Imaging Alcohol Induced Dopamine Release in the Human Brain: a PET/[\textsuperscript{11}C]-(+)-PHNO Study

by

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

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ABSTRACT

Findings from previous positron emission tomography studies exploring the effect of acute alcohol on dopamine levels within the reward circuitry are inconsistent. However, the recently developed radiotracer, \textsuperscript{[11]}C-(+)\textsuperscript{-}propyl-hexahydro-naphtho-oxazin (\textsuperscript{[11]}C-(+)\textsuperscript{-}PHNO), may detect fluctuations in dopamine levels with greater sensitivity than previously used radiotracers. Seven social drinkers underwent two \textsuperscript{[11]}C-(+)\textsuperscript{-}PHNO PET scans following alcohol or placebo consumption in a randomized, single blind, crossover design. The non-displaceable binding potential ($BP_{\text{ND}}$) was examined in the striatum and extra-striatal regions (substantia nigra, globus pallidus, ventral pallidum) and compared between the two scans. There were no significant reductions in $BP_{\text{ND}}$ in the alcohol scan compared to the placebo scan in any regions of interest, indicating no significant changes in dopamine levels. This is the first study to explore the alcohol-induced dopaminergic response in social drinkers using \textsuperscript{[11]}C-(+)\textsuperscript{-}PHNO. Our findings suggest that this response may be heterogeneous and the various factors influencing this response warrant further investigation.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Statement of Problem</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Purpose</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>1.3.1 Dopamine elevation in drug reinforcement</td>
<td>3</td>
</tr>
<tr>
<td>1.3.2 Dopamine and alcohol consumption/seeking: Animal Studies</td>
<td>7</td>
</tr>
<tr>
<td>1.3.3 Dopamine receptors and alcohol consumption/seeking: Animal Studies</td>
<td>10</td>
</tr>
<tr>
<td>1.3.4 Vulnerability to alcohol consumption/seeking: Animal Studies</td>
<td>14</td>
</tr>
<tr>
<td>1.3.5 Vulnerability to alcohol consumption/seeking: Human Studies</td>
<td>16</td>
</tr>
<tr>
<td>1.3.5.1 Vulnerability phenotypes</td>
<td>16</td>
</tr>
<tr>
<td>1.3.5.2 DRD2 and DRD3 polymorphisms</td>
<td>20</td>
</tr>
<tr>
<td>1.3.6 Brain imaging in drug administration studies</td>
<td>22</td>
</tr>
<tr>
<td>1.3.6.1 Hemodynamic and glucose metabolism imaging</td>
<td>22</td>
</tr>
<tr>
<td>1.3.6.2 Receptor and neurotransmitter imaging</td>
<td>23</td>
</tr>
<tr>
<td>1.3.7 Brain imaging of acute alcohol administration</td>
<td>27</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.3.7.1 Functional magnetic resonance imaging (fMRI) studies</td>
<td>27</td>
</tr>
<tr>
<td>1.3.7.2 Positron emission tomography imaging (PET) studies</td>
<td>28</td>
</tr>
<tr>
<td>2. OBJECTIVES AND HYPOTHESES</td>
<td>32</td>
</tr>
<tr>
<td>2.1 Rationale</td>
<td>32</td>
</tr>
<tr>
<td>2.2 Main Objective and Hypothesis</td>
<td>32</td>
</tr>
<tr>
<td>2.3 Exploratory Objective and Hypothesis</td>
<td>33</td>
</tr>
<tr>
<td>3. METHODS</td>
<td>34</td>
</tr>
<tr>
<td>3.1 Participant Selection</td>
<td>34</td>
</tr>
<tr>
<td>3.2 Participant Recruitment</td>
<td>35</td>
</tr>
<tr>
<td>3.3 Sample Size Calculation</td>
<td>35</td>
</tr>
<tr>
<td>3.4 Study Design</td>
<td>36</td>
</tr>
<tr>
<td>3.5 Study Procedures</td>
<td>37</td>
</tr>
<tr>
<td>3.6 Materials</td>
<td>45</td>
</tr>
<tr>
<td>3.7 PET Image Acquisition</td>
<td>47</td>
</tr>
<tr>
<td>3.8 MR Image Acquisition</td>
<td>48</td>
</tr>
<tr>
<td>3.9 PET Image Analysis</td>
<td>48</td>
</tr>
<tr>
<td>3.10 Statistical Analysis</td>
<td>49</td>
</tr>
<tr>
<td>4. RESULTS</td>
<td>52</td>
</tr>
<tr>
<td>4.1 Participant Demographics</td>
<td>52</td>
</tr>
<tr>
<td>4.2 Baseline Questionnaires</td>
<td>53</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

7-OH-DPAT: (+/-)-7-hydroxy-N,N-di-n-propyl-2-aminotetralin

$[^{11}\text{C}](+)$-PHNO: $[^{11}\text{C}](+)$-propyl-hexahydro-naphtho-oxazin

$[^{18}\text{F}]-\text{FDG}$: $[^{18}\text{F}]$-fluorodeoxyglucose

AST: Associative striatum

AUC: Area under the curve

AUD: Alcohol use disorder

AUDIT: Alcohol Use Disorders Identification Test

AUQ: Alcohol Urge Questionnaire

ASL: arterial spin labelling

BAC: Blood alcohol concentration

BAES: Biphasic Alcohol Effects Scale

BDI: Beck Depression Inventory

BMI: Body mass index

BP: Binding potential

$BP_{\text{ND}}$: Non-displaceable binding potential

BOLD: Blood oxygenation level dependent

CAMH: Centre for Addiction and Mental Health

CO: Carbon monoxide

CBF: Cerebral blood flow

CPP: Conditioned place preference

DRD1: Dopamine receptor D1

DRD2: Dopamine receptor D2

DRD3: Dopamine receptor D3
DRD4: Dopamine receptor D4
DRD5: Dopamine receptor D5
DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition-Revised
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EKG: Electrocardiogram
fMRI: Functional magnetic resonance imaging
GABA: Gamma-aminobutyric acid
GP: Globus pallidus
LST: Limbic striatum
MRI: Magnetic resonance imaging
NAc: Nucleus accumbens
NEO PI-R: Revised NEO Personality Inventory
PD: Proton density
PET: Positron emission tomography
POMS: Profile of Mood States
ROI(s): Region(s) of interest
ROMI: Regions of mental interest
SMST: Sensorimotor striatum
SN: Substantia nigra
SUV: Standard uptake value
TAC: Time activity curve
TBW: Total body water
TLFB: Timeline Followback
USP: United States Pharmacopeia
UPPS-P: UPPS Impulsive Behavior Scale

VP: Ventral pallidum

VTA: Ventral tegmental area
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table 4.1 Participant demographics</th>
<th>52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.2 NEO PI-R and UPPS-P scores</td>
<td>53</td>
</tr>
<tr>
<td>Table 4.4 Scan parameters of alcohol and placebo scan</td>
<td>54</td>
</tr>
<tr>
<td>Table 4.7 Percent change of $BP_{ND}$ and Z-scores of Wilcoxon signed rank test of alcohol scan versus placebo scan</td>
<td>66</td>
</tr>
<tr>
<td>Table 4.8 Spearman’s rank correlation coefficients</td>
<td>70</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>Flow diagram of all study visits</td>
<td>36</td>
</tr>
<tr>
<td>3.5.3</td>
<td>PET scan timeline</td>
<td>43</td>
</tr>
<tr>
<td>4.3</td>
<td>Boxplots of blood alcohol concentration (BAC) for alcohol beverage scan</td>
<td>54</td>
</tr>
<tr>
<td>4.5.1</td>
<td>Boxplots of BAES total sedation and total sedation change scores (AUC)</td>
<td>55</td>
</tr>
<tr>
<td>4.5.2</td>
<td>Boxplots of AUQ total urge change scores (AUC)</td>
<td>57</td>
</tr>
<tr>
<td>4.5.3</td>
<td>Boxplots of blood cortisol change scores (AUC)</td>
<td>57</td>
</tr>
<tr>
<td>4.5.4.1</td>
<td>Boxplots of systolic blood pressure change scores (AUC)</td>
<td>58</td>
</tr>
<tr>
<td>4.5.4.2</td>
<td>Boxplots of diastolic blood pressure change scores (AUC)</td>
<td>58</td>
</tr>
<tr>
<td>4.5.5</td>
<td>Boxplots of heart rate change scores (AUC)</td>
<td>59</td>
</tr>
<tr>
<td>4.6.1.1</td>
<td>Boxplots of change scores of “Excited” BAES item</td>
<td>62</td>
</tr>
<tr>
<td>4.6.1.2</td>
<td>Boxplots of change scores of “Sedated” BAES item</td>
<td>62</td>
</tr>
<tr>
<td>4.6.1.3</td>
<td>Boxplots of change scores of “Talkative” BAES item</td>
<td>63</td>
</tr>
<tr>
<td>4.6.2</td>
<td>Boxplots of change scores of “it would be difficult to turn down a drink” AUQ item</td>
<td>63</td>
</tr>
<tr>
<td>4.6.3</td>
<td>Boxplots of change scores of blood cortisol levels</td>
<td>64</td>
</tr>
<tr>
<td>4.6.4</td>
<td>Boxplots of change scores of diastolic blood pressure</td>
<td>64</td>
</tr>
<tr>
<td>4.7.1</td>
<td>Cerebellum time activity curves for alcohol and placebo scan</td>
<td>66</td>
</tr>
<tr>
<td>4.7.2</td>
<td>Scatter plot of individual participants’ BPND for alcohol and placebo scan</td>
<td>67</td>
</tr>
<tr>
<td>4.7.3</td>
<td>Boxplots of BPND for alcohol and placebo scan</td>
<td>67</td>
</tr>
<tr>
<td>4.8.1</td>
<td>Scatter plot for spearman’s rank correlation of ΔBPND in LST and scan order</td>
<td>68</td>
</tr>
<tr>
<td>4.8.2</td>
<td>Scatter plot for spearman’s rank correlation of ΔBPND in AST and peak BAC</td>
<td>72</td>
</tr>
<tr>
<td>4.8.3</td>
<td>Scatter plot for spearman’s rank correlation of ΔBPND in VP and systolic blood pressure (AUC of change from baseline)</td>
<td>72</td>
</tr>
</tbody>
</table>
Figure 4.8.4 Scatter plot for spearman’s rank correlation of $\Delta BP_{ND}$ in GP and UPPS-P Positive Urgency score

Figure 4.8.5 Scatter plot for spearman’s rank correlation of $\Delta BP_{ND}$ in GP and NEO PI-R Agreeableness factor
1. INTRODUCTION

1.1 Statement of Problem

In a recent national survey, the prevalence of alcohol use in the last year was reported to be 78.0% of Canadians 15 years and older (Health Canada 2012). This is comparable to older prevalence rates of 79.3% among the same age group (Adlaf, Begin et al. 2005). The results of these surveys indicate not only a high prevalence of alcohol use in Canada over the past decade, but also, a sizable proportion of hazardous drinking among our drinkers: In 2012, 18.6% and 13% of drinkers exceeded Canada’s Low Risk Guidelines for Alcohol Drinking (Stockwell, Butt et al. 2012) recommendations for the avoidance of chronic and acute health risks, respectively (Health Canada 2012). As noted by these guidelines, such drinking patterns can increase the risk for the development of alcohol use disorders (AUD). Alcohol use disorders encompass the diagnosis of alcohol abuse and alcohol dependence and represent a considerable global burden. In 2004, a 3.6% global prevalence of AUD contributed to 36% of all global neuropsychiatric disability adjusted life years (DALY), a measure of burden of disease (Rehm, Mathers et al. 2009).

Alcohol use disorders are widely recognized to be complex diseases involving an interplay of various different genetic, environmental and neurobiological factors (Weiss and Porrino 2002). It is for this reason that the neurobiological mechanisms underpinning excessive use of alcohol, and the eventual development of alcohol abuse and dependence, remains unclear. However, the need for a greater understanding of what neurobiological processes influences initial alcohol liking, continual alcohol seeking and finally, alcohol addiction, can be suggested by the limited availability of pharmacotherapies for AUD. The two frontline medications – opioid antagonist naltrexone, and the glutamate and gamma-aminobutyric acid (GABA) mediator
acamprosate – have revealed only modest effects on drinking outcomes in recent clinical trials (Kranzler and Van Kirk 2001, Maisel, Blodgett et al. 2013).

Although alcohol’s pharmacological effects are numerous, acting on a multitude of receptors and down-stream messenger systems (Vengeliene, Bilbao et al. 2008), much of these effects converge on the dopaminergic reward system, both directly and indirectly. Dopamine has been highly implicated in the reinforcing properties of various drugs of abuse, and considerable recent animal work suggests that both the D3 (DRD3) and D2 (DRD2) dopamine receptor subtypes participate in alcohol seeking. However, further delineation of the precise role of dopamine and its receptors in the response to acute alcohol is necessary to better inform the development of future treatments for alcohol abuse and dependence.

1.2 Purpose

There is a paucity of positron emission tomography (PET) imaging studies exploring the acute dopaminergic response to a dose of alcohol in human participants. The results of the current imaging studies have been inconsistent, with only some reporting a significant increase in dopamine at the level of the striatum, with varying explanations for what this response may signify. In these studies, increases in dopamine release following consumption have been associated with subjective and physiological responses, personality traits, as well as genetic proclivity to alcohol abuse. One explanation for the lack consistent findings in studies pairing alcohol administration with PET imaging may in fact be a heterogeneous effect of alcohol on the human striatum. Further, most previous alcohol administration studies to measure this response have relied on a single antagonist radiotracer, $[^{11}\text{C}]$-raclopride. As $[^{11}\text{C}]$-raclopride binds to the dopamine receptor D2 (DRD2) and dopamine receptor D3 (DRD3) with similar affinity
Seeman, Wilson et al. 2006, little can be said about a possible differential role of each receptor in alcohol induced dopamine changes in humans. Thus, an alternate explanation for the incongruent findings thus far may be the technical limitations in the previously employed $[^{11}C]$-raclopride. With the recent development of the novel dopamine agonist radiotracer, $[^{11}C]$-(-)-propyl-hexahydro-naphtho-oxazin ($[^{11}C]$-(-)-PHNO), it may be possible to examine the dopaminergic response at the level of each receptor separately, owing to the radiotracer’s greater affinity for DRD3 (Gallezot, Beaver et al. 2012). Specifically, investigation of regions where the $[^{11}C]$-(-)-PHNO signal reflects mostly DRD3 (ex., substantia nigra) versus regions where the signal reflects mainly DRD2 (ex., dorsal striatum) can provide insight into action of drugs on the different receptor subtypes (Tziortzi, Searle et al. 2011). Most importantly, $[^{11}C]$-(-)-PHNO may additionally be more sensitive than previously used radiotracers in detecting this dopaminergic response (Gallezot, Kloczynski et al. 2013). The present study was undertaken to be the first to explore the dopaminergic response induced by an acute moderate to high dose of oral alcohol, and the possible factors influencing it, in healthy social drinkers using the novel radiotracer $[^{11}C]$-(-)-PHNO.

1.3 Literature Review

1.3.1 Dopamine elevation in drug reinforcement

In discussing the significance of dopamine elevation in drug reinforcement, it should be noted that the brain dopaminergic systems implicated in drug taking are understood to be highly dynamic and vulnerable to neuroadaptive change with the onset of chronic drug taking and abuse. There are various different lines of work exploring the eventual sensitization of the dopaminergic system with chronic drug use (Thomas, Kalivas et al. 2008), the gradual depletion of basal dopamine in the dopamine depletion hypothesis (Salamone, Cousins et al. 1997) and
overall dysregulation of the reward system in allostasis models, which attempt to incorporate both of the above neuroadaptations (Koob and Le Moal 2001). The following discussion will cover current proposed roles of dopamine specifically in response to acute drug administration in early phases of drug taking, prior to the onset of complete dependence.

The role of dopamine in drug taking processes has been explored extensively in the last few decades. Dopamine’s importance was gradually supported with increasing evidence that drugs with abuse liability – drugs for which dependence may develop – share the common mechanism of eliciting an elevation of dopamine levels in the nucleus accumbens (NAc)/ventral striatum, within the basal ganglia (Di Chiara and Imperato 1988). The NAc is a part of a well-defined network known as the mesolimbic dopaminergic pathway, receiving dopamine neuronal projections from the ventral tegmental area (VTA) of the midbrain, one of the primary dopamine producing nuclei of the brain (Swanson 1982). Dopamine is also involved in the VTA projections to cortical regions (prefrontal cortex, orbitofrontal cortex) in the mesocortical pathway, and also in the substantia nigra (SN) projections to the dorsal striatal regions (caudate, putamen) in the nigrostriatal or mesostriatal pathway.

In light of initial work establishing the mesolimbic pathway as a consistent area of electrical self-stimulation in animals (Olds and Milner 1954, Heath 1972) and also, evidence that natural rewards of sex and food work on this same pathway (Damsma, Pfaus et al. 1992, Schultz 1998), it has been widely proposed that this characteristic elevation in dopamine functions in the reinforcing properties of drugs of abuse.
A possible role for dopamine elevation may be the coding of hedonic pleasure, or the rewarding effects of drugs, serving as the primary drive for repeated drug seeking and consumption. Indeed, as the mesolimbic pathway has come to be known as the “reward” pathway of the brain, drugs which act on this centre may be rewarding. Extensive work using drug self-administration paradigms in animals has further supported this idea. In intracranial self-stimulation studies, whereby electrical stimulation to “reward” brain regions are self-administered by animals, the threshold for stimulation is lowered by prior administration of various drugs of abuse (Kornetsky and Esposito 1979, Bespalov, Lebedev et al. 1999). One interpretation of this observation is that drugs of abuse, by acting on the reward pathway, provide sufficient pleasure and thereby decrease seeking for further stimulation. Furthermore, interference with dopamine transmission within the mesolimbic neural substrate, either through lesion or through pharmacological blockade, results in significant attenuation of self-administration in animals previously administering cocaine, morphine and nicotine (Zito, Vickers et al. 1985, Corrigall, Franklin et al. 1992). Thus, without a functional reward pathway, it appears that previously rewarding drugs appear no longer rewarding.

Findings in human imaging studies also support a role for dopamine elevations in eliciting hedonic pleasure in humans. Namely, elevation of dopamine in striatal regions in response to drug administration has been correlated with drug induced euphoria and high (Drevets, Gautier et al. 2001, Barrett, Boileau et al. 2004). Furthermore, findings from Volkow et al. (Volkow, Wang et al. 1999, Volkow, Wang et al. 2002) suggests that the baseline levels of DRD2 receptors in both abusers and non-abusers of drugs may also be related to “drug liking” of intravenously administered psychostimulants.
Recently however, more complex roles for dopamine in addiction processes have been espoused. In contrast to simple liking of a drug’s effects, a passive state, elevations in dopamine may instead mediate a more activated state of incentive learning and motivation for the drug. Underpinning these newer roles for dopamine is associative learning, which can subsequently influence and shape future behaviour (Wise 2004). The associative learning model assumes that drugs of abuse are inherently pharmacologically rewarding, with learning initially serving to reinforce drug taking behaviour through its association with the intrinsic drug reward. Associative learning can also explain how initially neutral stimuli or cues related to drug taking may eventually become rewarding themselves. In this view, dopamine can signal as reward prediction, initially firing in a phasic manner in response to unpredicted rewards (initial drug taking). Eventually, this firing can shift to cues which are related to drug delivery (Schultz 1998). Indeed, in a recent seminal study employing temporally precise optogenetic tools, artificial activation of VTA dopamine neurons during the time of initial natural reward consumption has been shown to trigger the learning of the reward value of new cues (evidenced by cue-elicited behavioural responses) (Steinberg, Keiflin et al. 2013). With regular drug taking, it is possible that such associations may strengthen, leading to aberrant dopamine responses to cues in the absence of the actual drug reward (Di Chiara and Bassareo 2007).

Robinson and Berridge have also incorporated such associative learning in their incentive salience model of dopamine (Robinson and Berridge 2001) to explain how motivation for drug taking develops. Briefly, this construct separates hedonic effects (liking) from motivated drug seeking (wanting) on the basis that drugs of abuse are administered, even in the absence of a clear liking of the drug (Berridge and Robinson 1998). Moreover, manipulating the dopaminergic transmission has been clearly shown to influence goal-directed drug taking
behaviour without influencing the hedonic component of drug taking (Robinson and Berridge 2001, Robinson, Sandstrom et al. 2005). In this view, increases in dopamine code the salience of the drug. Attribution of salience to drug taking and its associated sensory cues renders them attractive and thereby guides seeking towards them. Also, sensitization of the dopaminergic neural systems during the addiction process can greatly heighten salience of drug taking and associated cues, possibly leading to compulsive drug taking behaviour (Robinson and Berridge 2001).

In summary, the specific role of dopamine elevations in drug reinforcement is a complicated one at best, with implications for reward, simple associative learning and incentive salience learning. More recently, a number of investigations have suggested a greater role for dopamine in motivation for drug taking or drug-seeking, rather than simple liking of the drug (Le Foll, Gallo et al. 2009). Overall however, these varying theories taken together suggest an important role for dopamine in drug reinforcement.

1.3.2 Dopamine and alcohol consumption/seeking - Animal studies

As ethanol is readily administered by laboratory animals, similar to other drugs of abuse, it is understood to have reinforcing properties. In much of animal literature, these reinforcing properties have been explored through different models of alcohol seeking including operant self-administration of oral ethanol, a 2-bottle choice procedure to assess alcohol preference, conditioned place preference (CPP), and cue-induced relapse drinking following extinction of self-administration. In line with the previous discussion, the use of these different models may be employed to assess the varying facets of drug reinforcement, with models like CPP providing greater insight into incentive salience or associative learning.
The role of dopamine in alcohol seeking as suggested by animal studies is contradictory. There is some evidence to support that dopamine, particularly within the mesolimbic system, plays a role in the reinforcing properties of alcohol. Indeed, rats have been shown to self-administer ethanol directly into the VTA, with intracranial self-administration either ameliorated or reinstated through the use of dopamine receptor agonists and antagonists (Rodd, Melendez et al. 2004). However, there are a sizable number of studies which have reported that the blocking or deletion of key dopaminergic substrates does not effect ethanol reinforcement. In fact, the severe lesioning of the NAc and olfactory tubercle leading to a nearly 95% dopamine depletion in one study did not result in significant changes in the number of operant responses for oral alcohol (Rassnick, Stinus et al. 1993). Thus, while playing some role, the neurotransmitter dopamine may not be entirely critical for alcohol seeking.

In line with other drugs of abuse however, various methods of ethanol administration have been demonstrated to elicit elevations in synaptic dopaminergic levels within the mesolimbic system of rats (Di Chiara and Imperato 1988) and primates (Bradberry 2002). These increases are apparent in both the ventral striatum and the dorsal striatum of the brain. Specifically, ethanol has been proposed to increase dopamine levels in the NAc/ventral striatum, as well as other limbic targets, through increasing the firing rate of VTA dopaminergic projections (Gessa, Muntoni et al. 1985, Brodie, Shefner et al. 1990, Brodie, Pesold et al. 1999). What this elevation signifies with respect to the reinforcing properties of ethanol essentially remains unclear however.
However, not all animal studies exploring this effect have observed an elevation in dopamine in response to alcohol at the level of the striatum. Specifically, either no effect on dopamine release (Budygin, Phillips et al. 2001) or a decrease in dopamine release has been previously reported (Blanchard, Steindorf et al. 1993, Budygin, Phillips et al. 2001, Jones, Mathews et al. 2006). These studies reveal that the dopaminergic effect by alcohol may be dose dependent, with low to moderate doses eliciting an increase in evoked dopamine and higher doses eliciting a decrease (Blanchard, Steindorf et al. 1993). A possible mechanism through which decreases in extracellular dopamine in the striatum may occur is through enhanced inhibitory GABAergic influence on the VTA neurons elicited by alcohol (Theile, Morikawa et al. 2008, Theile, Morikawa et al. 2009, Theile, Morikawa et al. 2011).

Microdialysis studies attempting to explore this dopaminergic response separately during operant alcohol seeking through lever pressing and during oral ethanol consumption, have found that increases in accumbal dopamine are not present during alcohol seeking lever pressing period. Rather, elevated dopamine is only evident within the initial periods following initiation of ethanol consumption. Furthermore, the elevations of dopamine do not appear to parallel the time course of elevation of blood ethanol, highlighting the importance of initial sensory cues in the dopaminergic response (Doyon, York et al. 2003). Similarly, another study by Gonzales and Weiss has demonstrated that in rats trained to self-administer ethanol, substantial increases in dopamine are even apparent during a “waiting period” in the previously trained operant chamber prior to access to ethanol. Following the onset of ethanol self-administration in these rats, only a modest increase in dopamine levels are revealed from that measured during the waiting period. As discussed by the authors, this is suggestive of a greater role of dopamine elevations in the incentive salience of the operant chamber compared to the pharmacological properties of alcohol.
Additional support for this possibility lies in recent findings that in alcohol administration in naïve rats, only the second day administration of ethanol significantly increases accumbal dopamine during the first 5 minutes of consumption, while no such increases in the first day administration are evidenced. Thus, the enhanced dopaminergic signal may in fact code for reward prediction following the acquisition of the associative relationship between ethanol cues and the inherent rewarding property of ethanol (Carrillo and Gonzales 2011). This dopamine elevation related to reward prediction or incentive salience has also been shown to mediate ethanol seeking in animals. That is, stimuli predicting the delivery of ethanol not only works to enhance dopamine efflux after a period of extinction, but also significantly recovers responding for alcohol in rats (Katner and Weiss 1999).

Taken together, these studies suggest that while dopamine may not be entirely integral for alcohol seeking in animals, it is certainly an important neurotransmitter for alcohol reinforcement overall. Specifically, there is some work to suggest that dopamine may be more so involved in the prediction of ethanol availability or the salience of ethanol related cues in animals.

1.3.3 Dopamine receptors and alcohol consumption/seeking – Animal studies

The five subtypes of G-protein coupled dopamine receptors are often categorized into the two families of D1-like and D2-like, which differ in their physiological actions. The D1-like family includes DRD1 and DRD5, while the D2-like family comprises of DRD2, DRD3, and DRD4. The D2-like family has been explored extensively in the reward literature, and exists both pre-synaptically as autoreceptors, as well as post-synaptically. However, owing to a general lack of available pharmacological agents with varying receptor selectivity, it has proven difficult
to assess the separate contributions of the individual receptors in drug taking (Heidbreder, Andreoli et al. 2004). As elaborated in recent reviews (Le Foll, Goldberg et al. 2005, Heidbreder and Newman 2010), the distribution of DRD3 in the reward circuitry of the forebrain (including the mesolimbic, mesocortical and mesostriatal systems, and other midbrain structures including the ventral pallidum and globus pallidus) is suggestive of a potentially important role of DRD3 in drug reinforcement. Of note, in the rat forebrain, the distribution of DRD2 and DRD3 are generally non-overlapping, with DRD2 predominance in the dorsal striatum and DRD3 predominance in the ventral striatum (Murray, Ryoo et al. 1994). Thus, a distinct role of these two receptors may in fact exist.

The DRD2 has been specifically shown to be relevant to alcohol consumption and seeking in animals. Antagonism of this receptor leads to greater ethanol self-administration in alcohol preferring rats (Dyr, McBride et al. 1993), while mice lacking DRD2 display an overall low preference and intake of alcohol (Risinger, Freeman et al. 2000). With adrenoviral transfer of these receptors into the NAc for the purpose of overexpression, there are significant reductions in alcohol preference and intake in alcohol preferring (Thanos, Taintor et al. 2004) and self-administering non-preferring rats (Thanos, Volkow et al. 2001). When adrenoviral transfer is undertaken with mice lacking the DRD2 receptor however, an increase in preference and intake results (Thanos, Rivera et al. 2005). Collectively, these findings are suggestive of a crucial role for the presence of DRD2 in alcohol taking, with a hypofunctioning DRD2 system indicative of pre-disposed alcohol preference leading to aberrant drinking.

With respect to DRD3, DRD3 preferential agonists (including 7-OH-DPAT, quinpirole, quinelorane, PD 128907 and (+)3PPP) are shown to dose-dependently decrease ethanol taking in
self-administration and free-choice paradigms (Cohen, Perrault et al. 1998). The authors of this former study have speculated that these agonists may work to decrease responding for ethanol via a decrease of dopamine transmission through presynaptic receptors. This conclusion is corroborated by another finding in the same study that using DRD2/DRD3 antagonist haloperidol (at a dose thought to block postsynaptic receptors) also decreases responding dose-dependently. A further compelling conclusion of these authors’ is that ethanol reinforcement may be mitigated with decreased dopaminergic transmission through the enhancement of DRD3 activity on the pre-synaptic level and blockade of both DRD2 and DRD3 on the post-synaptic level. Similarly, ethanol induced hyperactivity, another potential measure of ethanol reinforcement, also demonstrates attenuation by DRD3 agonist quinpirole and DRD2/DRD3 antagonist haloperidol, presumably through the decreased dopaminergic transmission discussed above (Cohen, Perrault et al. 1997). Indeed, it has been shown that direct injection of quinpirole into the VTA, where it can directly decrease dopaminergic firing by action on DRD3 autoreceptors, decreases responding for ethanol (Hodge, Haraguchi et al. 1993). Finally, in animals with stronger alcohol seeking - alcohol preferring and high drinking rats - DRD3 agonism also attenuates ethanol drinking (Russell, McBride et al. 1996).

With respect to DRD3 receptor antagonism, earlier studies using the DRD3-preferring antagonist U99194A suggest only a limited role of DRD3 in ethanol consumption. Specifically, intraperitoneal injections of this antagonist, although appearing to enhance the conditioned place preference acquired for ethanol (Boyce and Risinger 2000, Boyce and Risinger 2002), has no influence on ethanol self-administration. However, as conditioned place preference paradigms (CPP) are consistently used to evaluate reward properties of drugs, the authors of these studies have concluded that these results are indicative of increases of ethanol reward with receptor
blockade. This may be in line with the results of DRD3 agonists, owing that U99194A is acting on DRD3 autoreceptors. However, more recently, opposite effects of DRD3 antagonism have also been evidenced. Selective antagonism using SB-277011-A significantly attenuates ethanol preference and consumption in both alcohol preferring and non-preferring rats (Thanos, Katana et al. 2005). This somewhat discrepant finding compared to the effects of U99194A may be explained by a greater selectivity of SB-277011-A versus U99194A for DRD3, 100-fold versus 20-fold respectively (Barth, Need et al. 2013). However, DRD3 antagonism using S33138, an antagonist with a comparable preference for DRD3 as U99194A (25-fold), also works to attenuate ethanol drinking in a restricted binge paradigm (Rice, Patrick et al. 2012). Thus, an alternative explanation for the incongruent findings of DRD3 antagonism may be that the predominant action of SB-277011-A and S33138 are on post-synaptic DRD3 instead of autoreceptors, resulting in decreased dopaminergic transmission in the limbic targets. Indeed, these studies have relied on systemic injection, it is certainly possible that that differing behavioural effects may be seen with activation of DRD3 in different brain regions (Jeanblanc, He et al. 2006).

Finally, in animals with long term drinking experience, DRD3 may have a specific role in craving for alcohol, relevant to later processes in addiction. That is, in animals having undergone alcohol deprivation, SB-277011-A dose dependently decreases both relapse drinking and alcohol seeking following cue exposure in rats (Vengeliene, Leonardi-Essmann et al. 2006) as well as mice (Heidbreder, Andreoli et al. 2007).

It should be noted that in a recent genetic deletion study of the DRD3 receptor, a lack of significant differences between mice lacking this receptor and wild-type mice on a number of
ethanol consumption paradigms has been suggested, although ethanol metabolism appears slower in the former mice (McQuade, Xu et al. 2003). Indeed, when investigating separately ethanol consumption and reinforcement of ethanol through operant self-administration, DRD3 knock-out mice do not differ from C57BL/6J alcohol preferring mice on either factor (Boyce-Rustay and Risinger 2003). However, the interpretation of results from knock-out studies are difficult in light of possible compensatory changes which may emerge, possibly obscuring any role of the abolished receptor (Heidbreder, Andreoli et al. 2004).

Overall, animal studies involving receptor knock-out animals and selective receptor agonism and antagonism suggest important roles for both DRD2 and DRD3 in alcohol consumption and seeking. However, further exploration of how these receptors function in the dopaminergic elevation in response to ethanol is necessary to better understand how each receptor may influence ethanol seeking behaviour separately.

1.3.4 Vulnerability to alcohol consumption/seeking – Animal studies

The majority of ethanol administration studies in animals have employed various lines of alcohol preferring rats (Gonzales, Job et al. 2004). These rats, bred for a greater proclivity towards alcohol seeking, display a heightened sensitivity to the stimulation effects of alcohol through increases in heart rate (Bell, Rodd-Henricks et al. 2002) as well as overall greater behavioural impulsivity compared to heterogeneous rats (Oberlin and Grahame 2009). Exploring dopamine function in such rats provides further insight into the importance of elevated dopamine levels in alcohol seeking. Most importantly, such studies reveal that the characteristic dopaminergic response to acute alcohol is not consistent across all strains.
Indeed, while heterogeneous strains do in fact display an alcohol-induced rise in dopamine following ethanol administration, this increase is significantly more pronounced in alcohol preferring rats (Weiss, Lorang et al. 1993). Specifically, a two-fold greater elevation of dopamine in alcohol preferring rats versus abstainer rats was evidenced in one study (Bustamante, Quintanilla et al. 2008). However, other studies suggest that greater alcohol preference may instead be related to a lower ethanol induced dopamine release as measured by microdialysis (Ramachandra, Phuc et al. 2007) and animal PET imaging (Sullivan, Risacher et al. 2011). The reasons behind these discrepant findings are unclear. Notably however, the anticipation of alcohol availability may also be more heightened in alcohol preferring rats than heterogeneous strains as it is primarily these rats which display significant elevations of accumbal dopamine (Katner, Kerr et al. 1996) and increases in locomotor activity in response in alcohol related stimuli (Melendez, Rodd-Henricks et al. 2002). As alcohol preferring rats are in fact characterized with lower basal dopamine concentrations (Murphy, McBride et al. 1982, Li, Lumeng et al. 1993) and lower baseline dopamine receptors (McBride, Chernet et al. 1993), the enhanced acute dopaminergic response in response to drug administration and drug related cues may be interpreted as compensatory behaviour for an underlying reward deficiency.

In summary, alcohol preferring animals demonstrate robust dopaminergic elevations in response to alcohol and alcohol related cues which provide further support for the role of dopamine in alcohol seeking. Moreover, the heightened dopaminergic response in alcohol preferring rats compared to heterogeneous rats may underlie greater alcohol reinforcement experienced in alcohol preferring animals, which in turn may drive greater seeking. This also suggests that the alcohol induced dopamine response is not a consistent one, and may be more relevant to strains with a propensity for alcohol abuse.
1.3.5 Vulnerability to alcohol consumption/seeking – Human Studies

1.3.5.1 Vulnerability phenotypes

In the alcohol literature, the link between a propensity for alcohol consumption and seeking and a greater risk for alcohol abuse and dependence have been well explored in a number of human studies. Several phenotypes have been proposed which appear to confer increased vulnerability for developing an AUD in individuals. Firstly, many of these studies have implicated the personality traits and behavioural correlates of impulsivity, novelty and sensation seeking and risk taking with this increased vulnerability. Impulsivity appears to have a genetic basis, with children of alcoholics reported to be more likely to exhibit externalizing and impulsive behaviour (Marmorstein, Iacono et al. 2009). A recent meta-analysis of the influence of various impulsivity traits on drinking outcomes in adolescents reported that all measured traits were related to both consumption and alcohol related problems (Stautz and Cooper 2013), with sensation seeking most highly related to the former and positive and negative urgency (forms of impulsivity) most related to the latter. In support of these traits existing prior to any alcohol initiation, novelty seeking measured as early as in infancy was also shown to predict development of alcohol abuse in early adulthood (Kirisci, Tarter et al. 2006). Moreover, the traits of sensation seeking and impulsivity measured in high school not only predicted heavy drinking in college years, but also increases in the intensity of these traits, highlighting the existence of a bidirectional relationship (Quinn, Stappenbeck et al. 2011).

While trait measurements of impulsivity are usually acquired through self-report, cognitive or behavioural impulsivity requires laboratory testing through the use of various tasks, often with response inhibition or delay discounting as the outcome measure. In a recent study exploring cognitive impulsivity in individuals participating in harmful drinking, poorer ability to
withhold impulse for an immediate reward in the face of monetary loss was seen using a Go/No Go task (Rossiter, Thompson et al. 2012). It is possible that increased impulsivity through a decreased sensitivity for punishment may underlie harmful drinking patterns. However, as a caveat of all cross-sectional studies, it is unclear whether such insensitivity preceded chronic alcohol use or instead resulted from excessive drinking. Prospective studies in individuals who later went on to develop heavy drinking patterns have yielded mixed results, with response inhibition not emerging as a reliable predictor of increased frequency of drinking and alcohol dependence during follow-up (Rubio, Jimenez et al. 2008, Goudriaan, Grekin et al. 2011). Of note however, a recent prospective study reported that all the explored behavioural impulsivity measures of disinhibition, delay discounting and risk-taking were related to alcohol involvement in a group of 12 and 13 year olds at a 6 month follow-up (Fernie, Peeters et al. 2013). Thus, greater trait impulsivity may render individuals vulnerable to earlier alcohol exposure as well.

Other suggested phenotypes conferring increased vulnerability include characteristic subjective and objective responses to an acute dose of alcohol. How exactly subjective responses describing alcohol induced feelings of stimulation and sedation differ across the vulnerability spectrum still remains highly debated (Quinn and Fromme 2011). However, as described by Quinn et al., two prevailing theories as described below seek to distinguish the nature of these subjective responses in those at higher risk from those at a lower risk.

Perhaps more so relevant in individuals with a familial vulnerability for increased alcohol abuse and dependence, a lower sensitivity to the effects of alcohol forms the basis of the low-level response theory (LLRT) advanced by Schuckit and Smith (Schuckit and Smith 2000). In a number of large prospective studies, lower responses on the subjective high assessment scale
(SHAS), a scale assessing the degree of intoxication, were related to future alcohol related problems or alcoholism (Schuckit 1994, Schuckit, Smith et al. 2004). Thus, those experiencing lower levels of sedation may be more likely to drink chronically, perhaps through the need for greater amounts of alcohol to receive the desired effects. Alternatively, the differential model (DM) (Newlin and Thomson 1990) posits that while vulnerable individuals are indeed less sensitive to the sedative effects of alcohol, they are also more sensitive to the stimulatory effects. The differential model does not appear to be limited to those with a family positive history of alcoholism, as heavy drinkers compared to light drinkers have also been shown to respond to an alcohol challenge as described by this model (King, Houle et al. 2002). Importantly, in one large prospective study, greater stimulation effects and lower sedative effects in response to alcohol increased the probability of binging during a future follow-up period (King, de Wit et al. 2011).

Increased cardiovascular reactivity as measured by increased heart rate from a baseline in response to alcohol is yet another proposed phenotype of vulnerability. This increased heart rate has been shown to be characteristic in individuals with a family history of alcoholism (Peterson, Pihl et al. 1996), with those who display a marked increase more likely to consume more alcohol in both a laboratory setting and on a weekly basis (Pihl, Giancola et al. 1994). Heightened heart rate from baseline, particularly during the ascending limb of the alcohol curve, may confer vulnerability through an exaggerated sensitivity to the stimulation properties of alcohol (Conrod, Peterson et al. 1997, Conrod, Peterson et al. 2001). In support, in a group of heavy drinkers, increases in heart rate were negatively correlated with alcohol induced sedation and subjective intoxication (Ray, McGeeary et al. 2006). Of interest, increases in heart rate were also related to impulsivity and subjective scores in this same group, suggesting the possibility that the various different vulnerability phenotypes may not be acting singularly. Furthermore, the enhanced
vulnerability to develop alcohol abuse with this phenotype was related to a greater sensitivity to
reward, as measured by higher scores on a standardized reward scale (Brunelle, Assaad et al.
2004).

In contrast to increased cardiovascular activity, cortisol release in response to drug
administration has been a less consistent phenotype for vulnerability, with not all studies
reporting a difference in cortisol reactivity between family history groups (Gianoulakis,
Krishnan et al. 1996, Munro, McCaul et al. 2006). A number of acute alcohol administration
studies have generally evidenced both lower baseline stress hormones as well as blunted stress
response in individuals with a positive family history of alcoholism (Schuckit, Gold et al. 1987).
Others have revealed a trend in the opposite direction, with alcohol induced increase in cortisol
in those with a positive family history compared to individuals with a negative history (Lex,
Ellingboe et al. 1991). Further, in heavy drinking individuals deemed vulnerable for alcohol
abuse and dependence as a result of their drinking history, reduced cortisol activity was also
evidenced (King, Munisamy et al. 2006, King, de Wit et al. 2011). However, reduced cortisol
was not a direct predictor of increased binge drinking and dependence during follow-up in this
study (King, de Wit et al. 2011). While not directly studied with alcohol administration, this
lowered cortisol response may be indicative of poorer feedback mechanisms between the limbic
system and the hypothalamus during stress, thereby conferring elevated abuse potential of
alcohol as a means of stress reduction (Lovallo 2006).

There is a possibility that increased vulnerability to developing an AUD through the
above proposed phenotypes above may result from their association with the dopaminergic
reward system. There is some work to suggest this association. Indeed, in social drinkers,
increased heart rate and trait impulsivity was related to greater dopamine release in the ventral striatum in response to alcohol, supporting a role of these phenotypes in sensitivity to reward and the stimulating properties of alcohol (Boileau, Assaad et al. 2003).

1.3.5.2 DRD2 and DRD3 polymorphisms

In line with existing roles for both DRD2 and DRD3 in alcohol seeking in animals, there have been a number of human genetic studies investigating the role of polymorphisms of DRD2 and DRD3 genes in increased vulnerability for alcohol abuse and dependence. The functional TaqI A1 allele associated with the DRD2 receptor, conferring a lower density of this receptor, has been shown to be modestly associated with alcohol dependence (Le Foll, Gallo et al. 2009). In a recent pilot trial, there were greater relapse rates following 1.5 years of treatment in alcohol dependent individuals who are also carriers of TaqI A1 allele, compared to non-carriers (Dahlgren, Wargelius et al. 2011). Moreover, this allele was further associated with frequency and problematic drinking in non-dependent individuals (Meyers, Nyman et al. 2013). Similarly, the presence of another polymorphism of the DRD2 gene, the-141C Ins/Del allele, also was shown to confer increased risk for the development of alcoholism (Prasad, Ambekar et al. 2010). In contrast, results of association studies of mutations in the DRD3 gene with alcoholism have not suggested a reliable relationship. With the Bal I polymorphism of the DRD3 gene, little association with outcomes of inpatient treatment for alcoholism (Wiesbeck, Weijers et al. 2003), or even susceptibility to alcoholism (Lee and Ryu 2002) was evidenced. Such studies have further suggested that, rather than involvement in vulnerability for drug abuse and dependence specifically, a possible role for the Bal I polymorphism, or DRD3 in general, may be in impulsivity traits like sensation seeking (Gorwood, Limosin et al. 2001). In this way, DRD3 may
be related to increased vulnerability for alcohol abuse, as well as a number of other neuropsychiatric disorders.

In summary, polymorphisms associated with DRD2 appear to be associated with problematic alcohol use, alcohol dependence risk and relapse vulnerability to a much greater degree than polymorphisms associated with DRD3. Increased vulnerability through variation of dopamine receptor function and expression also lends some support to the possibility that the vulnerability phenotypes of impulsivity and response to acute alcohol may be mediated by differences in the dopaminergic response. However, while receptor polymorphism association studies do allow for investigation of the influence of receptor variation in humans, little can be said about how these receptors function specifically in actual alcohol taking behaviour as has been established with animal studies. Further examination using tools such as neuroimaging are thus necessary to draw more exacting conclusions.

Overall, there are potentially several important differences between those at a higher risk and those at a low risk for the development of alcohol abuse and dependence which merit consideration in alcohol administration studies. These differences may include the possession of the following phenotypes: personality traits of vulnerability and sensation seeking and characteristic subjective and objective responses to alcohol. An interesting possibility is that these phenotypes of vulnerability may converge on the dopaminergic alcohol reinforcement system to modulate reinforcement received from alcohol and alcohol cues. In some support of this, variation of dopamine receptor function through possession of a specific dopamine receptor gene polymorphism also appears to confer some increased impulsivity. However, more direct
PET studies are needed to explore how neural activity and changes in neurotransmitters, specifically in the mesolimbic pathway, functions in relation to these traits.

1.3.6 Brain imaging in drug administration studies

1.3.6.1 Hemodynamic and glucose metabolism imaging

Functional magnetic resonance imaging (fMRI) has been a reliable tool in providing measures of neural activity across brain regions through changes in cerebral blood flow (CBF). Such changes can be determined by the relative increases and decreases in oxy-hemoglobin and deoxy-hemoglobin in the blood, which generates a blood oxygenation level dependent (BOLD) signal. An increased BOLD signal is interpreted as greater activation of neural activity in a specific region, reflecting a decrease in regional deoxy-hemoglobin through increases in CBF (Detre and Wang 2002). Alternatively, blood flow can be measured with magnetically labelled arterial blood water, which generates an arterial spin labelling (ASL) signal. Using ASL, fMRI images are produced through the decay of the labelled blood as it flows into the brain tissue. Cerebral blood flow is then determined by assessing the difference in images generated with labelled blood and unlabelled blood (Detre and Wang 2002).

Similar to the ASL method of fMRI, positron emission tomography (PET) has also served as a hemodynamic neuroimaging tool to assess changes in regional CBF. With PET however, water in blood is labelled with a radioactive tracer (often $^{15}$O) which decays through positron emission and subsequent generation of photons to produce an image of the radioactivity. In a similar way, increases in regional energy utilization by brain tissue has also been assessed through radioactive labelling of glucose (fluorine-18 labelled fludeoxyglucose) in $^{18}$F-FDG.
PET. Local increased uptake of glucose in brain tissue, another surrogate for increased neural activity, is revealed with images of increased radioactivity in these regions.

With both fMRI and PET to measure hemodynamic and energy utilization changes, the effects of acute drug administration on regional neural activity can be explored by comparing images obtained during a baseline or placebo administration and during active drug administration.

1.3.6.2 Receptor and neurotransmitter imaging

In many drug administration studies, PET has also proven invaluable for the in vivo measurement of various receptors and neurotransmitters of interest. As outlined by Martinez and Narendran, either antagonists or agonists of the receptor in question can be labelled with a radioactive radionuclide which then binds to a receptor. As these radionuclides are receptor bound, the process of tracer decay allows for an assessment of the levels of receptors in various regions (Martinez and Narendran 2010). Specifically, one main outcome of PET imaging of receptors is the receptors available for binding, or binding potential (BP). This term encompasses the concentration of available receptors and the affinity for the radionuclide for the receptor (Yoder, Kareken et al. 2011).

Comparable to determining changes in hemodynamics or energy utilization, the completion of two scans is utilized for determining changes in neurotransmitter levels. One scan occurs under baseline conditions or the administration of a placebo, and the second scan involves the administration of an active drug. Determining the difference in BP between these two scans will provide an estimation of changes in endogenous levels of a neurotransmitter which the
radionuclide is competitive with. It is in this way that the levels of dopamine following drug administration have been imaged in the human brain. In comparison to the baseline scans, decreases in binding potential during the active scan can be suggestive of increases in endogenous dopamine levels (which displace the radionuclide) while increases in binding potential during the active scan may be suggestive of decreases in dopamine levels (which make more receptors available for binding with the radionuclide).

The majority of the literature of PET imaging paired with drug taking has relied on radiolabelling DRD2/DRD3 antagonists. Of these antagonists, \([^{11}C]\) labelled raclopride has been most employed following early demonstration of its sensitivity to compete with endogenous dopamine (Seeman, Guan et al. 1989). \([^{11}C]\)-raclopride has been noted to have a relatively low signal to noise ratio however, which limits its use to only regions of high DRD2/DRD3 receptor density, such as the striatum. The other commonly used DRD2/DRD3 antagonists, \([^{18}F]\)-fallypride and \([^{11}C]\)-FLB457 (FLB), have higher signal to noise ratios than \([^{11}C]\)-raclopride (Vandehey, Moirano et al. 2010). Thus, they are more suitable for the assessment of extra-striatal regions implicated in drug taking such as cortex and amygdala. However, the slow wash rate of FLB renders this radiotracer more suitable for regions of lower receptor density than the striatum, where the washout phase may be reached in time for accurate quantitative imaging (Slifstein, Kegeles et al. 2010). Moreover, while \([^{18}F]\)-fallypride may be used to image both striatal and extra-striatal structures (Slifstein, Kegeles et al. 2010), it does not appear to be as sensitive as \([^{11}C]\)-raclopride within the striatum (Morris and Yoder 2007).

More recently, DRD2/DRD3 agonists have been explored in drug taking studies, including the recently developed agonist ligand, \([^{11}C]\)-(+)-propyl-hydroxy-naphthoxazine
This novel radiotracer may be superior to the currently available tracers for a number of reasons. In a direct comparison study in healthy humans, baseline receptor binding of $[^{11}\text{C}](+)$-PHNO and that of $[^{11}\text{C}]$-raclopride were comparable, but with notable differences. Firstly, $[^{11}\text{C}](+)$-PHNO labelling of the globus pallidus was notably greater than that of the antagonist $[^{11}\text{C}]$-raclopride, and $[^{11}\text{C}](+)$-PHNO labelling was also observed in the substantia nigra/ventral tegmental regions. Secondly, the $[^{11}\text{C}](+)$-PHNO signal was also more pronounced in the ventral-medial portions of the striatum, compared to the dorsal striatal labelling of $[^{11}\text{C}]$-raclopride (Willeit, Ginovart et al. 2006). Similar findings were reported by Graff-Guerrero et al., where $[^{11}\text{C}](+)$-PHNO showed preferential binding to the ventral striatum and globus pallidus and $[^{11}\text{C}]$-raclopride revealed preferential dorsal striatal binding (Graff-Guerrero, Willeit et al. 2008). Taken together, these results suggest that $[^{11}\text{C}](+)$-PHNO may be a valuable tracer for the imaging of both striatal and relevant extra-striatal regions. Furthermore, in light of $[^{11}\text{C}](+)$-PHNO’s 20 to 50 fold preferential binding to DRD3 compared to DRD2 in vivo (Searle, Beaver et al. 2010, Tziortzi, Searle et al. 2011, Gallezot, Beaver et al. 2012), the binding distribution noted above may possibly reflect binding to DRD3. Indeed, in the baboon brain following treatment with DRD3 antagonist SB-277011, the $[^{11}\text{C}](+)$-PHNO signal was nearly abolished in the midbrain regions of the substantia nigra/ventral tegmental area, thalamus and globus pallidus but preserved in the dorsal caudate and putamen (dorsal striatum) (Rabiner, Slifstein et al. 2009). This has similarly been shown in humans, where DRD3 binding appears to contribute to nearly 100% of $[^{11}\text{C}](+)$-PHNO binding in the substantia nigra and hypothalamus, 26% of binding in the limbic striatum, 65% in the globus pallidus, 75% in the ventral pallidum and 6% and virtually 0% of binding in the putamen and caudate, respectively (Tziortzi, Searle et al. 2011).
A comparison of the sensitivity to compete with endogenous dopamine of $[^{11}\text{C}]-(+)$-PHNO versus $[^{11}\text{C}]-\text{raclopride}$ has also resulted in interesting findings. In cats treated with various doses of intravenous amphetamine, dopamine release in response to the highest dose of the psychostimulant resulted in an approximately 85% reduction of striatal $[^{11}\text{C}]-(+)$-PHNO binding potential, but only a 56% reduction of striatal $[^{11}\text{C}]-\text{raclopride}$ binding potential (Ginovart, Galineau et al. 2006). In humans as well, an amphetamine challenge displaced $[^{11}\text{C}]-$(+)-PHNO binding to a greater degree (average 1.5 fold) than $[^{11}\text{C}]-\text{raclopride}$ in different portions of the striatum, including the ventral and dorsal portions (Shotbolt, Tziortzi et al. 2012). Most recently, $[^{11}\text{C}]-$(+)-PHNO was shown to significantly detect the smaller dopamine signal induced by nicotine in rhesus monkeys, while $[^{11}\text{C}]-\text{raclopride}$ failed to detect any effect (Gallezot, Kloczynski et al. 2013). Thus, $[^{11}\text{C}]-$(+)-PHNO may also be more sensitive to competition with endogenous dopamine than previously used antagonist radiotracer $[^{11}\text{C}]-\text{raclopride}$, and thus be more suitable to be used for drugs with smaller dopamine signals.

One explanation for this increased sensitivity of $[^{11}\text{C}]-$(+)-PHNO to competition with endogenous dopamine may be primary binding of this agonist radiotracer to the functionally active DRD2-high state to which dopamine also binds (Martinez and Narendran 2010). This would be in contrast to $[^{11}\text{C}]-\text{raclopride}$ and generally to dopamine antagonist radiotracers, where binding occurs equally with the functionally inert DRD2-low state (Laruelle 2000). However, considerable growing evidence indicating that $[^{11}\text{C}]-$(+)-PHNO is not selective for the high state, but also binds to the low state (McCormick, Kapur et al. 2008, Seeman 2012), renders this an explanation of ongoing debate.
In summary, neuroimaging through fMRI and PET provides a valuable tool for the measurement of acute drug administration effects on the human brain. Specifically, the use of PET can allow for the assessment of changes in various neurotransmitters of interest, including dopamine. While different PET radiotracers do exist to measure changes in dopamine at the DRD2/DRD3 level, the recently developed radiotracer $[^{11}\text{C}](+)$-PHNO may be more sensitive to competition with dopamine, and thus more ideal, than the previously used antagonist tracers, including $[^{11}\text{C}]$-raclopride.

1.3.7 Brain imaging of acute alcohol administration

1.3.7.1 Functional magnetic resonance imaging (fMRI) studies

Function magnetic resonance imaging (fMRI) studies have been helpful in delineating the neural substrates involved in mediating the acute effects of alcohol. In line with animal studies suggesting the importance of the mesolimbic system in mediating the effects of alcohol, intravenous administration of alcohol has been shown to significantly increase the BOLD signal in striatal reward areas, specifically in the NAc and the caudate in social drinkers (Gilman, Ramchandani et al. 2008). Moreover, greater BOLD signal in these regions appear to be correlated with both increased subjective effects of intoxication (Gilman, Ramchandani et al. 2008) and to risky decision making relative to safe choices (Gilman, Smith et al. 2012). However, this increased BOLD activation following alcohol intake is somewhat inconsistent, as a heavy drinking group in one study not demonstrate this response (Gilman, Ramchandani et al. 2012). To explain this, the authors of this study cited the existence of a complex neural response of alcohol in the human brain, which is influenced by several factors.
1.3.7.2 Positron emission tomography (PET) imaging studies

To date, there have only been a limited number of positron emission tomography studies involving alcohol administration in humans. Parallel to the findings of fMRI studies, PET studies exploring glucose metabolism have revealed changes in the mesolimbic neural substrate in response to acute alcohol. Through the use of FDG-PET, a moderate dose of oral alcohol has been evidenced to decrease the relative energy metabolism in areas of the cerebellum and occipital cortex while increasing the relative metabolism in limbic areas including the ventral and dorsal striatum (Schreckenberger, Amberg et al. 2004, Volkow, Ma et al. 2008). PET studies exploring changes in cerebral blood flow (CBF) in response to alcohol consumption have also reported increased perfusion in the neural substrate of reward system at higher doses of alcohol (Volkow, Mullani et al. 1988, Ingvar, Ghatan et al. 1998).

Of note, the majority of PET studies involving alcohol administration have explored changes in dopamine levels in the reward circuitry. The results of these studies are notably equivocal, however. Increases in dopamine levels in response to acute alcohol, primarily in the ventral portion of the striatum as seen in animals, have been evidenced in some human studies. In an early study of healthy males, a 1.0 ml/kg dose of alcohol was shown to elicit a 15% decrease in binding potential in the NAc and a 13% decrease in the ventral putamen (Boileau, Assaad et al. 2003), signaling an increase in dopamine in these regions. These changes were moreover associated with increases in heart rate following alcohol administration, as well as baseline measures of trait impulsivity in participants. In another heterogeneous group of healthy males and females, a similar dose of alcohol elicited decreases in binding potential in all of the striatal regions, with the most sizable decrease seen in the ventral striatum (Urban, Kegeles et al. 2010). When further grouped by sex, a significantly greater change in binding potential was seen.
in males in this former study, with this change positively correlated with drug induced subjective activation and negatively correlated with frequency of drinking a reported maximum number of alcoholic drinks within 24 hours.

Conversely, an initial study by Salonen et al. reported no elevation in dopamine at the level of striatal DRD2/DRD3 in response to a high dose of oral alcohol (Salonen, Hietala et al. 1997). An important caveat of this study was that the images were analyzed across the entire striatum, which may have masked a possible effect in specific striatal regions, namely the ventral striatum. However, intravenous administration of a moderate dose of alcohol designed to result in a steady state blood alcohol concentration of 0.06% also did not significantly increase endogenous dopamine levels compared to a baseline state (Yoder, Kareken et al. 2005). When the same participants were reanalyzed using a voxel extraction method of the striatum, changes in dopamine were shown to be anatomically heterogeneous, with some participants reporting increases in dopamine levels, and others reporting decreases in different regions (Yoder, Constantinescu et al. 2007). In voxels demonstrating increases in dopamine, the magnitude of the increase was further positively correlated with the level of intoxication.

This heterogeneous alcohol-induced dopaminergic response reported by Yoder et al. (Yoder, Constantinescu et al. 2007) alcohol may be explained by the recent findings that elevated dopamine following alcohol consumption was seen exclusively in those vulnerable to developing an AUD. Namely, a significant increase in endogenous dopamine in much of the striatum was seen only in individuals at high risk for developing an AUD as measured as having a lower subjective response to alcohol and higher reported sensation seeking traits (Setiawan, Pihl et al. 2013). In individuals at low risk for developing AUD, the binding potential differences
between alcohol and placebo scans were indicative of a decrease in endogenous dopamine in striatal regions. With the use of an intravenous administration route of alcohol as well, there is some evidence to suggest that striatal dopamine release may be further related to a genetic predisposition to alcoholism, specifically through the possession of the mu opioid OPRM1 A118G polymorphism. That is, intravenous alcohol administration to a steady state of 0.08% elicited significant increases in dopamine release compared to intravenous saline at the level of anterior and posterior ventral striatum, but only in those positive for the polymorphism (Ramchandani, Umhau et al. 2011). In contrast, carriers of normal allele did not show dopaminergic increases in any regions of interest. It is thus plausible that dopamine elevation in ventral striatal regions following alcohol consumption may be relevant to only those at risk for developing an AUD, representing a marker of vulnerability. This is certainly congruent with findings in animal studies of a heightened dopaminergic response in alcohol preferring rats compared to heterogeneous rats, while incongruent with other animal findings of increased dopamine in heterogeneous rats not bred for alcohol seeking.

Adding to this complication, changes in dopamine levels may also result from alcohol cues leading to reward expectation as evidenced by some studies. In social drinkers of varying family histories of alcoholism, consumption of a drink with beer flavoring and only a miniscule amount of alcohol elicited dopamine releases in right ventral striatum compared to a sports drink, with this effect most notable in those with a positive family history (Oberlin, Dzemidzic et al. 2013). Conversely, another study which predicted an elevation in striatal dopamine following the presentation of alcohol cues found an opposite effect; a decrease in dopamine levels after the presentation of alcohol cues followed by intravenous saline administration (Yoder, Morris et al. 2009). Interestingly, significant dopamine release was noted in the condition where alcohol
intravenous administration took place following neutral cues. These results were interpreted in terms of the error prediction theory, whereby a negative error (no reward following reward expectation) will decrease firing of dopamine, and positive error will increase firing.

Taken together, the results of these alcohol administration PET studies are generally inconsistent with respect to whether alcohol induces elevations in dopamine at the level of the striatum. Moreover, there are also varying proposals of what this release may signify. The heterogeneity of the dopaminergic response as evidenced by these studies may be suggestive of its selectivity to only those predisposed to developing alcohol abuse and dependence. Alternatively, in considering that all of these investigations have relied on the use of $[^{11}\text{C}]$-raclopride to measure changes in dopamine, it is possible that this radiotracer may lack the sensitivity necessary to observe changes in individuals who less vulnerable, if such changes do exist.
2. OBJECTIVES AND HYPOTHESES

2.1 Rationale

Previous attempts at detecting alcohol induced changes in dopamine levels within the human brain have yielded equivocal results, with only some studies reporting an elevation in dopamine at the level of the striatum. Furthermore, differing reports of what may influence this dopamine response have been presented. With the recent development of the agonist radiotracer, $[^{11}\text{C}]-(+)$-PHNO, possible fluctuations in dopamine levels may be detected with a greater sensitivity than the previously used radiotracer, $[^{11}\text{C}]-\text{raclopride}$. Furthermore, $[^{11}\text{C}]-(+)$-PHNO allows for the detection of changes in dopamine levels in DRD3 rich extra-striatal areas, which have largely been unexplored in existing human alcohol administration literature. Taken together, this will provide a greater understanding of the role of dopamine in human alcohol consumption.

2.2 Main Objective and Hypothesis

**Objective:** To examine the effects of a moderate to high dose of oral alcohol administration on dopamine levels in healthy social drinkers using the novel PET radiotracer, $[^{11}\text{C}]-(+)$-PHNO.

**Hypothesis:** In light of the substantiated role of the limbic striatum in the reinforcing properties of alcohol, we hypothesize that if alcohol increases dopamine levels in healthy social drinkers, then a decrease in the radiotracer binding potential in the alcohol scan compared to the placebo scan will be seen in the limbic striatum.
2.3 Exploratory Objective and Hypothesis

**Objective:** To determine the potential associations of dopamine level changes with measures of drinking history, impulsivity and personality, subjective responses to alcohol and objective responses to alcohol.

**Hypothesis:** In light of the existing literature which suggests that possession of a heavier drinking history, greater impulsivity, and characteristic subjective and objective responses to alcohol may confer greater vulnerability to AUD, we hypothesize that any changes in dopamine levels observed will correlate with these variables.
3. METHODS

3.1 Participant Selection

Participants were included into the study if the following inclusion criteria were met: 1) between the ages of 21 and 45; 2) experienced at least 2 heavy drinking occasions in the past 30 days (defined as ≥ 5 drinks for males, or ≥ 4 drinks for females); 3) capable and willing to provide informed consent; 4) possession of a good command of the English language as determined through the phone screen.

Participants were not included if the following exclusion criteria were met: 1) DSM-IV diagnosis of alcohol dependence and/or treatment for alcohol dependence; 2) taking medications or having any medical condition for which alcohol is contraindicated; 3) any medical conditions which require immediate attention or treatment; 4) previous head trauma or neurological condition or intracranial surgery; 5) Beck Depression Inventory Score (BDI) of > 16; 6) current or past suicidal ideation; 7) pregnancy or lactation; 8) current DSM-IV diagnosis of any Axis I psychiatric disorder; 9) regular use of any therapeutic or recreational psychoactive drug during the last three months (with the exception of nicotine and alcohol) or any other substance use disorder (including nicotine); 10) abnormal body mass as defined as not within 20% of normal BMI; 11) current, past or anticipated exposure to radiation exceeding 20 mSv in the last year; 12) metal implants or paramagnetic objects within the body which may interfere with the MRI; 13) claustrophobia or a history of panic attacks; 14) Abnormal clinical laboratory findings or pre-trial EKG results demonstrating clinical significant abnormality.
3.2 Participant Recruitment

Participants were recruited through the use of local advertisement in Toronto newspapers, word of mouth and through posters distributed around the CAMH and the University of Toronto campuses. Advertisements were also posted on online venues such as craigslist and NowToronto.

Of all the individuals expressing interest to participate, 187 individuals were available to be phone screened. Of the 68 individuals eligible at this point, 19 were invited to complete the in-person Screening Assessment Day. Reasons for not inviting the remaining individuals included lack of interest expressed, scheduling difficulty and loss to follow-up. A total of 12 participants were enrolled in the study, of which 4 dropped out; 1 due to scheduling difficulties following the Baseline Day, and the remaining 3 due to transient claustrophobia or general apprehension of scanning procedures. One participant was excluded following study completion as her PET images could not be accurately analysed due to an interruption during emission acquisition. This resulted in a total of 7 completers.

3.3 Sample Size Calculation

It must be noted that this study was undertaken as a pilot study. However, to observe comparable differences in radiotracer binding potential between beverage conditions as noted with similar previous studies reporting significant increases in dopamine levels (average Cohen’s $d = 1.08$) (Boileau, Assaad et al. 2003, Urban, Kegeles et al. 2010), a sample size of 10 is necessary with a power of 80% and an alpha set at 0.05.
3.4 Study Design

This was a randomized, single blind, crossover study. In total, participants completed five visits to CAMH. Following initial telephone screening, potential participants were invited to an in-person Screening Assessment Day for a full screen. Participants were enrolled in the study if deemed eligible at this point. A Baseline Day followed whereby they completed various baseline questionnaire and tasks. Two PET scans were then completed on two separate days, following alcoholic beverage consumption on one day and placebo beverage consumption on the other day. The administration of the beverage condition was randomized and counterbalanced across participants, with participants blinded to which beverage they were receiving. A final separate visit was made for the completion of a MRI scan. The study design is illustrated in Figure 3.4.

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**Figure 3.4** Flow diagram of all study visits. PET scans were at least one week apart.
3.5 Study Procedures

3.5.1 Screening Assessment Day Procedures

Participants were initially screened with a brief telephone screen to assess for a subset of the inclusion and exclusion criteria. If deemed potentially eligible upon initial screenings, participants were invited to an in-person Screening Assessment Day. The Screening Assessment Day began with an initial description of all study procedures, including a thorough description of the PET scan procedures. Participants were also shown an example thermoplastic immobilization mask to be used in the PET scanner. Participants then signed the informed consent if they remained interested.

For descriptive purposes, participants received a baseline breath alcohol concentration measurement using the Alco-Sensor FST®, and an expired breath carbon monoxide measurement using the Micro+™ Smokerlyzer®. Participants were then asked questions regarding demographics and safety to undergo a MRI scan necessary for analysis purposes. To assess for mental health, the Mini International Neuropsychiatric Interview (MINI) (Sheehan, Lecrubier et al. 1998) was administered. To assess for nicotine dependence in smokers, the Fagerstrom Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski et al. 1991) and Minnesota Nicotine Withdrawal Scale (M-NWS) (Hughes and Hatsukami 1986) were administered. To determine the presence of depressive symptoms in participants, the Beck Depression Inventory (BDI) was administered (Beck and Steer 1987). In addition, for descriptive purposes, the following questionnaires were also administered to gather information regarding tobacco and alcohol use:
(a) **Alcohol Use Disorders Identification Test (AUDIT):**

The AUDIT was used to help characterize the degree of risky or harmful patterns of drinking reported by participants. The AUDIT consists of 10 questions regarding recent alcohol use, alcohol related problems and alcohol dependence (Saunders, Aasland et al. 1993) in the past year. A score of 8 or more has been suggested to be a good indicator of hazardous drinking (Bohn, Babor et al. 1995).

(b) **Timeline Followback (TLFB):**

The Timeline Followback (TLFB) was used to assess the level of alcohol and nicotine use in the past 90 days. The TLFB has been shown to be effective in the determination of both smoking (Harris, Golbeck et al. 2009) and alcohol intake (Sobell, Sobell et al. 1988). Briefly, participants were guided to recall, through the use of anchored events, the days in the past 90 days during which alcohol or nicotine was consumed. The number of standard drinks and cigarettes during each of these days were also recorded. From the TLFB, a number of variables were generated including the number of drinks per week, the number of drinks per drinking day, the maximum number of drinks per drinking day and the frequency of maximum number of drinks.

Following the completion of questionnaires, participants received a general physical examination by a study physician and were then taken to the CAMH Clinical Laboratory where they provided a urine sample for toxicology and blood samples for basic indicators including pregnancy in females. A 12-lead EKG was also performed. Following receipt of the lab results, the participant’s folder was reviewed for eligibility. If eligible, participants were scheduled for a Baseline Day.
3.5.2 Baseline Day Procedures

Upon arrival at CAMH, participants received a 10-panel urine drug screen rapid cup test and an expired carbon monoxide measurement to ensure compliance to protocol instructions to refrain from all illicit drugs, as well as nicotine. The baseline day served to assess differences that may influence response to alcohol during the PET scan days, namely family history of alcoholism, personality traits and levels of impulsivity. Participants completed the following assessments:

(a) The Family History Assessment Module (FHAM) of the Semi-structured Assessment for the Genetics of Alcoholism (SSAGA) II

The Family History Assessment Module was employed to assess the presence of a positive family history of alcoholism based on DSM III-R criteria (Rice, Reich et al. 1995). Positive family history was defined as having a first degree relative with alcoholism. Specifically, participants were initially administered a Screener to determine if, to their knowledge, any relatives had previous or current problems with alcohol use. This was followed by an Individual Assessment Module to determine the degree of problematic use, and the presence of a diagnosis of alcohol dependence.

(b) The Revised NEO Personality Inventory (NEO PI-R)

This widely used 240 item inventory was created to assess the five major personality factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness and Conscientiousness (McCrae and Costa 1987). Participants in this study completed this inventory
as a self-report, with each item graded on a 5-point Likert scale ranging from “Strongly agree” to “Strongly disagree.” A total score was then generated for each personality factor.

(c) **UPPS-P Impulsive Behavior Scale**

Unlike various impulsivity questionnaires which attempt to measure trait impulsivity, the UPPS-P Impulsive Behavior Scale assesses pathways leading to impulsive behaviour. The original UPPS scale assesses 4 pathways - urgency, lack of premeditation, lack of perseverance, and sensation seeking (Whiteside and Lynam 2001). This revised version further assesses positive urgency, which is differentiated from the previous negative urgency pathway (Cyders, Smith et al. 2007). Participants completed this scale as a self-report, on a 4-point Likert scale ranging from “Strongly agree” to “Strongly disagree”. An average total score was then generated for each pathway.

**3.5.3 PET Scan Procedures**

All PET scans were scheduled for either noon or mid-afternoon. PET scans were also scheduled at least a week apart. The day before the scheduled PET scans, participants were reminded to refrain from drinking for 24 hours prior to coming in the next day. To standardize time since last meal, participants were asked to fast approximately 4 hours from the time they were scheduled to receive their experimental beverage. Participants were further asked to limit their caffeine consumption to one cup in the morning of their PET scan to limit interference with scan accuracy.

Upon arriving at the PET Centre at CAMH, a breath alcohol measurement and an expired carbon monoxide (CO) reading was taken to assess for compliance to study instructions. A
breath alcohol reading of 0.00% and an expired CO reading of <10 ppm were expected. In addition, a 10-panel urine drug screen cup test was administered, along with a urine pregnancy test for female participants. These tests were required to be negative to proceed with study procedures.

To determine the participants’ mood prior to scanning on each scan day, participants completed the following:

**Profile of Moods States (POMS)**

The POMS is a 65 item questionnaire regularly employed to assess recent affective mood states. Specifically, participants were asked to report how they were feeling in the last week, including the current time, on a 5-point Likert Scale ranging from “Not at all” to “Extremely”. The individual items were then grouped into the six different affective states: Tension-Anxiety, Anger-Hostility, Fatigue-Inertia, Depression-Dejection, Vigor-Activity, Confusion-Bewilderment (McNair 1971). A Total Mood Disturbance score was generated from the totals of all the subscales.

Furthermore, for female participants, a discussion of where the participant was in her menstrual cycle took place. This information was collected for descriptive purposes.

Participants were then fitted with an intravenous catheter in the antecubital vein of their arm by a trained PET Centre technician, and then asked to lie down in the scanner. A thermoplastic mask was fitted using the Orbit mask fitting systems.
Instructions were then given to consume the beverage through a straw over a 15 minute period, with consumption spaced equally across the consumption period. Immediately following beverage completion, participants received an initial transmission scan. This was followed by an injection of the radiotracer, $[^{11}\text{C}]-(+)$-PHNO. The PET scan began at this point.

Several subjective and objective measures were taken throughout both of the PET Scans. These measures were acquired together during the following approximate times: 0 minutes (prior to drink initiation), 10 minutes post drink completion, 25 minutes drink post completion, 40 minutes post drink completion, 70 minutes post drink completion, and 100 minutes post drink completion (Figure 3.5.3).
Figure 3.5.3 PET scan timeline. Blue circles denote measurement of objective (blood samples for cortisol and alcohol concentration, blood pressure, heart rate) and subjective measures (Alcohol Urge Questionnaire (AUQ), Biphasic Alcohol Effects Scale (BAES)) at the different time points (T1-T6). Red arrow denotes [11C]-(+)-PHNO injection.
**Objective Measures**

(a) **Blood Samples**

Two 5.0 mL vials of blood were taken by a trained PET Centre Technician at each of the time points. To assess the level of stress hormones, one vial was used to determine the blood cortisol concentration throughout each of the two scan days. The second vial was used to determine blood alcohol concentration following beverage consumption on each of the scan days.

(b) **Heart Rate and Blood Pressure**

Systolic and diastolic blood pressures as well as heart rate were measured at each time point with an automatic cardiovascular monitor from the PET Centre. Specifically, the Propaq monitor (Welch Allyn Propaq CS, Model 242) was employed.

**Subjective Measures**

(a) **Alcohol Urge Questionnaire (AUQ)**

The Alcohol Urge Questionnaire (AUQ) is an 8-item questionnaire used to assess one’s feelings regarding drinking alcoholic beverages (Bohn, Krahn et al. 1995). Specifically, participants were asked to determine how they thought that the 8 statements of alcohol craving agreed with their feeling at each of the time points. They answered on a 7-point Likert scale which ranged from “Strongly disagree” to “Strongly agree”. A total score using the average of the responses was generated.
(b) Biphasic Alcohol Effects Scale (BAES)

The Biphasic Alcohol Effects Scale (BAES) was used to assess the direct subjective effects of alcohol. This 14-item adjective checklist incorporates both the stimulant and sedative effects of alcohol. Participants were asked to respond on an 11-point scale ranging from “Not at all” to “Extremely”. A total score was determined for the two subscales of stimulation and sedation by summing the respective items (Martin, Earleywine et al. 1993).

3.5.4 Magnetic Resonance Imaging (MRI) Scan

MRI scans were completed at the Imaging Centre at CAMH. Prior to the scan, participants received a 10-panel urine drug screen cup test and an expired carbon monoxide measurement to determine compliance to protocol instructions. Furthermore, females received a urine pregnancy test.

3.5.5 Randomization Considerations

The CAMH research pharmacy oversaw the randomization process of participants in this study. A randomization table was created with 3 blocks of 4. The code for this randomization was kept confidential in the research pharmacy.

3.6 Materials

3.6.1 Alcoholic and Placebo Beverages

The alcoholic beverage comprised of 95% USP ethyl alcohol from Commercial Alcohols. This was given at a dose of 1.5 g/L of body water (approximately 0.80g/kg of body weight; (Evans and Bisaga 2009). This is roughly equivalent to 3-5 drinks in an average woman.
weighing 66 kg and average man weighing 83 kg (Gilmore 1999). The amount of total body water (TBW) was determined using the equations outlined by Watson et al. (Watson, Watson et al. 1980). These equations (below) have been used in previous studies involving alcohol administration (Curtin and Fairchild 2003, Urban, Kegeles et al. 2010).

**Total body water (TBW) equations:**

**Males:**

\[
2.447 - (0.09156 \times \text{_____ Yrs}) + (0.1074 \times \text{_____ cm}) + (0.3362 \times \text{_____ Kg}) = \text{_____ L}
\]

Age \hspace{1cm} Height \hspace{1cm} Weight \hspace{1cm} TBW

**Females:**

\[
-2.097 + (0.1069 \times \text{_____ cm}) + (0.2466 \times \text{_____ Kg}) = \text{_____ L}
\]

Height \hspace{1cm} Weight \hspace{1cm} TBW

The alcohol was mixed with orange juice and tonic water with the following ratio: 1 part alcohol to 2 parts Tropicana Pure Premium® Original orange juice and 3 parts Schweppes™ Tonic Water.

The placebo beverages were identical to the alcoholic beverage (including volume), with the exception of an additional part of tonic water substituted for the 1 part alcohol. The final ratio of the placebo beverage was 4 parts Schweppes™ Tonic Water and 2 parts Tropicana Pure Premium®.

Both beverages had 2 mL of Absolut Vodka® floated on top and were capped and chilled.
3.6.2 $[^{11}\text{C}]-(-)-\text{PHNO}$ synthesis

The radiosynthesis and evaluation of $[^{11}\text{C}]-(-)-4$-Propyl-3,4,4a,5,6,10b-hexahydro-2$H$-naphtho[1,2-$b$][1,4]oxazin-9-ol ($[^{11}\text{C}]-(-)-\text{PHNO}$) has been described elsewhere (Wilson, McCormick et al. 2005). Briefly, $[^{11}\text{C}]-\text{Propionyl chloride}$ in THF was added to a vial containing 9-hydroxynaphthoxazine in THF. Following this reaction which produces an $[^{11}\text{C}]-\text{amide}$, the reducing agent LiAlH$_4$ was added to the mixture. Purification by high performance liquid chromatography and formulation in saline followed. This resulted in a radiochemically pure $[^{11}\text{C}]-(-)-\text{PHNO}$ as a sterile, pyrogen-free solution suitable for human studies.

3.7 PET Image Acquisition

PET scans were performed using a second-generation high-resolution tomograph (Siemens-HRRT; Siemens Molecular Imaging) consisting of 8 panel detectors, each composed of 117 (lutetium oxyorthosilicate / lutetium yttrium oxyorthosilicate) LSO/LYSO phoswiches detectors, arranged 13 axial by 9 radial. The CPS-HRRT tomograph samples 207 slices spaced 1.2mm, covering a transaxial width of 31.2 cm, and an axial extent of 25.4 cm in 3D mode.

Participants were scanned supine with their head held in place using a custom-made thermoplastic facemask fixation system (Orfit Industries). Transmission scans were acquired immediately after drink completion, using a single-photon $^{137}\text{Cesium}$ ($E_{\gamma} = 662$ keV) point-source, and used to correct the emission scans for the attenuation of 511 keV photons through tissue and head support. After transmission, $[^{11}\text{C}]-(-)-\text{PHNO}$ was injected as a bolus into the intravenous line placed in an antecubital vein. The mean mass of $[^{11}\text{C}]-(-)-\text{PHNO}$ was 2.01 µg (SD: ±0.42 µg; range: 1.35 – 2.54 µg), with a mean injected activity of 9.26 mCi (SD: ± 0.99 mCi; range: 7.34 - 10.61 mCi) and a mean specific radioactivity of the injected dose was 1202.71 mCi/µmol (SD:
366.65 mCi/µmol; range: 764.96 - 1920.53 mCi/µmol). This initiated the scan, and data were acquired for 90 minutes after the injection. Data were reconstructed by the Fourier rebinning (FORE) filtered back projection algorithms (Defrise, Kinahan et al. 1997).

3.8 MR Image Acquisition

For regions of interest (ROI) delineation during PET image analysis, a proton density (PD) image was acquired on the General Electric MR750 3T scanner at CAMH. The scan parameters were a repetition time of 6000 ms, a slice thickness of 2 mm, number of excitations (NEX) of 1, field of view (FOV) of 22 cm, 256 x 196 acquisition matrix, and echo time of 8 ms.

3.9 PET Image Analysis

Regions of Mental Interest (ROMI), an automated software for the analysis of PET data, was used for regions of interest (ROI) delineation and time activity curve (TAC) generation as previously described elsewhere (Rusjan, Mamo et al. 2006). Firstly, a standardized brain template with the ROIs already defined was transformed onto the participant’s proton density weighted MR image. Specifically, the functional striatal regions of the sensorimotor striatum (SMST), the associative striatum (AST) and the limbic striatum (LST) were segmented as described by Martinez et al (Martinez, Slifstein et al. 2003). This automated procedure was also used for the delineation of the extra-striatal regions of the globus pallidus (GP; (Rusjan, Mizrahi et al. 2006)) and the substantia nigra (SN; (Rusjan, Mamo et al. 2006)). The ventral pallidum (VP) was further automatically segmented using a pre-existing template. In ROMI, a refinement step followed region delineation based on the probability of grey matter of voxels in the MR image. The MR image was then co-registered to the PET image, allowing for the generation of $[^{11}\text{C}]-(+)-\text{PHNO}$ TACs in each ROI. Specific binding potential estimation was completed using
PMOD (version 2.8.5; PMOD Technologies) to generate non-displaceable binding potential (BP\textsubscript{ND}) values. Specifically, the TACs were extracted into this software, and a simplified reference tissue model (SRTM) (Lammertsma and Hume 1996) was applied. With this model, the cerebellum cortex was used as a reference region due to a lack of binding sites for $[^{11}\text{C}]-$ (+)-PHNO (Ginovart, Galineau et al. 2006, Ginovart, Willeit et al. 2007). Motion correction was applied to one participant for the placebo scan due to considerable head motion resulting from the use of a bed plan during scanning. The correction technique was implemented using ROMI, and is based on a realignment process as described by Mawlawi et al. (Mawlawi, Martinez et al. 2001).

3.10 Statistical Analysis

In light of the small sample size, only non-parametric statistical tests were employed.

Firstly, to compare the standardized radioactivity in the reference region, the area under the curve (AUC) of the TACs in the cerebellum (standard uptake values (SUV); calculated as [radioactivity concentration]/[injected radioactivity/body weight]) were compared between the placebo and alcohol scan using the Wilcoxon signed rank test. The AUC values were calculated using the trapezoidal method. To explore changes in dopamine levels following alcohol consumption compared to placebo, the percent change in binding potential was generated using the following formula: $[\text{BP}_{\text{ND}} \text{alcohol} - \text{BP}_{\text{ND}} \text{placebo}]/[\text{BP}_{\text{ND}} \text{placebo}] \times 100$. Differences in $[^{11}\text{C}]-$ (+)-PHNO BP\textsubscript{ND} in the different ROIs between beverages conditions were analyzed using the Wilcoxon signed rank test.
For repeated subjective (AUQ, BAES) and objective data (heart rate, systolic and diastolic blood pressure, blood cortisol levels) collected during the PET scans, variables were firstly transformed into change scores by subtracting T1 baseline values from the values obtained in subsequent time points (T2 to T6). These change scores were analyzed in two separate ways: i) as AUC using the trapezoidal rule and compared between scans using Wilcoxon signed rank test, ii) and simply compared between scans using Wilcoxon signed rank test at each specific time point. For subjective measures (AUQ, BAES), both total scores and individual scale items were analyzed using the above two methods. The AUC analysis allowed for a global measurement of each variable throughout the duration of each PET scan, while comparisons at each time point allowed for greater delineation of any effect. Corrections for multiple comparisons were not applied given the pilot nature of this study.

To explore the possibility of an order effect, the main outcome variable of ΔBPND in the various ROIs were correlated with scan order using bivariate Spearman’s rank correlation coefficients. The following variables were also correlated with ΔBPND in each region of interest using Spearman’s rank correlation coefficients: drinking history, baseline variables (NEO PI-R subscale scores and UPPS-P subscale scores), PET scan objective variables (peak blood alcohol level, change in blood cortisol, change in heart rate and change in blood pressure) and PET scan subjective variables (change in total urges AUQ score and change in total stimulation and total sedation BAES scores). With the exception of peak alcohol concentration, all variables collected during the PET scans were calculated as ΔAUC scores using the following formula: AUC variable (alcohol scan) – AUC variable (placebo scan).
Significance was defined as $p < 0.05$ and trend level was set to be $0.5 \leq p < 0.10$. All tests were performed using the statistical software package SPSS 20.0 (SPSS Inc., Chicago IL, USA).
4. RESULTS

4.1 Participant Demographics

The demographics of the 7 participants, including drinking history in the past 90 days, are shown below (Table 4.1). Two participants were occasional smokers; one participant smoked 6 cigarettes over 90 days while the other participant smoked 1 cigarette over 90 days.

Table 4.1 Participant demographics.
M=male, F=female; HSD = high-school degree, PSD= post-secondary degree, GD=graduate degree; FP=family history positive, FN=family history negative, FA=family history ambiguous

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>7 (4 M, 3 F)</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.43 (4.43)</td>
<td>23.00 (22.00, 29.00)</td>
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<tr>
<td>Highest Education level</td>
<td>HSD (n=2), PSD (n=4), GD (n=1)</td>
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<td>Body Mass Index (BMI)</td>
<td>23.39 (2.47)</td>
<td>22.50 (21.60, 26.00)</td>
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<td>-</td>
</tr>
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<td>Number drinks per week (past 90 days)</td>
<td>6.99 (3.16)</td>
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<tr>
<td>Number drinks per drinking day (past 90 days)</td>
<td>4.27 (1.43)</td>
<td>5.00 (2.77, 5.32)</td>
</tr>
<tr>
<td>Max number drinks per drinking day (past 90 days)</td>
<td>8.57 (1.99)</td>
<td>8.00 (7.00, 10.00)</td>
</tr>
<tr>
<td>Frequency of max drinks (past 90 days)</td>
<td>1.29 (0.76)</td>
<td>1.00 (1.00, 1.00)</td>
</tr>
</tbody>
</table>
4.2 Baseline Questionnaires

The subscale scores of the personality and impulsivity baseline questionnaires are shown in Table 4.2 below. For the Revised NEO Personality Inventory, results for one participant were not available due to a technical issue with the questionnaire following data collection (n=6).

<table>
<thead>
<tr>
<th>Table 4.2 NEO PI-R and UPPS-P scores.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>UPPS-P Impulsive Behavior Scale</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>Revised NEO Personality Inventory</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

4.3 Blood Alcohol Levels

On average, participants received 58.40 g of pure alcohol (SD: 9.91; Mdn (IQR): 58.73 g (49.33, 67.19)). Peak blood alcohol concentration ((Mean (SD): 0.096% (0.015); Mdn (IQR): 0.099% (0.09, 0.11)) was reached 100 minutes following beverage completion for 5 of the participants. For the remaining 2 participants, peak concentration was reached 70 and 40 minutes following beverage completion. The blood alcohol curve is shown in Figure 4.3 below.
Figure 4.3 Boxplots of blood alcohol concentration (BAC) for alcohol beverage scan. Medians are presented as horizontal lines. Means are denoted by black crosses.

4.4 Scan Parameters
Table 4.4 Scan parameters of alcohol and placebo scan.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Alcohol</th>
<th>Placebo</th>
<th>Z-score (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline POMS (Total Mood Disturbance)</td>
<td>53.57 (23.31)</td>
<td>62.00 (33.00, 74.00)</td>
<td>54.86 (29.25)</td>
</tr>
<tr>
<td>Mass Radioligand Injected (µg)</td>
<td>2.09 (0.41)</td>
<td>2.22 (1.75, 2.43)</td>
<td>1.93 (0.44)</td>
</tr>
<tr>
<td>Injected activity (mCi)</td>
<td>8.89 (1.06)</td>
<td>9.07 (7.78, 9.57)</td>
<td>9.58 (0.84)</td>
</tr>
<tr>
<td>Specific Activity at time of injection (mCi/µmol)</td>
<td>1119.48 (406.75)</td>
<td>1011.88 (783.94, 1351.64)</td>
<td>1285.95 (331.15)</td>
</tr>
</tbody>
</table>
There were no significant differences in the PET scan radiotracer parameters between the two scan conditions (Table 4.4). Notably, participants did not differ in their level of mood disturbance prior to each scan, as measured by the POMS (z = -0.42; p=0.67).

4.5 Subjective and Objective Scan Measures – Area Under the Curve (AUC)

4.5.1 Biphasic Alcohol Effects Scale AUC

Participants did not show a significant difference in the AUC of the total stimulation change scores (z = -1.01, p = 0.31) between the two scans. The AUC of the total sedation change scores also did not differ significantly (z=-0.51, p=0.61) (Figure 4.5.1). Furthermore, none of the AUC of the change scores for the 14 individual BAES items were significantly different between scans.

Figure 4.5.1 Boxplots of BAES total stimulation and total sedation change scores (AUC). No significant differences were noted in the AUC for either subscale. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.
4.5.2 Alcohol Urges Questionnaire AUC

No significant difference was noted between the two scans in the AUC of the total urge change score ($z = -0.34, p=0.74$) (Figure 4.5.2). Furthermore, none of the AUC of the change scores for the 8 individual items of the AUQ were significantly different between the two scans.

4.5.3 Blood Cortisol AUC

No significant difference was noted between the two scans in the AUC of blood cortisol level change scores ($z = -0.51, p=0.61$) (Figure 4.5.3).

4.5.4 Blood Pressure AUC

For systolic blood pressure, no significant difference was noted between the two scans in the AUC of change scores ($z = 0.00, p=1.00$) (Figure 4.5.4.1). For diastolic blood pressure, the AUC for pressure change scores was statistically different between the two scans ($z=-2.20, p<0.05$), with a greater AUC for the placebo scan (Mean (SD): 7.29 (21.73); Mdn (IQR): 12.5 (-15.00, 27.00)) compared to the alcohol scan (Mean (SD): -25.93 (24.13); Mdn (IQR): -30.00 (-44.50, -9.50)). This is shown in Figure 4.5.4.2.

4.5.5 Heart Rate AUC

No significant difference was noted between the two scans in the AUC of heart rate change scores ($z = -0.17, p=0.87$) (Figure 4.5.5).
Figure 4.5.2 Boxplots of AUQ total urge change scores (AUC). No significant differences were noted in the AUC between scans. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.

Figure 4.5.3 Boxplots of blood cortisol change scores (AUC). No significant differences were noted in the AUC between scans. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.
Figure 4.5.4.1 Boxplots of systolic blood pressure change scores (AUC). No significant differences were noted in the AUC between scans. Medians are presented as horizontal lines. Means are denoted by black crosses.

Figure 4.5.4.2 Boxplots of diastolic blood pressure change scores (AUC). Significant difference indicated (p<0.05) during alcohol scan compared to placebo scan. Medians are presented as horizontal lines. Means are denoted by black crosses.
Figure 4.5.5 Boxplots of heart rate change scores (AUC). No significant differences were noted in the AUC between scan Medians are presented as horizontal lines. Means are denoted by black crosses.

4.6 Subjective and Objective Scan Measures – Change scores

Change scores of all subjective and objective measures taken during the PET scans were compared between scans for each of the time points (25 minutes – 115 minutes).

4.6.1 Biphasic Alcohol Effects Scale change scores

There were no significant differences between scans in the BAES total stimulation and total sedation change scores at any of the time points. However, when the individual BAES items were explored, there were significant differences between scans in the item “Excited” at 25 minutes (z= -2.06, p < 0.05) and 55 minutes (z= -1.98, p<0.05). Specifically, at 25 minutes, greater excitement scores from baseline were reported during the alcohol scan (Mn (SD): 1.29 (1.80); Mdn (IQR): 2.00 (-1.00, 2.00)) compared to the placebo scan (Mn (SD): -1.00 (1.00); Mdn (IQR): -1.00 (-1.00, 0.00)). Similarly, at 55 minutes, excitement scores from baseline were greater for the alcohol scan (Mn (SD): -0.29 (1.38); Mdn (IQR): 0.00 (-1.00, 1.00)) compared to
placebo (Mn (SD): -1.86 (1.07); Mdn (IQR): -2.00 (-3.00, -1.00)). This is illustrated in Figure 4.6.1.1.

Some trend level differences in the individual BAES items were also noted. Specifically, for the BAES item “Sedated”, participants reported greater sedation from baseline ($z = -1.81$, $p=0.07$) during the alcohol scan (Mean (SD): 2.86 (3.39); Mdn (IQR): 4.00 (2.00, 5.00)) compared to the placebo scan (Mean (SD): 1.29 (2.87); Mdn (IQR): 1.00 (0.00, 3.00)) at 40 minutes (Figure 4.6.1.2). Finally, a trend level difference was noted in the item “Talkative” at 25 minutes ($z=-1.80$, $p=0.07$), with greater scores reported during the alcohol scan (Mean (SD): 0.57 (1.72); Mdn (IQR): 0.00 (-1.00, 3.00)) compared to the placebo scan (Mean (SD): -1.14 (1.35); Mdn (IQR): -1.00 (-3.00, 0.00). This is shown in Figure 4.6.1.3.

4.6.2 Alcohol Urge Questionnaire change scores

There were no significant differences between scans in the total urge change score or the individual AUQ items change scores, at any of the time points. However, there was a trend in the AUQ item “it would be difficult to turn down a drink this minute” ($z = -1.73$, $p=0.08$) at 115 minutes. Participants reported greater difficulty in turning down a drink from baseline during the alcohol scan (Mean (SD): 0.57 (0.79); Mdn (IQR): 0.00 (0.00, 1.00)) compared to the placebo scan (Mean (SD): 0.14 (0.38); Mdn (IQR): 0.00 (0.00, 0.00)). This is shown in Figure 4.6.2.

4.6.3 Blood cortisol change score

While a significant difference between the alcohol and placebo scan in the blood cortisol change score was not evident in any of the time points, there was a notable trend level difference at 85 minutes ($z = -1.69$, $p=0.09$) (Figure 4.6.3). Greater blood cortisol levels from baseline were
noted during the placebo scan (Mean (SD): 133.00 (115.48); Mdn (IQR): 111.00 (64.00, 214.00)) compared to alcohol scan (Mean (SD): 83.14 (79.82); Mdn (IQR): 94.00 (31.00, 154.00)).

4.6.4 Blood pressure change scores

For systolic blood pressure, no significant or trend level difference in blood pressure changes were evident between scans at any of the time points. However, there was a trend level difference in the change in diastolic blood pressure from baseline at 40 minutes ($z = -1.78$, $p = 0.08$), and significant differences at 55 minutes ($z = -2.02$, $p < 0.05$) and 85 minutes ($z = -2.02$, $p < 0.05$). At 55 minutes, participants had a greater decrease in diastolic blood pressure from baseline during the alcohol scan (Mean (SD): -4.57 (4.20); Mdn (IQR): -5.00 (-8.00, -1.00)) compared to the placebo scan (Mean (SD): 0.43 (4.35); Mdn (IQR): -2.00 (-3.00, 5.00)). Similarly, at 85 minutes, greater decreases in pressure from baseline was evident during the alcohol scan (Mean (SD): -5.14 (5.05); Mdn (IQR): -6.00 (-8.00, -1.00)) compared to the placebo scan (Mean (SD): 2.29 (4.72); Mdn (IQR): 2.00 (-2.00, 6.00)). These results are shown in Figure 4.6.4.

4.6.5 Heart rate change scores

There were no significant differences in change in heart rate at any of the time points explored.
Figure 4.6.1.1 Boxplots of change scores of “Excited” BAES item. Significant differences at 25 minutes (p<0.5) and 55 minutes (p<0.05) noted. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.

Figure 4.6.1.2 Boxplots of change scores of “Sedated” BAES item. Trend level difference at 40 minutes noted. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.
Figure 4.6.1.3 Boxplots of change scores of “Talkative” BAES item. Trend level difference at 25 minutes noted. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.

Figure 4.6.2 Boxplots of change scores of “It would be difficult to turn down a drink” AUQ item. Trend level difference at 115 minutes noted. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.
Figure 4.6.3 Boxplots of change scores of blood cortisol levels. Trend level differences at 85 minutes noted. Medians are presented as horizontal lines. Means are denoted by black crosses. Outliers are denoted by individual dots.

Figure 4.6.4 Boxplots of change scores of diastolic blood pressure. Trend level difference at 40 minutes, and significant differences at 55 minutes (p<0.05) and 85 minutes (p<0.05) noted. Medians are presented as horizontal lines. Means are denoted by black crosses.
4.7 PET Data

The AUC of the TACs in the reference region of the cerebellum did not differ significantly (p=0.40) between the placebo and alcohol scans. The time activity curves for the two scan conditions is illustrated in Figure 4.7.1. The binding potential in the globus pallidus (GP) was undetectable in one participant. In another participant, there was considerable noise leading to unusually low binding potential (BP\textsubscript{ND}= 0.13) in the placebo scan within the GP. This left only 5 cases to be analyzed for this region. None of the regions of interest revealed a significant difference in \([^{11}\text{C}]-(+)-\text{PHNO}\) binding potentials between the two scans. Inspection of the average percent change in binding potential of the alcohol scan relative to the placebo scan revealed a decrease in \([^{11}\text{C}]-(+)-\text{PHNO}\) binding potential in the limbic striatum (LST), globus pallidus (GP), ventral pallidum (VP) and to a small degree, the associative striatum (AST). The other regions – substantia nigra (SN) and sensorimotor striatum (SMST) - revealed an increase in binding potential of the alcohol scan relative to the placebo scan. The percent change in binding potential for each of the regions, along with the Z-scores and p-values for the Wilcoxon signed rank test, are displayed in Table 4.7. Figure 4.7.2 and Figure 4.7.3 illustrate the measured binding potentials for the two scans.
Figure 4.7.1. Cerebellum time activity curves for alcohol and placebo scan, represented as standard uptake values (SUV). Mean ±SD are presented.

Table 4.7 Percent change of $B_{ND}$ and Z-scores of Wilcoxon signed rank test of alcohol scan versus placebo scan. LST = limbic striatum; AST = associative striatum; SMST = sensorimotor striatum; GP = globus pallidus (n=5); SN = substantia nigra

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Percent Change $B_{ND}$ (%)</th>
<th>Z-score (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>LST</td>
<td>-4.83 (12.92)</td>
<td>0.87 (-18.55, 7.10)</td>
</tr>
<tr>
<td>AST</td>
<td>-1.42 (14.44)</td>
<td>0.16 (-14.88, 6.95)</td>
</tr>
<tr>
<td>SMST</td>
<td>4.84 (20.83)</td>
<td>8.26 (-14.09, 12.00)</td>
</tr>
<tr>
<td>GP</td>
<td>-4.90 (18.33)</td>
<td>-2.36 (-21.81, 10.75)</td>
</tr>
<tr>
<td>SN</td>
<td>6.96 (44.46)</td>
<td>2.01 (-24.58, 25.29)</td>
</tr>
<tr>
<td>VP</td>
<td>-12.22 (22.60)</td>
<td>-25.73 (-27.24, 12.30)</td>
</tr>
</tbody>
</table>
Figure 4.7.2. Scatter plot of individual participants’ BP$_{ND}$ for alcohol and placebo scan. Each participant is represented by a different shape. Medians are presented as horizontal lines. GP: n=5.

Figure 4.7.3. Boxplots of BP$_{ND}$ for alcohol and placebo scan. Medians are presented as horizontal. Means are denoted by black crosses. Outliers are denoted by individual dots. GP: n=5.
4.8 Correlations

There was a significant positive correlation with $\Delta BP_{ND}$ in the LST and scan order ($\rho (5) = 0.87, p<0.05$). This is shown in Figure 4.8.1. A trend level positive correlation was also seen with $\Delta BP_{ND}$ in the GP and scan order ($\rho (3) = 0.87, p=0.06$). No other ROIs revealed either significant or trend level correlations with scan order.

The correlations of $\Delta BP_{ND}$ in the ROIs and the various variables of interest are presented in Table 4.8. Significant and trend level significant correlations are noted.

The $\Delta BP_{ND}$ in the AST was significantly positively correlated with peak blood alcohol concentration ($\rho (5) = 0.89, p<0.01$). This is shown in Figure 4.8.2. The $\Delta BP_{ND}$ in the VP was significantly negatively correlated with AUC of systolic blood pressure from baseline ($\rho (5) = \ldots$). 

Figure 4.8.1 Scatter plot for spearman’s rank correlation of $\Delta BP_{ND}$ in LST and scan order. Spearman’s rho = 0.87, p<0.05. Two data points are overlapping with scan order=2 and $\Delta BP_{ND} = \sim 0.0.$
-0.79, p<0.05). This is shown in Figure 4.8.3. The ΔBP_{ND} in the GP was significantly negatively correlated with the following UPPS-P subscale scores: (lack of) Perseverance (ρ (3) = -0.90, p<0.05); Positive Urgency (ρ (3) = -0.98, p<0.01); and Negative Urgency (ρ (3) = -1.00, p<0.001). The ΔBP_{ND} in the GP was further negatively correlated with the NEO PI-R Neuroticism score (ρ (2) = -1.00, p<0.001) and positively correlated with the NEO PI-R Agreeableness score (ρ (2) =1.00, p<0.001) and Conscientiousness score (ρ (2) = 1.00, p<0.001). Figure 4.8.4 illustrates the correlation between ΔBP_{ND} in the GP and the UPPS-P Positive Urgency score. Figure 4.8.5 illustrates the correlation between ΔBP_{ND} in the GP and the NEO PI-R Agreeableness score.

Trend level significant correlations included: the positive correlation of ΔBP_{ND} in the SN and the NEO PI-R Conscientiousness score (ρ (4) = 0.77, p = 0.07); the positive correlation of ΔBP_{ND} in the SMST and number of drinks per week (ρ (5) = 0.71, p = 0.07); the positive correlation of ΔBP_{ND} in the VP and the AUC of BAES total sedation score from baseline (ρ (5) = 0.75, p = 0.05); the positive correlation of ΔBP_{ND} in the LST and peak blood alcohol concentration (ρ (5) = 0.68, p = 0.09); and the positive correlation of ΔBP_{ND} in the SN and peak blood alcohol concentration (ρ (5) = 0.71, p = 0.07).
Table 4.8 Spearman’s rank correlation coefficients. ρ = rho (correlation coefficient); df = degrees of freedom; * = trend level significance (0.05 ≤ p < 0.10); ** = significant (p < 0.05)

<table>
<thead>
<tr>
<th>Variable</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; LST ρ (df), p value</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; AST ρ (df), p value</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; SMST ρ (df), p value</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; GP ρ (df), p value</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; SN ρ (df), p value</th>
<th>ΔBP&lt;sub&gt;ND&lt;/sub&gt; VP ρ (df), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UPPS-P Impulsivity Scale</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Urgency</td>
<td>-0.54 (5), p = 0.22</td>
<td>-0.25 (5), p = 0.60</td>
<td>-0.46 (5), p = 0.29</td>
<td>-1.00 (3), p &lt; 0.001**</td>
<td>-0.42 (5), p = 0.34</td>
<td>-0.07 (5), p = 0.88</td>
</tr>
<tr>
<td>(lack of) Premeditation</td>
<td>0.04 (5), p = 0.94</td>
<td>-0.14 (5), p = 0.76</td>
<td>0.21 (5), p = 0.65</td>
<td>0.30 (3), p = 0.60</td>
<td>-0.25 (5), p = 0.59</td>
<td>0.18 (5), p = 0.70</td>
</tr>
<tr>
<td>(lack of) Perseverance</td>
<td>-0.32 (5), p = 0.49</td>
<td>-0.22 (5), p = 0.64</td>
<td>0.20 (5), p = 0.67</td>
<td>-0.90 (3), p = 0.04**</td>
<td>-0.20 (5), p = 0.67</td>
<td>0.11 (5), p = 0.82</td>
</tr>
<tr>
<td>Sensation Seeking</td>
<td>-0.09 (5), p = 0.85</td>
<td>-0.07 (5), p = 0.88</td>
<td>0.49 (5), p = 0.27</td>
<td>-0.10 (3), p = 0.87</td>
<td>0.40 (5), p = 0.38</td>
<td>0.23 (5), p = 0.61</td>
</tr>
<tr>
<td>Positive Urgency</td>
<td>-0.49 (5), p = 0.27</td>
<td>-0.13 (5), p = 0.79</td>
<td>-0.36 (5), p = 0.43</td>
<td>-0.98 (3), p = 0.005**</td>
<td>-0.20 (5), p = 0.67</td>
<td>0.13 (5), p = 0.79</td>
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<tr>
<td><strong>Revised NEO Personality Inventory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism</td>
<td>-0.37 (4), p = 0.47</td>
<td>-0.09 (4), p = 0.87</td>
<td>-0.43 (4), p = 0.40</td>
<td>-1.00 (2), p &lt; 0.001**</td>
<td>-0.43 (4), p = 0.40</td>
<td>0.03 (4), p = 0.96</td>
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<tr>
<td>Extraversion</td>
<td>-0.23 (4), p = 0.66</td>
<td>-0.41 (4), p = 0.43</td>
<td>0.12 (4), p = 0.83</td>
<td>0.40 (2), p = 0.60</td>
<td>-0.38 (4), p = 0.46</td>
<td>-0.52 (4), p = 0.29</td>
</tr>
<tr>
<td>Openness</td>
<td>-0.52 (4), p = 0.29</td>
<td>-0.61 (4), p = 0.20</td>
<td>-0.03 (4), p = 0.96</td>
<td>-0.74 (2), p = 0.76</td>
<td>-0.73 (4), p = 0.10</td>
<td>-0.61 (4), p = 0.20</td>
</tr>
<tr>
<td>Agreeableness</td>
<td>0.31 (4), p = 0.54</td>
<td>0.03 (4), p = 0.96</td>
<td>0.37 (4), p = 0.47</td>
<td>1.00 (2), p &lt; 0.001**</td>
<td>0.37 (4), p = 0.47</td>
<td>-0.09 (4), p = 0.87</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>0.60 (4), p = 0.21</td>
<td>0.49 (4), p = 0.33</td>
<td>0.26 (4), p = 0.62</td>
<td>1.00 (2), p &lt; 0.001**</td>
<td>0.77 (4), p = 0.07*</td>
<td>0.37 (4), p = 0.47</td>
</tr>
<tr>
<td>Drinking History</td>
<td>AUDIT</td>
<td>Number of binge episodes</td>
<td>Number drinks per week</td>
<td>Number drinks per drinking day</td>
<td>Max number drinks per drinking day</td>
<td>Frequency of max drinks</td>
</tr>
<tr>
<td>------------------</td>
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<td></td>
<td>0.49 (5), p = 0.27</td>
<td>0.31 (5), p = 0.50</td>
<td>0.36 (5), p = 0.43</td>
<td>0.50 (5), p = 0.25</td>
<td>0.31 (5), p = 0.16</td>
<td>-0.20 (5), p = 0.66</td>
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<td>0.11 (5), p = 0.82</td>
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<td>0.07 (5), p = 0.88</td>
<td>0.31 (5), p = 0.50</td>
<td>0.20 (5), p = 0.88</td>
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<td></td>
<td>0.51 (5), p = 0.25</td>
<td>0.44 (5), p = 0.33</td>
<td>0.71 (5), p = 0.07</td>
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<td>0.41 (5), p = 0.66</td>
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<td></td>
<td>0.50 (3), p = 0.39</td>
<td>0.70 (3), p = 0.20</td>
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<td>0.30 (3), p = 0.62</td>
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<td>0.35 (3), p = 0.56</td>
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<td></td>
<td>0.07 (5), p = 0.88</td>
<td>-0.04 (5), p = 0.94</td>
<td>0.25 (5), p = 0.59</td>
<td>-0.18 (5), p = 0.70</td>
<td>-0.16 (5), p = 0.73</td>
<td>0.61 (5), p = 0.14</td>
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<td></td>
<td>0.09 (5), p = 0.85</td>
<td>-0.27 (5), p = 0.55</td>
<td>-0.07 (5), p = 0.88</td>
<td>-0.25 (5), p = 0.59</td>
<td>-0.16 (5), p = 0.73</td>
<td>0.41 (5), p = 0.36</td>
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<table>
<thead>
<tr>
<th>PET Scan Subjective Measures</th>
<th>AUQ total urge score</th>
<th>BAES total stimulation score</th>
<th>BAES total sedation score</th>
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**Correlation indicates a strong relationship between variables.**
Figure 4.8.2. Scatter plot for spearman’s rank correlation of $\Delta \text{BP}_{ND}$ in AST and peak BAC. Spearman’s rho = 0.89, p<0.01.

Figure 4.8.3. Scatter plot for spearman’s rank correlation of $\Delta \text{BP}_{ND}$ in VP and systolic blood pressure (AUC of change from baseline). Spearman’s rho = -0.79, p<0.05.
Figure 4.8.4. Scatterplot for spearman’s rank correlation of $\Delta BP_{ND}$ in GP and UPPS-P Positive Urgency score. Spearman’s rho = -0.98, p<0.01.

Figure 4.8.5. Scatterplot for spearman’s rank correlation of $\Delta BP_{ND}$ in GP and NEO PI-R Agreeableness factor. Spearman’s rho = 1.00, p<0.001.
5. DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS

5.1 General Discussion

To our knowledge, this is the first study to explore the dopaminergic response to an acute dose of alcohol in a group of healthy social drinkers using the novel radiotracer, $^{[11]}$C-($\pm$)-PHNO. In the current study population, an acute moderate to high dose of oral alcohol did not result in lower BP_{ND} in the alcohol scan compared to the placebo scan, in the limbic striatum (LST), or in any of the following regions of interest: the associative striatum (AST), the sensorimotor striatum (SMST), or the extra-striatal regions of the substantia nigra (SN), the ventral pallidum (VP) and the globus pallidus (GP). While lower BP_{ND} in the alcohol scan relative to the placebo scan was noted in the LST ($\% \Delta BP_{ND} = -4.83\%$), this result was not significant. Furthermore, correlation analysis revealed that $\Delta BP_{ND}$ in these regions may be related to a number of factors, although these results must be approached with caution as correction for multiple comparisons was not performed in light of the pilot nature of this study. Regardless, such results do provide some insight into the possible factors influencing the dopaminergic response in humans. Taken together, the results of this current study suggest an overall heterogeneity of the dopaminergic response to alcohol observable in all regions, which is congruent with some of the previous literature.

One of the first studies to explore alcohol-induced dopamine changes in humans similarly did not report significant changes in dopamine levels in a group of healthy male volunteers (Salonen, Hietala et al. 1997). However, the image analysis in the Salonen et al. study was limited to the striatum as a whole and therefore, directs comparisons with the present results in the different striatal and extra-striatal regions cannot be made. Of note however, these authors further reported that the participant who demonstrated the greatest elevation in striatal dopamine
was the only individual with a positive family history of alcoholism. This is suggestive of the possibility that enhancement of dopamine levels, or elevated dopamine release, may be relevant to only those with a vulnerability for problematic drinking. In line with this, Setiawan et al. recently reported that increased dopamine release in the left ventral striatum (\(\% \Delta \text{BP}_{\text{ND}} = -8.0\%\)) after an alcoholic beverage intake was seen only in individuals characterized as having a high risk for the development of AUD, and not in low risk individuals (Setiawan, Pihl et al. 2013). In this former study, high risk individuals were deemed vulnerable based on lower reported subjective effects to alcohol (in line with the low level response theory) and greater traits of sensation seeking, and also tended to have a more positive family history of alcoholism. Similar findings were reported by Ramchandani et al. using intravenous alcohol administration whereby significant dopamine release was noted in the anterior and posterior (\(\% \Delta \text{BP}_{\text{ND}} = -9\%\) and -7%, respectively) ventral striatum in only the carriers of the mu opioid receptor OPRM1 A118G polymorphism (Ramchandani, Umhau et al. 2011), and not in non-carriers. This polymorphism may confer greater susceptibility to the development of alcohol abuse through possible heightened reinforcement as evidenced by greater self-report of “high” after receiving alcohol (Ray and Hutchison 2007).

Interestingly, in the current study, the only participant with a positive family history of alcoholism demonstrated the greatest negative \(\Delta \text{BP}_{\text{ND}}\) in all regions of interest, including the LST (\(\% \Delta \text{BP}_{\text{ND}} = -22.5\%\)). However, due to the small sample size in our study, we were not able to explore any group effects of increased vulnerability for problematic drinking (impulsivity and sensation seeking scores, objective and subjective PET responses, drinking history) on dopamine level changes in any region of interest. Our sample is therefore generally not characterized for increased vulnerability. However, the lack of significant increases in heart rate from baseline in
response to the alcohol administration – a proposed indicator of propensity for abuse (Conrod, Peterson et al. 1997)– is somewhat consistent with lower vulnerability in this current group as a whole. In addition, while the current group as a whole demonstrated an average AUDIT score (Mean (SD): 8.43 (4.12), Mdn (IQR): 7.00 (6.00, 10.00)) above the suggested cutoff for hazardous drinking, the majority of our participants had AUDIT scores below this cutoff. Thus, the possibility stands that a significant dopaminergic response was not observed due to a general invulnerability for alcohol abuse and dependence in the current study population.

While on average, the negative ΔBP\textsubscript{ND} in the LST of the present results may be suggestive of a slight, albeit non-significant, elevation of dopamine levels, two of the participants displayed marked positive ΔBP\textsubscript{ND}. This is a possible reflection of non-significant decreases in dopamine in response to alcohol. This degree of heterogeneity in the dopaminergic response to acute alcohol in humans has been reported elsewhere (Yoder, Constantinescu et al. 2007). Also, in the Ramchandani et al. study noted above, the non-carriers of the mu opioid polymorphism in the study also revealed non-significant decreases in dopamine in the ventral striatum in response to intravenous alcohol (Ramchandani, Umhau et al. 2011). Notably, in the Setiawan et al. study, the reported decreases in striatal dopamine following alcohol consumption in individuals at low risk for AUD were statistically significant (Setiawan, Pihl et al. 2013).

As suggested by Setiawan et al., one explanation for this generally observed decrease in dopamine following acute alcohol may be through the direct pharmacological action of alcohol on the reward system (Setiawan, Pihl et al. 2013). While decreased dopamine firing in response to an acute dose of ethanol may challenge a substantial body of animal research revealing direct excitation of VTA dopamine neurons to elicit dopamine release in the limbic targets (Gessa,
Muntoni et al. 1985, Brodie, Pesold et al. 1999), ethanol has also been shown to work acutely on the inhibitory GABAergic VTA neurons to depress VTA dopaminergic firing (Theile, Morikawa et al. 2008, Theile, Morikawa et al. 2009, Theile, Morikawa et al. 2011). Together, these studies demonstrate that acute exposure to alcohol in rats increases GABA neurotransmitter release onto the VTA dopaminergic neurons, enhancing overall GABA tone to these neurons. Ethanol can therefore modulate VTA firing, or dopamine release, through both direct excitatory action on VTA dopaminergic neurons and inhibitory action through GABAergic receptors on these neurons. As noted by the authors of these studies, greater GABA stimulation by ethanol and the subsequent decreased dopamine levels can be less reinforcing or linked with greater sedative effects of alcohol, and would be observed in individuals less susceptible to excessive alcohol consumption. On the other hand, vulnerable individuals may have less GABA tone and would demonstrate greater alcohol-induced levels of dopamine and possibly experience greater stimulant effects of alcohol, as a result. This notion is in line with the previously discussed studies demonstrating decreased dopamine in non-vulnerable individuals, as well as the current study’s results revealing a heterogeneous dopaminergic response.

Of interest, the participants in the current study who demonstrated the greatest positive $\Delta B_{\text{ND}}$ (higher $B_{\text{ND}}$ in the alcohol scan relative to the placebo scan) within the LST also demonstrated positive $\Delta B_{\text{ND}}$ within the AST. A similar trend was seen in the SMST in response to acute alcohol in this study. Again, this trend, possibly suggestive of decreases in striatal dopamine levels, is in agreement with previous work (Ramchandani, Umhau et al. 2011, Setiawan, Pihl et al. 2013). The significance of decreased dopamine in the dorsal striatal regions which are understood to be critical in habit formation and highly relevant to compulsive alcohol taking in later stage addiction processes (Corbit, Nie et al. 2012), is unclear. One hypothesis can
be drawn from the results of in vivo microdialysis and electrochemistry investigations in rats (Blanchard, Steindorf et al. 1993, Budygin, Phillips et al. 2001), in which the mesostriatal dopaminergic pathway is implicated in the behavioural effects of ethanol. In these studies, dose-dependent decreases in dopamine efflux were noted in the caudate-putamen regions with systemic administration of increasingly sedative doses of alcohol. Furthermore, the administration of a dopamine uptake inhibitor reversed both the dopamine efflux attenuation as well as the righting reflex reflective of sedation (Budygin, Phillips et al. 2001), suggesting that decreases in dopamine in these regions were in fact related to ethanol sedation. In the current study, there was a trend level significance of a greater “sedation” BAES score from baseline in the alcohol scan relative to the placebo soon after drink completion, and immediately following reports of greater “excitement”. While subjective effects of sedation have previously been suggested to occur mainly during the descending limb of the blood alcohol curve (Martin, Earleywine et al. 1993), more recent studies have demonstrated self-report of sedation early in the rising limb in some individuals, particularly with higher doses of alcohol (King, Houle et al. 2002, King, de Wit et al. 2011). These same individuals have also been shown to find such doses of alcohol less stimulating and to like the drug effect less than those with lower levels of sedation and later onset of sedation (King, de Wit et al. 2011). Therefore, the trend towards lower levels of dopamine following alcohol consumption in some participants in the current study, particularly within the dorsal striatal regions, may be due to heightened sedation.

It should be noted that the “sedation” BAES item was only higher in the alcohol scan relative to placebo at trend level (0.05≤p<0.10), and thus should be interpreted with caution. In addition, this increased subjective sedation was only notable at 40 minutes and not at any of the subsequent time points. However, the observed significant decreases in diastolic blood pressure in the alcohol scan relative to the placebo scan, an effect seen to begin at 40 minutes and persist,
may also reflect increased sedation. Indeed, decreases in diastolic blood pressure following acute alcohol, likely through its peripheral vasodilation effects (Johnson, Eisenhofer et al. 1986), have been noted in a number of previous studies (Ireland, Vandongen et al. 1984, Rosito, Fuchs et al. 1999, Mahmud and Feely 2002, Bau, Bau et al. 2005). The emergence and persistence of depressed blood pressure may generally be reflective of increasing intoxication. In one study, this decrease in diastolic pressure appeared during the descending limb where sedation is often reported, but only after a transient increase in systolic blood pressure during the ascending limb (Ireland, Vandongen et al. 1984). While we did not observe any significant differences in the changes in systolic blood pressure, and while it appears that the BAC continued to increase or plateau throughout the entire scan, the depressed diastolic blood pressure in the alcohol scan relative to the placebo scan may still signal the onset of sedation in our participants.

Greater sedation and thereby an overall lack of significant differences in BPND between scans following alcohol consumption in our participants may have resulted from the use of too high a dose of alcohol in the current study. Indeed, as noted previously, there is some work to suggest that moderate to high doses of alcohol can decrease dopamine levels in the rat NAc (Jones, Mathews et al. 2006). In fact, a biphasic trend has been evidenced in some of the literature, with lower doses of ethanol eliciting significant increases in dopamine and higher doses decreasing dopamine levels (Blanchard, Steindorf et al. 1993).

However, two previous studies which used a similar dose of alcohol (~0.80 g/kg body weight or 1 ml/kg body weight), found marked and significant increases in dopamine in the ventral striatum (Boileau, Assaad et al. 2003, Urban, Kegeles et al. 2010). Regardless, there are noticeable differences between these studies and the current one. As noted by Setiawan et al.
(Setiawan, Pihl et al. 2013), while the drinking history of the participants in the Boileau et al. study was uncharacterized, the participants did exhibit increases in heart rate from baseline suggestive of greater vulnerability (Conrod, Peterson et al. 1997), whereas this was not seen in our study. In light of this, the sample in the former study may have been a more vulnerable one than that of the current study, based on their physiological reactivity to alcohol, with possibly a heavier drinking history. The significant increases in dopamine observed in this former study may have therefore been the result of a greater vulnerability to developing AUD. Similarly, in the Urban et al. study, the participants were notably heavier drinkers with an average standard drinks per week of approximately 15, a value nearly twice as much as reported in the current study. Lower overall levels of sedation in response to a single dose of alcohol, or tolerance to sedation during the ascending limb, have been suggested both for individuals with a vulnerability to developing AUD (Schuckit and Smith 2000) as well as heavier drinkers (King, Houle et al. 2002, King, de Wit et al. 2011). Thus, the increased dopamine release evidenced in these former studies, despite using a similar dose of alcohol as in the current study, may have been driven by lower experienced sedation. In support of this, while the peak BAES sedation score in the current study was similar to that reported by Urban et al., subjective sedation peaked considerably earlier in the current study (25 minutes post drink vs. 50 minutes post drink).

The positive correlation with peak BAC and $\Delta BP_{ND}$ in the AST observed in this study may serve to further corroborate the possibility that the stimulating dopaminergic response to alcohol may occur with lower levels of blood alcohol concentration, possibly promoted by lower doses. That is, greater peak BAC appears to be associated with more positive $\Delta BP_{ND}$ in the AST, possibly reflecting a decrease in dopamine levels. Similar trend level correlations were also seen
with peak BAC and $\Delta BP_{ND}$ in the LST as well as the extra-striatal region of the SN, suggesting that this biphasic dose dependent effect may not be limited to only the striatum.

There were some novel findings in this study with respect to the extra-striatal regions of the SN, the GP and VP, which we were able to explore using the novel radiotracer, $[^{11}\text{C}](+)$-PHNO. Due to the high attribution of the $[^{11}\text{C}](+)$-PHNO signal to DRD3 in these regions (Tziortzi, Searle et al. 2011), it is tempting to ascribe these findings to the specific role of DRD3.

Of most interest is that the VP displayed the most marked average negative $\Delta BP_{ND}$, revealing a potential trend towards increases in dopamine levels. The VP has received considerable recent interest as a crucial neural substrate for reward and motivation owing it’s high connectivity with the mesolimbic, mesostriatal and mesocortical systems (Smith, Tindell et al. 2009). Specifically, the VP receives dense projections from both VTA and NAc, making it highly relevant to the processes of mesolimbic system (Groenewegen, Berendse et al. 1993). These substrates together form the ventral portion of the striatopallidal pathway, which have been suggested to be critical circuit for incentive salience attribution (Depue and Collins 1999).

A previous animal study using intraperitoneal injections of high doses of alcohol demonstrated significant increases in extracellular dopamine in the VP (Melendez, Rodd-Henricks et al. 2003), somewhat in line with our observation of non-significant greater dopamine levels in this region. In related studies, the same authors revealed that dopamine increases in the VP were evident during the anticipatory phase in the operant chamber as well as during the ethanol self-administration (Melendez, Rodd et al. 2004), paralleling results seen in the NAc (Melendez, Rodd-Henricks et al. 2002). Similarly, antagonism of this area using the DRD2/DRD3 antagonist sulpiride increased ethanol intake in alcohol preferring rats (Melendez, Rodd et al. 2005),
possibly through action on cell body autoreceptors, a finding which has also been observed with sulpiride antagonism of the NAc (Levy, Murphy et al. 1991). Taken together, the VP appears to be involved in the general reinforcing properties of alcohol, with a specific role in incentive salience and possibly ethanol reward, akin to the role played by the NAc. As a considerable degree of the [\textsuperscript{11}C]-(+)-PHNO in the VP is from DRD3, increases in dopamine in this region by most of our participants lends some support for the possible role of this specific receptor in alcohol reinforcement. Further exploration of the VP is warranted in future human drug administration studies as changes in dopamine within this region has also been evidenced with nicotine administration (Le Foll, Guranda et al. 2014).

Interestingly, while there were no notable differences in systolic blood pressure from baseline between scans as was seen with diastolic blood pressure, the current study observed a negative correlation between the difference in systolic blood pressure changes between the alcohol and placebo scan and ΔBP\textsubscript{ND} in the VP. That is, individuals with more negative changes in systolic blood pressure from baseline in the alcohol scan compared to the placebo scan tended to have more positive changes in binding potential. Some previous studies exploring systolic blood pressure following acute alcohol report only a transient increase relative to control drinks (Ireland, Vandongen et al. 1984), with other studies reporting general decreases in systolic blood pressure (Adesso, Ritchie et al. 1990) compared to control. Decreased systolic blood pressure during the alcohol scan, similar to our observation of decreased diastolic blood pressure, may also signal greater levels of sedation with increasing BAC. Indeed, we also observed a trend positive correlation of ΔBP\textsubscript{ND} in the VP and total subjective sedation. If interpreted in this light, these correlations are also in line with the positive significant association of peak BAC and ΔBP\textsubscript{ND} in the AST.
In contrast to the VP, the SN revealed an average positive $\Delta BP_{ND}$, which again may be suggestive of a trend towards decreases in dopamine levels. Of note however, there was also considerable variability in the direction of $BP_{ND}$ changes within this region similar to the variability previously noted within the striatal regions. With respect to the role of the SN in drug reinforcement and seeking, there has been minimal investigation, in spite of earlier observations of elevated DRD3 mRNA following chronic exposure to morphine and cocaine (Staley and Mash 1996, Spangler, Goddard et al. 2003). The significance of this increased DRD3 has yet to be fully elucidated, but may highlight the importance of DRD3 in the SN in later drug processes (i.e., development of sensitization or dependence). A recent PET study using $[^{11}\text{C}]+$-PHNO observed marked significant dopamine release in this region following amphetamine administration in non-human primates (Gallezot, Kloczynski et al. 2013), which is not in line with the current study’s observations of non-significant decreases in SN dopamine following alcohol intake. As noted by Gallezot et al., the smaller size of the SN combined with a dopamine signal less robust than that of amphetamine may render the detection of such dopamine changes within this region difficult (Gallezot, Kloczynski et al. 2013).

With respect to the GP, one study of ethanol administration in rats demonstrated the GP no significant increases in dopamine levels in response to high doses of ethanol (Melendez, Rodd-Henricks et al. 2002). The current study did not observe significant or trend level differences in $BP_{ND}$ between scans in this region, and thus may be in line with this previous finding. The GP receives projections from the SN (Prensa and Parent 2001) as well as afferents from the dorsal portions of the striatum (Zahm and Heimer 1990). It has been proposed that these structures together, forming the dorsal portion of striatopallidal pathway, may be less relevant to
the reinforcing properties of alcohol than the ventral striatopallidal pathway described above (Melendez, Rodd-Henricks et al. 2003). In some agreement with this proposal, participants as a whole demonstrated the most negative $\Delta BP_{ND}$ within the VP of the ventral striatopallidal pathway, and overall most positive $\Delta BP_{ND}$ within regions of the dorsal striatopallidal pathway. 

Of interest, a number of impulsivity scores appeared to be related to $\Delta BP_{ND}$ in the GP, including negative correlations with the (lack of) Perseverance, Negative Urgency and Positive Urgency. That is, greater tendency to give up in the midst of fatiguing exercises despite a desire to persist, as well as greater tendency towards rash impulses under positive and negative affect, may be associated with greater dopamine levels in this region. There is some work to suggest a role for the GP in general impulsivity. Specifically, higher impulsivity has been previously associated with reduced activation of the GP in response to anticipation of loss in both healthy controls and alcoholics in an fMRI study of monetary incentive delay (Beck, Schlagenhauf et al. 2009). As noted by the authors, as the GP is a crucial region for behavioural control and motivation, reduced activation can conceivably be associated with increased impulsivity in individuals. While the (lack of) Perseverance subscale appears to be least related to alcohol use and alcohol related problems in adolescents, Urgency appears to be consistently related to both alcohol frequency and problems, likely through interaction with excessive emotional reactivity (Shin, Hong et al. 2012). Interestingly, the current study also found that $\Delta BP_{ND}$ in the GP was negatively correlated with Neuroticism of the NEO PI-R, a subscale considered to reflect increased affective reactivity. The correlations of more positive $\Delta BP_{ND}$ with the higher subscale scores of Conscientiousness and Agreeableness (measures of self-discipline) lends further support to the possibility that greater dopamine levels in the globus pallidus following alcohol may be relevant to individuals who are lower in self-control/self-discipline. In considering that
the only other region to reveal a trend level correlation with the NEO PI-R personality trait of Conscientiousness is the SN, another area with a high DRD3 signal, one possibility is that general impulsivity or low self-control may be mediated by DRD3. Again, the preliminary nature of these results requires that these correlations be interpreted with caution. Interestingly, a polymorphism of the DRD3 gene has been shown to confer greater impulsivity in individuals (Limosin, Romo et al. 2005), which lends some support to this exciting possibility.

An unanticipated effect in the current study was a significant scan order effect on $\Delta BP_{\text{ND}}$ in the LST. Specifically, participants who received alcohol for the first scan displayed more negative $\Delta BP_{\text{ND}}$ in this region compared to those who received alcohol for the second scan. Such an effect has also been reported in a previous alcohol administration study (Urban, Kegeles et al. 2010). This may be attributable to heightened novelty during the first day as evidenced by significant lower $BP_{\text{ND}}$ on the first scan day compared to the second scan day regardless of beverage type (data not shown). The changes in dopamine levels seen specifically in this region, and at trend level in the GP, appear to encompass a combined effect of alcohol together with novelty of scanning procedures which the participants were previously unexposed to. This suggests the importance of interaction of alcohol with the situation in which it is consumed for alcohol reinforcement.

5.2 Limitations

This study is not without limitations. Firstly, the sample size of n=7 is notably small. There were a considerable number of trend level significant observations. Also, with a sample size this small, we were not able explore group effects of family history, impulsivity level and objective responses to alcohol (heightened heart rate, attenuated cortisol release). However, as
these were exploratory aims, correlations were appropriate for the purpose of detecting possible relationships. Such correlations serve to inform the decision to conduct in future larger studies, either within the same group or within an entire population characterized by their impulsivity or their physiological response to alcohol.

We did not control for multiple comparisons for any statistical test performed, rendering a possible inflation of our Type 1 error rate due to the large number of comparisons made. The decision to not make such adjustments was undertaken in light of the pilot nature of this study. That is, for an exploratory pilot study meant to inform the development of further in-depth studies through evidenced trends towards differences in binding between scan conditions, adjustments for multiple comparisons would appear inappropriate. However, through the use of rather conservative adjustments such as the Bonferroni correction to the set p-value, the majority of significant correlations found in this study would not have met threshold and thus should be interpreted with caution. Despite this, our results do provide some insight into possible factors which may influence dopamine levels in response to alcohol.

In light of the possible contribution of novelty to our findings of dopamine level changes in certain regions, this current study would have benefited from the simulation of scan procedures prior to the first scan day. Specifically, participants could have been introduced to the PET Centre environment and scanner with a tour prior to initiation of data collection, and have undergone a practice of actual scanning procedures.

While we attempted to minimize head motion during scanning through the use of a thermoplastic masks, we did not correct for motion across all participants. The only correction
for head motion was performed in one scan for one participant where we felt considerable motion could have resulted from the use of a bed pan during scanning. As motion can introduce noise to the TACs, this does serve as a limitation in the study.

Another limitation was the lack of a true baseline scan in this study. Not having a baseline scan without the administration of a placebo did not permit for the exploration of possible relationships between baseline receptor levels and various variables of interest. In particular, the level of baseline DRD2/DRD3 levels is evidenced to be associated with the subjective responses to alcohol, with those having higher levels experiencing greater intoxication and high (Yoder, Kareken et al. 2005). This may explain why alcoholics, who tend to have lower baseline levels of DRD2/DRD3 (Volkow, Wang et al. 2002), consume more alcohol. Furthermore, high midbrain DRD3 levels have also been shown to be related to lower connectivity between critical cognitive regions indicative of poorer executive control and greater impulsivity. Exploration of baseline levels of dopamine receptors may have provided further insight into why some participants differed in their subjective responses and impulsivity scores.

Furthermore, we relied on the use of an oral administration of alcohol in this study. Such an administration paradigm is well understood to result in varying time courses of blood alcohol levels through both intra-individual and inter-individual variability in alcohol pharmacokinetics (Norberg, Gabrielsson et al. 2000). In consideration of this, an intravenous administration paradigm, designed to maintain a steady blood alcohol level, may have been more beneficial (Ramchandani and O'Connor 2006). Naturalistically however, alcohol is always consumed orally, which made alcohol administration in the form of a beverage more so relevant to our question of dopamine’s role in addiction processes as they occur in real life.
Of note, alcoholic beverages are accompanied by sensory cues which have previously been shown to elicit dopamine release on their own (Oberlin, Dzemidzic et al. 2013). We attempted to control for these sensory cues by maintaining similar sensory properties (same volume of drink, smell of vodka, and the bitterness of quinine in tonic water) in the placebo beverage as the alcohol beverage. However, alcohol cues in both the alcohol and placebo beverages may have elicited dopamine release due to expectancy of receiving a reward entirely independent of a pharmacological effect of the drug. Alternatively, in line with the negative error prediction interpretation by Yoder et al., there may have also been dopamine decrease in the placebo condition after realizing a lack of reward following the presentation of cues (Yoder, Morris et al. 2009). We did not formally ask participants regarding which beverage they believed they were receiving on each of the scan days to determine whether the placebo cues allowed for a valid blind. Furthermore, without at a true baseline scan, the presence of expectancy effects cannot be ascertained.

It is further possible that the notably unpleasant effects of the scanning procedure may have confounded self-reports of drug effects, particularly with the BAES. Nausea, a common side effect of \([^{11}\text{C}](+)-\text{PHNO}\) (Mizrahi, Houle et al. 2010), was reported by some participants in the current study. This nausea was transient for all participants however. While participants received comparable masses of the radiotracer, nausea as well as discomfort from the thermoplastic mask and overall scanning procedures may have masked subjective stimulation experienced from the alcohol beverage.
As a final limitation, no arterial data were recorded for an input function to determine the $[^{11}\text{C}]$-\(+\)-PHNO binding potential in this study. The use of the simplified reference tissue method, although deemed appropriate, appears to underestimate the true binding potential as determined by a direct kinetic method, especially in regions with higher radiotracer binding potentials (i.e., the ventral pallidum and globus pallidus) (Ginovart, Willeit et al. 2007). However, as noted by Ginovart et al., the underestimations are minor and still very highly related to the true binding potentials measured by full kinetics methods.

5.3 Conclusions

To our knowledge, this is the first study to explore the effects of an acute dose of alcohol on the dopaminergic response in healthy social drinkers using the novel DRD3 selective radiotracer, $[^{11}\text{C}]$-\(+\)-PHNO. Moreover, this work assessed the dopaminergic response not only at the level of the striatum, as previously done, but also in extra-striatal regions. This study did not find that a moderate to high dose of oral alcohol significantly altered $B\text{P}_{\text{ND}}$ between scans in the limbic striatum of healthy drinkers, but did evidence variable responses in dopamine levels across participants. While alcohol significantly increased scores of “excited” from baseline and decreased diastolic blood pressure from baseline, it did not have any notable effects on overall measures of stimulation, sedation, alcohol craving or other objective parameters of heart rate and blood cortisol. The various different factors which can influence the dopaminergic response, including increased vulnerability for alcoholism, warrant further investigation using $[^{11}\text{C}]$-\(+\)-PHNO. Taken together, this work lends some support to the possible existence of a heterogeneous response to alcohol. Future studies with larger cohorts will be necessarily to draw more exacting conclusions.
5.4 Future Directions

This study was initially undertaken as a pilot study, and in this light, we are underpowered. Data from more participants should be obtained in the future to further validate the results of this study, namely the trend level significant associations observed with the various variables of interest, and to draw more confirmatory conclusions. To further characterize the level of vulnerability to developing alcohol abuse and dependence, and to possibly account for the variability of the dopaminergic response observed in this sample, we have collected whole blood samples for genotyping analysis which we may perform in the future. Potential polymorphisms to be analyzed for will include the TaqI A1 and -141C Ins/Del associated with the DRD2 gene, the Bal I for the DRD3 gene and the OPRM1 A118G of the mu opioid receptor gene.

A region of interest approach was adopted to explore the changes in dopamine levels within defined anatomical regions which have been previously investigated in addiction literature. However, as outlined by Yoder et al., the possible existence of an anatomically heterogeneous effect of alcohol on the brain may render a voxel-wise approach more appropriate (Yoder, Constantinescu et al. 2007). This analysis may allow us to decipher more subtle effects of alcohol that may be limited to certain voxels or generally to smaller areas of the region of interest (for example, lateral).

The current study investigated the possible role of only trait impulsivity on the alcohol-induced dopaminergic response. However, data on cognitive impulsivity and related cognitive function have been collected and may be analyzed in the future. There is substantial recent support for the specific role of DRD3 neurotransmission in cognition (Nakajima, Gerretsen et al.
2013), which may be highly relevant to drug addiction processes. An interesting prospect will be the examination of how performance in various cognitive tasks of executive control, impulsivity and working memory may be related to the dopaminergic response to acute alcohol.

Elevated DRD3 levels following chronic stimulant drug exposure was reported initially with postmortem studies (Segal, Moraes et al. 1997, Spangler, Goddard et al. 2003) and more recently, with PET imaging using \([^{11}\text{C}]-\text{(+)}-\text{PHNO}\) (Boileau, Payer et al. 2012, Payer, Behzadi et al. 2014). In a recent study involving alcohol dependent participants, exploration of DRD3 levels using \([^{11}\text{C}]-\text{(+)}-\text{PHNO}\) evidenced increased DRD3 only on the level of the hypothalamus compared to non-dependent healthy controls (Erritzoe, Tziortzi et al. 2014). With DRD3 contributing nearly 100\% to the \([^{11}\text{C}]-\text{(+)}-\text{PHNO}\) signal in the hypothalamus (Tziortzi, Searle et al. 2011), this region may be important to explore alongside the substantia nigra for DRD3 effects.

No previous studies exploring the dose-dependent effects of alcohol on the dopaminergic response in humans exist, to our knowledge. All previous PET studies have relied on a similar moderate to high dose of alcohol (target BAC of 0.06 \% to 0.10\%), and no study has yet to characterize how differing doses may influence the level of dopamine released in the limbic targets in the same participants, as has been shown in some animal work. It is possible that an elevated dopaminergic response to alcohol does exist in all drinkers, but is dependent on the dose of alcohol. The dose at which the most stimulation occurs for each individual may be further related to the participant’s genetic predisposition and history of alcohol and drug intake. Such a dose-dependent investigation would help uncover this possibility.
6. REFERENCES


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