhibitory Neurotransmission

In the last study of the PhD thesis, it was demonstrated that acute alcohol consumption produces a reduction in N100 amplitude. The next step in this line of investigation will be to examine if there is a reduction in N100 amplitude in alcohol dependent individuals during withdrawal and during prolonged abstinence compared to healthy controls. This will help establish whether the N100 can be used as a marker for inhibitory neurotransmission in alcohol dependent individuals. Futhermore, future studies should examine whether the N100 is normalized following treatment for alcohol dependence. The N100 may be used as a marker to identify any residual deficits in inhibitory neurotransmission during treatment for alcohol dependence, so that these deficits can be targeted to improve treatment outcomes.

4.4 Conclusions

The studies that comprise this PhD thesis demonstrated that acute alcohol consumption produces an impairment in PAS-induced neuroplasticity in healthy drinkers that last up to 60 minutes following PAS administration in the motor cortex. This impairment in PAS-induced neuroplasticity in the motor cortex was no longer evident the day following acute alcohol consumption. Furthermore, acute alcohol consumption produced an impairment in PAS-induced neuroplasticity in the DLPFC of healthy drinkers. This impairment in PAS-induced neuroplasticity is associated with an impairment of the potentiation of θ-γ coupling that is seen following PAS administration to the DLPFC. Lastly, it was demonstrated that acute alcohol consumption produces a decrease in the N100 amplitude in response to TMS stimulation of the DLPFC, suggesting that acute alcohol consumption produces a decrease in $GABA_B$ receptor mediated neurotransmission. This last finding provides one possible mechanism by which alcohol consumption impairs neuroplasticity. While these studies have a number of limitations, they provide neurophysiological mechanisms by which alcohol consumption may impair motor learning and cognition. Furthermore, the impairment of neuroplasticity in the DLPFC by alcohol may contribute to the pathophysiology underlying the transition from social drinking to alcohol use disorders in some individuals.

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