The Relationship of Alcohol Dependence to Monoamine Oxidase A

by

Brittany Matthews

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Pharmacology and Toxicology
University of Toronto

© Copyright by Brittany Matthews 2015
The Relationship of Alcohol Dependence to Monoamine Oxidase A

Brittany Matthews

Doctor of Philosophy

Department of Pharmacology and Toxicology
University of Toronto

2015

Abstract

Background: Alcohol dependence (AD) is a substance abuse disorder characterized by compulsive alcohol seeking and intake, combined with a negative emotional state during withdrawal. AD is also associated with markers of apoptosis and/or oxidative stress across multiple organs. Monoamine oxidase A (MAO-A) is an important enzyme that participates in the cellular response to oxidative stress, and is elevated during major depressive episodes, somewhat more robustly in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC). It is unknown whether MAO-A levels are abnormal in AD. The current studies examine whether:

i) MAO-A level is elevated in the PFC and ACC in AD in humans.

ii) Chronic harman exposure, a MAO-A inhibitor found in alcoholic beverages, upregulates MAO-A in the PFC and ACC of rodents.

iii) Chronic alcohol exposure upregulates MAO-A in the PFC and ACC of rodents.

Methods:

i) Sixteen participants with AD underwent [$^{11}$C]-harmine positron emission tomography to determine MAO-A distribution volume (VT), an index of MAO-A level.
ii) Rats were treated with harman for 21 days (0, 2, 5, 15 mg/kg/day) via osmotic minipump. MAO-A protein and activity levels, measured with Western blot and a spectrophotometric assay respectively, were assessed immediately and after 8-hour withdrawal.

iii) Rats were exposed to alcohol vapor for 8 weeks, 17 hours per day. MAO-A protein/activity levels were assessed immediately and after 24-hour, 4-day, and 21-day withdrawal.

**Results:**

i) MAO-A $V_T$ was significantly greater in all brain regions analyzed in AD.

ii) MAO-A protein/activity levels were not altered by harman administration.

iii) MAO-A protein/activity levels were elevated in the PFC and ACC after 24-hour withdrawal from alcohol exposure. There were no changes at any other timepoints.

**Conclusion:** These findings identify elevated MAO-A level as a new pathological marker present in AD that is therapeutically targetable through direct inhibition. MAO-A protein/activity levels were not changed by harman administration, suggesting chronic MAO-A inhibition does not alter MAO-A level. The human PET findings, measured in early withdrawal from alcohol dependence, were replicated in the rodent model of chronic alcohol exposure in the PFC and ACC, suggesting that alcohol exposure likely led to elevated MAO-A in AD.
Acknowledgments

First, I would like to thank my supervisor Dr. Jeffrey Meyer and co-supervisor Dr. Stephen Kish for giving me the opportunity to work under their supervision at the Centre for Addiction and Mental Health. They have been excellent mentors and I am grateful for their guidance and encouragement throughout my doctoral program.

I would also like to thank my PhD supervisory committee members, Drs. Jose Nobrega, Isabelle Boileau, and Junchao Tong for their valuable insight and helpful suggestions. A special thank you to the members of both the Meyer and Kish lab, as well as the PET imaging technicians, who have shared their technical expertise and contributed to various parts of my PhD thesis.

Finally, I would like to thank my family and friends for their unconditional love and support, without which the completion of this thesis would not have been possible.
# Table of Contents

List of Tables .............................................................................................................................. viii

List of Figures ............................................................................................................................... ix

List of Abbreviations ................................................................................................................... xi

1 Introduction .............................................................................................................................. 1

1.1 Overview ............................................................................................................................ 1

1.1.1 Statement of the Problem ........................................................................................ 1

1.1.2 Purpose of the Study ............................................................................................... 2

1.2 Background ....................................................................................................................... 3

1.2.1 Importance of Alcohol Dependence ........................................................................ 3

1.2.2 Brief Review of Major Structural and Neurochemical Abnormalities in Alcohol Dependence .......................................................................................................................... 5

1.2.2.1 Structural Alterations ................................................................................ 5

1.2.2.2 Cellular Alterations .................................................................................. 6

1.2.2.3 Markers associated with Apoptosis in Alcohol Dependence ................... 8

1.2.2.4 Markers associated with Oxidative Stress in Alcohol Dependence .......... 9

1.2.2.5 Monoamine Receptor and Transporter Abnormalities ........................... 11

1.2.3 Alcohol Dependence and Mood ............................................................................ 14

1.2.4 Pathways and Common Pathologies of Comorbid Alcohol Dependence and Major Depressive Disorder ............................................................................................................ 18

1.2.5 Monoamine Oxidase A ......................................................................................... 20

1.2.5.1 General Properties of MAO-A .................................................................. 20

1.2.5.2 Substrates of MAO-A ............................................................................. 26

1.2.5.3 Radiotracers for Imaging MAO-A .............................................................. 28

1.2.6 Role of MAO-A in Mood Disorders ........................................................................ 31

1.2.7 Monoamine Oxidase A in Alcohol Dependence in Humans .............................. 33

1.2.8 Rationale for Investigating MAO-A in Alcohol Dependence .............................. 38
1.2.8.1 Alcohol Dependence and Dysphoric Mood ........................................... 38
1.2.8.2 Monoamine Oxidase A and Models of Alcohol Dependence in Rodents ................................................................. 38
1.2.8.3 Alcohol Dependence and Predisposition to Apoptosis ...................... 39
1.2.9 Role of Monoamine Oxidase A Inhibitor Harman in Addiction .............. 41

1.3 Study Objectives and Research Hypotheses .............................................. 47

2 Methods ........................................................................................................... 51

2.1 Human PET Imaging Study of MAO-A in Alcohol Dependence ............... 51
  2.1.1 Study Participants and Protocol for PET Imaging Scan ......................... 51
  2.1.2 PET Image Acquisition and Analysis ..................................................... 53
  2.1.3 Statistical Analysis ............................................................................. 56

2.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents ...... 57
  2.2.1 Animals ............................................................................................. 57
  2.2.2 Harman Osmotic Minipump Exposure .............................................. 57
  2.2.3 Ethanol Vapor Exposure .................................................................... 58
  2.2.4 Measurement of MAO Protein ............................................................ 60
  2.2.5 Measurement of MAO Activity ........................................................... 61
  2.2.6 Measurement of GFAP ................................................................. 62
  2.2.7 Statistical Analysis ........................................................................... 63

3 Results ............................................................................................................... 64

3.1 Human PET Imaging Study of MAO-A in Alcohol Dependence .............. 64
  3.1.1 Difference in MAO-A V_T between the Alcohol Dependent Group and Healthy Controls .................................................. 64
  3.1.2 Relationship between MAO-A V_T and Duration of Heavy Alcohol Use .... 67
  3.1.3 Relationship between MAO-A V_T and Other Clinical Features ................. 69
  3.1.4 Additional Characteristics of the Alcohol Dependent Group ....................... 73

3.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents ...... 74
3.2.1 Effect of Harman Exposure on MAO ................................................................. 74
3.2.2 Effect of Ethanol Exposure on MAO ............................................................... 79

4 Discussion .............................................................................................................. 90

4.1 Human PET Imaging Study of MAO-A in Alcohol Dependence ....................... 90

4.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents ...... 97

4.2.1 Effect of Harman Exposure on MAO .............................................................. 97

4.2.2 Effect of Ethanol Exposure on MAO ............................................................. 102

4.3 Implications for Treatment of Depressed Mood and Alcohol Dependence .......... 107

4.4 Recommendation for Future Investigations ...................................................... 113

4.5 Conclusion .......................................................................................................... 116

References .............................................................................................................. 118
List of Tables

Introduction

Table I. Substrate specificities of the monoamine oxidases in human cerebral cortex..................21

Table II. The amino acid sequence of human MAO......................................................................23

Table III. Comparison of PET radiotracers for monoamine oxidase A........................................30

Table IV. Comparison of post mortem studies of monoamine oxidase in alcohol
dependence......................................................................................................................................33

Table V. Comparison of studies of low concentrations of CSF 5-hydroxyindoleacetic acid
(5-HIAA) and impulsive aggressive behaviour..................................................................................36

Table VI. Relative inhibitory concentrations of harman.................................................................42

Results

Table VII. Demographic and Clinical Characteristics of Study Participants...............................65

Table VIII. Overview of Analyses of MAO-A $V_T$........................................................................71
List of Figures

Introduction

Figure 1. Regional distribution of MAO-A and MAO-B protein levels (mean ± SEM) in autopsied human brain ........................................................................................................................................................................... 22

Figure 2. Condensation products of acetaldehyde with several biogenic amines (and tryptophan) .............................................................................................................................................................................................................. 44

Results

Figure 3. Greater Monoamine Oxidase-A Vₜ Level in Alcohol Dependent Subjects Compared to Healthy Controls ..................................................................................................................................................................................................... 66

Figure 4. Greater Monoamine Oxidase-A Vₜ Level in Alcohol Dependent Subjects Correlates with Years of Heavy Alcohol Use ........................................................................................................................................................................................ 68

Figure 5. Greater Monoamine Oxidase-A Vₜ Level is associated with Elevated Depressed Mood and Angry-Hostility ...................................................................................................................................................................................... 70

Figure 6. Effect of 21 day osmotic minipump administration of harman, and 8 hour withdrawal of harman following 21 day administration, on plasma levels of harman ........................................................................................................................................................................... 74

Figure 7. Effect of 21 day harman administration on MAO-A activity level (a), 8 hour harman cessation on MAO-A activity level (b), 21 day harman administration on MAO-A protein level (c), and 8 hour harman cessation on MAO-A protein level (d) ............................................................................................................................ 76
Figure 8. Effect of 21 day harman administration on MAO-B activity level (a), 8 hour harman cessation on MAO-B activity level (b), 21 day harman administration on MAO-B protein level (c), and 8 hour harman cessation on MAO-B protein level (d). .......................................................... 78

Figure 9. Blood alcohol concentrations (BAC) obtained at the end of 17h exposure across the 8 week exposure period .................................................................................................................................................. 79

Figure 10. Effect of 8 week ethanol vapor exposure (no withdrawal) on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d)................ 81

Figure 11. Effect of 24 hour withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d)................................................................................................................................................................................. 83

Figure 12. Effect of 4 day withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d)......................................................................................................................................................................................................................... 85

Figure 13. Effect of 3 week withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d)................................................................................................................................................................................................................................................................................. 87

Figure 14. Protein levels of GFAP (glial fibrillary acidic protein) following 8 week ethanol vapor exposure (no withdrawal) (a), 24 hour withdrawal (b), 4 day withdrawal (c), and 3 week withdrawal (d)........................................................................................................................................................................................................................................................................................................... 89
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>AD</td>
<td>Alcohol dependence</td>
</tr>
<tr>
<td>ADS</td>
<td>Alcohol dependence scale</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>ARE</td>
<td>Androgen response element</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood alcohol concentration</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Beta cell lymphoma 2</td>
</tr>
<tr>
<td>[¹¹C]</td>
<td>Radiolabelled Carbon-11</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>CIWA-Ar</td>
<td>Clinical Institute Withdrawal Assessment for Alcohol, revised</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CRISPR</td>
<td>Clustered regularly interspaced short palindromic repeats</td>
</tr>
<tr>
<td>D₂</td>
<td>Dopamine 2 receptor</td>
</tr>
<tr>
<td>D₂/3</td>
<td>Dopamine 2/3 receptor</td>
</tr>
<tr>
<td>DASB</td>
<td>N,N-dimethyl-2-2-amino-4-cyanophenyli thiobenzylamine</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-Dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Effective dose; 50 % response in a biological system</td>
</tr>
<tr>
<td>ERK2</td>
<td>Extracellular signal-regulated kinase 2</td>
</tr>
<tr>
<td>[&lt;sup&gt;18&lt;/sup&gt;F]</td>
<td>Radiolabelled Fluoride-18</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GBq</td>
<td>Gigabecquerel</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>GRE</td>
<td>Glucocorticoid response element</td>
</tr>
<tr>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]</td>
<td>Tritium</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>H&lt;sub&gt;&amp;&lt;/sub&gt;L</td>
<td>Heavy chain and light chain</td>
</tr>
<tr>
<td>HAMD</td>
<td>Hamilton Rating Scale for Depression</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HVA</td>
<td>Homovanillic acid</td>
</tr>
<tr>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]</td>
<td>Radiolabelled iodine-123</td>
</tr>
<tr>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-beta-CIT</td>
<td>2-beta-carbomethoxy-3-beta-(4-iodophenyl)tropane</td>
</tr>
<tr>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-IBZM</td>
<td>Iodobenzamide</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>JNK</td>
<td>Jun N-terminal kinase</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>$K_i$</td>
<td>Inhibition constant</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis–Menten constant</td>
</tr>
<tr>
<td>KS</td>
<td>Korsakoff syndrome</td>
</tr>
<tr>
<td>M14 cells</td>
<td>Melanoma 14 cells</td>
</tr>
<tr>
<td>MANOVA</td>
<td>Multivariate analysis of variance</td>
</tr>
<tr>
<td>MANCOVA</td>
<td>Multivariate analysis of covariance</td>
</tr>
<tr>
<td>MAO-A</td>
<td>Monoamine oxidase A</td>
</tr>
<tr>
<td>MAO-B</td>
<td>Monoamine oxidase B</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen activated protein kinase</td>
</tr>
<tr>
<td>MBq</td>
<td>Megabecquerel</td>
</tr>
<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>MDE</td>
<td>Major depressive episode</td>
</tr>
<tr>
<td>MEK</td>
<td>Mitogen activated protein kinase/extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>MHPG</td>
<td>3-Methoxy-4-hydroxyphenylglycol</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute and Hospital</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial deoxyribonucleic acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>N</td>
<td>Normality</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NEO-Pi-R</td>
<td>Neuroticism-Extraversion-Openness Personality Inventory–Revised questionnaire</td>
</tr>
<tr>
<td>Nmol</td>
<td>Nanomole</td>
</tr>
<tr>
<td>NSE</td>
<td>Neuron specific enolase</td>
</tr>
<tr>
<td>PC12 cells</td>
<td>Pheochromocytoma 12 cells</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>pH</td>
<td>Power of hydrogen</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>Ras</td>
<td>Rat sarcoma</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>R1</td>
<td>Repressor protein 1</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SP</td>
<td>Specificity protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive species</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic antidepressant</td>
</tr>
<tr>
<td>TIEG2</td>
<td>Transforming growth factor-beta-inducible early gene</td>
</tr>
<tr>
<td>TIQ</td>
<td>Tetrahydroisoquinoline</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling</td>
</tr>
<tr>
<td>Ug</td>
<td>Micrograms</td>
</tr>
<tr>
<td>Umol</td>
<td>Micromole</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume/volume</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Vesicular monoamine transporter 2</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum rate of a reaction</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Total distribution volume</td>
</tr>
<tr>
<td>WE</td>
<td>Wernicke’s encephalopathy</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Overview

1.1.1 Statement of the Problem

Alcohol dependence (AD) is a complex psychiatric disorder characterized by compulsive alcohol seeking and intake, combined with a negative emotional state during withdrawal (Koob and Volkow 2010). This dysphoric mood state is proposed to contribute to addiction maintenance by generating a negative reinforcement pathway by which alcohol is used to alleviate these symptoms (Koob and Volkow 2010). Dysphoric mood during withdrawal also increases the risk of relapse, and is associated with greater risk of conversion to a major depressive episode (Glenn and Parsons 1991; Ramsey, Kahler et al. 2004). To date, there has not been identification of a neuropathology that is implicated in the initial onset of depressed mood in alcohol dependence. Monoamine oxidase A (MAO-A) is an enzyme that metabolizes serotonin, norepinephrine, and dopamine, participates in the cellular response to oxidative stress, and is elevated in the midst of major depressive episodes (Meyer, Ginovart et al. 2006; Youdim, Edmondson et al. 2006). There are three current unknowns in the literature:

1) whether MAO-A levels are altered in alcohol dependence in humans.

2) whether MAO-A levels are affected by harman (a MAO-A inhibitor found in alcoholic beverages).

3) whether MAO-A levels are affected by chronic alcohol administration.
1.1.2 Purpose of the Study

The purpose of the current series of studies was to determine whether MAO-A is elevated in alcohol dependence, whether harman affects MAO-A levels and/or activity, and whether alcohol exposure affects MAO-A levels and/or activity. Should MAO-A be elevated during alcohol dependence, this would identify MAO-A as the first therapeutically targetable marker implicated in the onset of depressive symptoms. The intent of the latter two studies was to identify if particular substances in alcohol containing beverages predispose towards elevated MAO-A levels and/or activity.
1.2 Background

1.2.1 Importance of Alcohol Dependence

Alcohol dependence is one of the most underappreciated addictive disorders in the world, affecting individual health and creating large social and economic burden. The World Health Organization estimates that 4% of global death and 5% of the global burden of disease are attributed to alcohol use disorders (Rehm, Mathers et al. 2009). It has been reported that 19% of men and 8% of women have been diagnosed with alcohol dependence at some point in their lives, as defined by the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM–IV) (First, Spitzer et al. 1995; Bierut, Schuckit et al. 2000). Alcohol dependence is defined by the DSM–IV as the repeated use of alcohol despite negative consequences, combined with physiological tolerance, withdrawal and the constant compulsion to drink. Alcohol withdrawal typically develops 6-24 hours after cessation of heavy alcohol use with the onset of autonomic hyperactivity. Symptoms of withdrawal include nausea, tremor, sweating, and tachycardia (Hall and Zador 1997; McIntosh and Chick 2004; McKeon, Frye et al. 2008), which peak at 10-30 hours after abstinence (McIntosh and Chick 2004). More severe withdrawal symptoms may develop depending on the level of intake, including epileptiform seizures at 12-48 hours abstinent, and delirium tremens at 48-72 hours abstinent (McIntosh and Chick 2004). The latter symptoms are more pronounced in those that suffer from severe alcohol dependence (Lechtenberg and Worner 1992; Mayo-Smith, Beecher et al. 2004). The relapse rate of this addiction is frequent, with 50–80% of individuals resuming hazardous alcohol use within a 12 month period after completing treatment (Naranjo and Kadlec 1991; McKenna, Chick et al. 1996; Monahan and Finney 1996; Miller, Walters et al. 2001; Boothby and Doering 2005).
The risks of alcohol dependence are three-fold, including harm to society, third parties, and to oneself. Economic burden develops through increased rates of crime and violence, increased need for law enforcement, and loss of productivity (Rehm, Mathers et al. 2009). Risks to third parties include those who become victim to traffic fatalities or are affected by fetal alcohol syndrome (Hunt 1993). Harm to oneself can occur from the development of diseases associated with alcohol consumption, including various forms of cancer, and alcohol-induced pancreatitis and liver disease (Rehm, Mathers et al. 2009). It is noteworthy to mention that cirrhosis of the liver was the ninth leading cause of death in the United States (Hunt 1993). Additionally, alcohol withdrawal can be very severe and if left untreated, death may occur from cardiovascular or respiratory complications (O'Brien 1996). Harm to oneself also includes significant neuropathological changes, detailed in Section 1.2.2, as well as the development of mood disorders and increased suicide risk, detailed in Section 1.2.3.
1.2.2 Brief Review of Major Structural and Neurochemical Abnormalities in Alcohol Dependence

Chronic, heavy alcohol use is associated with both structural and functional alterations in the central nervous system (Charness 1993). There are several different ways in which alcohol may affect the central nervous system (CNS), including direct neurotoxicity, the effects of chronic liver disease or other nutritional deficiencies, or toxicity of byproducts of alcohol metabolism (i.e. acetaldehyde) (Zahr, Kaufman et al. 2011). This section briefly outlines the major neuropathologies reported in AD in humans. This review is restricted to pathological changes that occur before the prominence of late stage neurological conditions such as Wernicke encephalopathy (WE) and Korsokoff syndrome (KS), as this earlier stage of alcohol dependence is the focus of the human investigation of MAO-A in the current thesis. For a detailed review, please see Zahr, Kaufman and Harper 2011 (Zahr, Kaufman et al. 2011).

1.2.2.1 Structural Alterations

One of the earliest studies of intracranial cavity volume in post mortem tissue of alcohol dependence demonstrated an elevation in mean pericerebral space from 8.3% of the total intracranial volume in healthy controls, to 11.3% in AD (Harper, Kril et al. 1985). This was interpreted as a loss of brain tissue and was hypothesized to occur due to shrinkage of white matter (de la Monte 1988), because reductions in white matter volume were seen in a variety of brain regions (prefrontal cortex, cerebellar vermis and corpus callosum) in post mortem tissue in AD (Phillips, Harper et al. 1987; Harper and Kril 1988; Tarnowska-Dziduszko, Bertrand et al. 1995; Kril, Halliday et al. 1997), especially in those with nutritional deficiencies (Lee, Jung et al. 2005). White matter volume loss also negatively correlated with daily alcohol intake (Kril,

In vivo studies employing quantitative magnetic resonance imaging (MRI) corroborate the post mortem findings, demonstrating significant white matter reductions in frontal cortex, cerebellum, and corpus callosum (Phillips, Harper et al. 1987; Pfefferbaum, Lim et al. 1992; Pfefferbaum, Lim et al. 1996; Estruch, Nicolas et al. 1997). Yet in contrast to the post mortem investigations, MRI studies also demonstrated volume reduction in grey matter regions of the brain, especially in the frontal cortex (Jernigan, Butters et al. 1991; Pfefferbaum, Sullivan et al. 1997; Cardenas, Studholme et al. 2007). Grey matter volume deficits were also identified in the anterior hippocampus (Sullivan, Marsh et al. 1995; Agartz, Momenan et al. 1999), while no reductions were seen in the caudate, putamen, or globus pallidus (Kril, Halliday et al. 1997). In vivo MRI analyses also allowed for the examination of brain volume loss into withdrawal, and there are some reports of reversal of structural changes with abstinence (Schroth, Naegele et al. 1988; O'Neill, Cardenas et al. 2001; Cardenas, Studholme et al. 2007). Specifically, in comparison to participants who relapsed, heavy drinkers who abstained from alcohol for 8 months had significant recovery of brain loss in the frontal cortex, parietal cortex, temporal cortex, thalamus, brainstem, cerebellum, corpus callosum, anterior cingulate, insula, and subcortical white matter (Cardenas, Studholme et al. 2007).

1.2.2.2 Cellular Alterations

Early microscopic studies revealed as much as a 25% reduction in the density of pyramidal neurons in the superior frontal gyrus, part of the dorsolateral prefrontal cortex in AD (Harper, Kril et al. 1987; Kril and Harper 1989; Kril, Halliday et al. 1997). This loss of pyramidal neurons
in the superior frontal gyrus was accompanied by reduced dendritic arborization (Harper and Corbett 1990). One study of subcortical brain regions such as the supraoptic and paraventricular nuclei of the hypothalamus also showed neuronal loss, and this correlated with daily alcohol intake (Harding, Halliday et al. 1996). Most studies suggest neuronal loss is specific to the prefrontal cortex, as there are no changes reported in the cerebellum, basal ganglia, hippocampus, or dorsal raphe nucleus (Baker, Halliday et al. 1996; Harding, Wong et al. 1997; Kril, Halliday et al. 1997; Harper, Dixon et al. 2003). More recently, the packing density of neurons of the orbitofrontal cortex was shown to be reduced in AD, and this correlated with the duration of alcohol dependence (Miguel-Hidalgo, Overholser et al. 2006). These studies collectively suggest progressive neuronal loss with chronic, heavy alcohol consumption.

An initial study of glial cells in the hippocampus of post mortem tissue revealed a substantial reduction in the number of glial cells in AD (Korbo 1999). Reductions in the density and size of glial cells in all cortical layers of the dorsolateral prefrontal cortex were also reported in AD (Miguel-Hidalgo, Wei et al. 2002). The packing density of glia was shown to be lowest in alcohol dependent subjects reporting depressive symptoms, revealing a relationship to major depressive disorder (MDD) (Miguel-Hidalgo, Wei et al. 2002). A reduction in glial cells was also reported in the orbitofrontal cortex, but did not correlate with duration of AD (Miguel-Hidalgo, Overholser et al. 2006). Levels of glial fibrillary acidic protein (GFAP; an astrocytic cytoskeletal marker) immunoreactivity were not altered in the dorsolateral prefrontal cortex or orbitofrontal cortex in AD (Miguel-Hidalgo, Wei et al. 2002; Miguel-Hidalgo, Waltzer et al. 2010). Interestingly, the packing density of astrocytes immunoreactive for GFAP was significantly reduced in alcohol preferring rodents without any alcohol exposure (Miguel-Hidalgo 2005). These studies collectively suggest that glial alterations appear early in AD, and
may be implicated in the onset of AD and/or contribute to neuronal loss (Miguel-Hidalgo, Overholser et al. 2006).

1.2.2.3 Markers associated with Apoptosis in Alcohol Dependence

A variety of markers associated with apoptosis have been identified in human and animal models of alcohol dependence. DNA fragmentation, measured through the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, was detected in the hippocampus and superior frontal cortex in post mortem brain tissue of alcohol dependent individuals (Ikegami, Goodenough et al. 2003). The DNA fragmentation co-localized with GFAP immunoreactivity, suggesting damage may occur in glial cells, consistent with previous reports of glial cell loss (Korbo 1999; Miguel-Hidalgo, Wei et al. 2002). To date, most studies of predisposition to apoptosis in brain have been investigated in rodents. For example, acute alcohol treatment (intraperitoneal injections, 2 g/kg each day for 3 days) led to a significant decrease in cell proliferation, a 134% increase in DNA fragmentation (number of TUNEL positive cells), and a 97% increase in apoptotic caspase 3, in the dentate gyrus (Jang, Shin et al. 2002). Acute alcohol administration (5 g/kg) also led to an approximate 50% depletion in mitochondrial DNA (mtDNA) 10 hours after treatment in mouse brain homogenate (Mansouri, Demeilliers et al. 2001). This depletion of mtDNA was prevented by 4-methylpyrazole, an inhibitor of alcohol dehydrogenase and CYP 2E1, and Vitamin E, an antioxidant, suggesting oxidative stress due to ethanol metabolism was likely involved in this effect (Mansouri, Demeilliers et al. 2001). Subchronic ethanol administration (intraperitoneal injections, 2 g/kg every other day for 15 days) increased cell degeneration (number of TUNEL positive cells) and reduced glial and neuronal cell densities in mouse hippocampus (Oliveira-da-Silva, Vieira et al. 2009). Chronic ethanol diet (14 g/kg per day for 28 days) increased transforming growth factor-beta-inducible early gene 2
(TIEG2; a transcription factor that induces cell death), decreased anti-apoptotic protein Bcl-2, and increased apoptotic protein caspase 3 in rat prefrontal cortex (Ou, Johnson et al. 2011). The finding of elevated protein caspase 3 was confirmed in another report of ethanol-fed rats (Valles, Blanco et al. 2004). Rodents fed an ethanol diet leading to steady state blood alcohol concentrations of 180-280 mg/dl (similar to human intoxication) for 40 days had significantly increased DNA strand breaks in the hippocampus and cerebellum (Renis, Calabrese et al. 1996). Collectively these studies suggest that alcohol induces predisposition to apoptosis, and it has been proposed that this is influential upon the structural and cellular changes observed in AD.

1.2.2.4 Markers associated with Oxidative Stress in Alcohol Dependence

For AD prior to onset of Wernicke encephalopathy and Korsokoff syndrome, alcohol induced oxidative stress and predisposition to apoptosis have been proposed to contribute to the mechanism underlying neurochemical alterations. Through chronic alcohol consumption, the excessive generation of free radicals can result in a state of oxidative stress. A class of oxygen containing free radicals known as reactive oxygen species (ROS) are formed via the reduction of \( \text{O}_2 \), and are particularly important in AD, as an accumulation of ROS can lead to damage or degradation of cellular macromolecules such as lipids, proteins, and DNA (Wu and Cederbaum 2003). Alcohol induced ROS production can occur through a variety of mechanisms that may contribute to a state of oxidative stress concurrently, including changes in the NAD\(^+\)/NADH ratio of the cell during alcohol metabolism, production of acetaldehyde during alcohol metabolism, increases in the activity of cytochrome enzyme CYP2E1, increases of levels of free iron in the cell, and decreases in antioxidant enzymes, particularly glutathione (Wu and Cederbaum 2003). In 1967, Di Luzio and Hartman demonstrated that administration of a large single dose of ethanol increased the content of lipid peroxides (a marker associated with
oxidative stress) in rodent liver, leading to the hypothesis that oxidative stress may contribute to
the pathogenesis of ethanol induced liver disease (Di Luzio and Hartman 1967). Since then,
many studies of ethanol induced liver disease in rodents have observed markers associated with
oxidative stress including enhanced lipid peroxidation, protein modification, and decreases in
hepatic mitochondria glutathione levels (Nanji, Zhao et al. 1994; Knecht, Adachi et al. 1995;
Tsukamoto and Lu 2001). These observations raised the possibility that ethanol induced
oxidative stress may occur in extrahepatic tissues, including brain. An early report demonstrated
enhanced lipid peroxidation in the cerebellum of rodents after administration of a single dose of
ethanol (Rouach, Park et al. 1987; Nordmann, Ribiere et al. 1990). This finding was followed up
in a rodent model of chronic alcohol consumption, where increases in lipid peroxidation (as
measured through an accumulation of thiobarbituric acid reactive species, TBARS) and reactive
oxygen species, as well as decreases in levels of glutathione, were reported in cortical
synaptosomes (Montoliu, Valles et al. 1994). Rodents fed an ethanol diet leading to steady state
blood alcohol concentrations of 180-280 mg/dl (similar to human intoxication) for 40 days had
significantly increased lipid peroxidation products in the cortex, hippocampus, striatum, and
cerebellum (Renis, Calabrese et al. 1996). Similarly, rodents fed an ethanol diet for 20 days
displayed increased lipid peroxidation and decreased glutathione levels in the cerebral cortex,
which were reduced by treatment with antioxidant Vitamin E, suggesting oxidative stress was
likely involved in this effect (Agar, Demir et al. 2003). The literature on markers associated with
oxidative stress in human alcohol dependence is limited, with a recent study revealing decreased
levels of antioxidant superoxide dismutase in the hippocampus of post mortem tissue in
uncomplicated alcoholism using a high throughput assay to determine protein expression profiles
(Matsuda-Matsumoto, Iwazaki et al. 2007). A reduction of superoxidase dismutase
immunoreactivity was also reported in the white matter of the substantia nigra in post mortem samples (Skuja, Groma et al. 2013).

1.2.2.5 Monoamine Receptor and Transporter Abnormalities

Many studies have examined the effect of alcohol dependence on monoamine receptors and transporters. Several studies have found decreased D$_{2/3}$ receptor binding potential, a measure of receptor density, in alcohol dependence compared to health in the whole striatum (Hietala, West et al. 1994; Volkow, Wang et al. 1996) and, more recently, in the caudate, putamen, and ventral striatum using PET radioligands $[^{11}\text{C}]$-raclopride and $[^{18}\text{F}]$-desmethoxyfallypride (Volkow, Wang et al. 2002; Heinz, Siessmeier et al. 2004; Heinz, Siessmeier et al. 2005; Martinez, Gil et al. 2005; Rominger, Cumming et al. 2012). Two early studies using SPECT ligands $[^{123}\text{I}]$-epidipride and $[^{123}\text{I}]$-IBZM reported a nonsignificant decrease of D$_{2/3}$ receptor binding in the whole striatum (Repo, Kuikka et al. 1999; Guardia, Catafau et al. 2000). Using functional magnetic resonance imaging (fMRI), low D$_{2/3}$ receptor binding potential was shown to correlate with prefrontal and anterior cingulate cortex activation in response to alcohol-related cues (Heinz, Siessmeier et al. 2004), craving (Heinz, Siessmeier et al. 2005), and quantity of alcohol intake (Martinez, Gil et al. 2005), suggesting a relationship to disease severity and relapse risk. This decrease in D$_{2/3}$ receptor binding potential did not recover after 1 to 4 months of abstinence, suggesting low D$_{2/3}$ receptor availability may reflect a predisposing factor (Volkow, Wang et al. 2002). In addition, stimulant induced dopamine release measured through $[^{11}\text{C}]$-raclopride displacement is consistently blunted in alcohol dependence in the ventral striatum, demonstrating a reduction in dopamine transmission that may underlie the decreased sensitivity to reward in addicted subjects (Martinez, Gil et al. 2005; Volkow, Wang et al. 2007). In healthy participants, acute alcohol administration was shown to reduce $[^{11}\text{C}]$-raclopride binding potential in the
ventral striatum/nucleus accumbens, which is indicative of increased extracellular dopamine and supports the hypothesis that the mesolimbic dopamine system is activated during alcohol intake, possibly mediating its reinforcing effects (Boileau, Assaad et al. 2003).

Two studies have found decreases in the dopamine transporter (DAT) in the striatum during early alcohol withdrawal using SPECT ligand \(^{123}\)I-beta-CIT, with no differences found later into abstinence (Tiihonen, Kuikka et al. 1995; Laine, Ahonen et al. 1999). This decrease in DAT correlated with depressive mood during withdrawal (Laine, Ahonen et al. 1999), suggesting transient changes in DAT may be important during alcohol withdrawal. Two other reports of DAT found no differences in alcohol dependent subjects who were abstinent for 2 to 3 months using PET ligand \(^{11}\)C-d-threo-methylphenidate (Volkow, Wang et al. 1996) or 3 to 5 weeks using SPECT ligand \(^{123}\)I-beta-CIT (Heinz, Ragan et al. 1998; Heinz, Jones et al. 2000). One study reported a significant reduction in the type-2 vesicular monoamine transporter (VMAT2) in the putaman, and a non significant reduction in the caudate using PET radioligand \(^{18}\)F-dihydrotetrabenazine (Gilman, Koepe et al. 1998). Post mortem studies are in accordance with these imaging studies, and demonstrate reductions in striatal D\(_2/D_3\) receptors and the dopamine transporter (Tupala, Hall et al. 2000; Tupala, Hall et al. 2001).

With regard to serotonin receptor based abnormalities in alcohol dependence, studies show decreased serotonin transporter (SERT) in the hippocampus, anterior cingulate, dorsal striatum, amygdala, and hypothalamus in post mortem samples (Chen, Casanova et al. 1991; Mantere, Tupala et al. 2002; Storvik, Tiihonen et al. 2006; Storvik, Tiihonen et al. 2007; Storvik, Haukijarvi et al. 2008). Decreased SERT binding was also reported in the midbrain using SPECT radioligand \(^{123}\)I-beta-CIT after 3 to 5 weeks of abstinence (Heinz, Ragan et al. 1998; Heinz, Jones et al. 2000; Heinz, Jones et al. 2002). Decreased SERT binding was associated with
lifetime alcohol consumption and severity of depression (Heinz, Ragan et al. 1998; Heinz, Jones et al. 2000; Heinz, Jones et al. 2002). Yet a study with PET radiotracer $[^{11}\text{C}]$-DASB, which has improved signal to noise ratio, found no differences in SERT in any brain region measured in AD (including midbrain, striatum, frontal and medial temporal cortex, thalamus, and cerebellum) (Brown, George et al. 2007). It is important to note that the SPECT ligand $[^{123}\text{I}]$-beta-CIT is nonselective, and has a near 1:1 affinity for serotonin to DAT (Laruelle, Giddings et al. 1994; Carroll, Kotian et al. 1995). A limited number of studies have examined serotonin receptor levels in AD, with one study reporting increased 5-HT$_{1B}$ binding in the ventral striatum (which agrees with rodent investigations) (Pandey, Piano et al. 1996; Hu, Henry et al. 2010), and unaltered 5-HT$_{1A}$ binding in the raphe nuclei and cortex (Martinez, Slifstein et al. 2009) (which differs from some rodent investigations) (Wong, Threlkeld et al. 1990; McBride, Guan et al. 1994).
1.2.3 Alcohol Dependence and Mood

Alcohol use leads to subjective ratings of euphoria across many different studies and a variety of rating scales (Freed 1978; Avery, Overall et al. 1982). Those without alcohol use disorders report feelings of happiness, carefree relaxation, and elation, which follow the time course of elevated blood alcohol concentration (Kelly 1970; Kelly, Myrsten et al. 1971; Muir, Pollitt et al. 1973; Rafaelsen, Chrstrup et al. 1973). This is in contrast to people with alcohol dependence, who report initial pleasurable effects directly after alcohol consumption, yet develop greater levels of anxiety and depression during withdrawal (Mayfield 1968; Mayfield 1968; Tamerin and Mendelson 1969; Tamerin, Tolor et al. 1974). Subjective reports from alcohol dependent individuals show lower mood levels than healthy populations after 12-24 hours of abstinence (Klett, Hollister et al. 1971; Freed, Riley et al. 1977; McMahon and Davidson 1986; Bokstrom, Balldin et al. 1989; Fahlke, Berggren et al. 1999). It is important to note that the lowered mood experienced during withdrawal in these studies is not a simple reduction from the euphoric effects of alcohol intake; it is lower than that of healthy subjects. The time course involved in the transition from normal to depressive emotional states correlates with the decline from peak intoxication (Kelly 1970; Kelly, Myrsten et al. 1971; Muir, Pollitt et al. 1973; Rafaelsen, Chrstrup et al. 1973; Madden 1993). These dysphoric mood ratings are highly predictive of relapse (Caster and Parsons 1977; Parsons, Schaeffer et al. 1990; Glenn and Parsons 1991), and it has been suggested that the expectation of alcohol to relieve patients of their anxious and depressed mood states contributes to the maintenance of alcohol dependence (Hershon 1977). Autonomic hyperactivity symptoms of early withdrawal can be managed with benzodiazepines, yet relapse rates are not reduced by this treatment (Mayo-Smith 1997), demonstrating that mood symptoms contribute to the high relapse rate of this disorder.
Alcohol dependence and major depressive disorder (MDD) are highly comorbid conditions, and often lead to difficulties in the effectiveness of patient therapy, including improper identification of illness and course of treatment, and increased chronicity of both disorders (Enns, Swenson et al. 2001). According to the US National Comorbidity Survey, the first large scale field survey of mental health in the United States, the lifetime prevalence rate of major depression was 24.3% and 48.5% among men and women with alcohol dependence, respectively (Kessler, Crum et al. 1997). A two-fold increase in the lifetime odds of depression is seen in those alcohol dependence (Kessler, Nelson et al. 1996). In Canada, 19.6% of the general population with alcohol dependence reported major depression in the past 12 months (Wang and El-Guebaly 2004). Similar rates were reported in the National Mental Health Survey in Canada; among those with alcohol use disorders, the lifetime prevalence of major depressive disorder was 16.3% (Currie, Patten et al. 2005).

Secondary depressive disorder in the context of substance abuse is defined as a depressive episode that occurs during periods of substance abuse/dependence, or within 4 weeks after the cessation of use (First, Spitzer et al. 1995). In a study of nearly 3000 alcohol dependent patients, 24.6% developed substance induced major depression (Schuckit, Tipp et al. 1997). Elevations in depressed mood are correlated with the frequency of intoxication, the consumption of large amounts of alcohol per occasion, and weekly intoxication (Bonin, McCreary et al. 2000; Hamalainen, Kaprio et al. 2001; Wang and Patten 2002; Graham, Massak et al. 2007). For example, hazardous drinkers, defined as men who have 14-27 drinks per week and women who have 7-13 drinks per week, are twice as likely to exceed clinical cutoffs of depressive symptoms compared to non-hazardous drinkers (Fillmore, Golding et al. 1998; Rodgers, Korten et al. 2000). Depressive symptoms and dysphoric mood are highly prevalent across both genders with alcohol dependence (Locke and Newcomb 2001), and it has been reported that alcohol abusing
men report lower levels of positive affect and greater depressed mood and anxiety, and women report increased levels of negative affect and depressed mood (Caldwell, Rodgers et al. 2002). Secondary depression has the same clinical symptoms as primary depressive disorder, and is hypothesized to occur due to the physiological reactions of intoxication or withdrawal, and/or the negative social and interpersonal consequences of alcohol abuse (Schuckit, Tipp et al. 1997; Schuckit, Tipp et al. 1997). In some cases, secondary depression may remit with abstinence (Brown and Schuckit 1988; Schuckit 1994), but there is increased risk of persistence of major depressive episodes (Ramsey, Kahler et al. 2004). As such, alcohol abuse is an important risk factor for suicidal behaviour, with a 60-120 times greater risk than the healthy population (Murphy and Wetzel 1990). This problem is inflated in those afflicted with comorbid depression (Cornelius, Salloum et al. 1995; Salloum, Mezzich et al. 1995).

In addition to suicide, another important clinical consequence that arises from the high prevalence of comorbid depression and alcohol abuse is the impact on treatment outcome. Depressed patients with a history of moderate alcohol use are more likely to drop out of pharmacological treatment for depression (Hoencamp, Haffmans et al. 1998), and those with diagnosed comorbid alcohol use disorders have increased time to reach remission and poorer treatment outcomes for MDD (Rae, Joyce et al. 2002; Davis, Wisniewski et al. 2010). Higher levels of baseline alcohol intake are associated with poorer response to antidepressants, even in patients without alcohol use disorders (Worthington, Fava et al. 1996). The presence of depressive symptoms at admission to a treatment program for alcohol dependence is associated with significantly higher rates of relapse in both genders (Glenn and Parsons 1991). Additionally, cases of comorbidity are associated with shorter time to rehospitalization after initial treatment for both addiction and depressive symptoms (Lin, Chen et al. 2007). Because depressive episodes among alcohol dependent patients are heterogeneous in causation and
clinical course, there is often great difficulty in selecting suitable treatment. Conventional therapy for depression alone may be less effective in a comorbid population (Schuckit, Tipp et al. 1997), suggesting a profound need for more beneficial treatment options. Left untreated, comorbid alcohol abuse and major depressive disorders are associated with increased risk of personal harm and grave economic burden (Kessler, Crum et al. 1997).
1.2.4 Pathways and Common Pathologies of Comorbid Alcohol Dependence and Major Depressive Disorder

It is well documented that AD and MDD are highly comorbid disorders (Kessler, Crum et al. 1997), with proposed pathways including shared vulnerability, greater likelihood of alcohol intake during MDD and greater risk of MDD consequent to AD. Most recent prospective studies suggest that a predominant mechanism for the high comorbidity is prolonged, heavy alcohol intake leading to the development of depressive symptoms (Wang and Patten 2002; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009). A nationally representative sample also concurred that prior alcohol dependence increases the risk of current MDD more than 4-fold (Hasin and Grant 2002). Despite these links between AD and MDD, there are very few investigations examining common neuropathologies between these two disorders. Reports of reduced hippocampal volume are seen in both AD and MDD (Sullivan, Marsh et al. 1995; Agartz, Momenan et al. 1999; Bremner, Narayan et al. 2000; MacQueen and Frodl 2011), suggesting this region may be involved in the pathological changes observed in these disorders. Yet this reduction in hippocampal volume is related to illness duration in MDD (MacQueen and Frodl 2011), and does not revert during remission (Neumeister, Wood et al. 2005), while several studies demonstrate both gray and white matter volume loss in AD (Jernigan, Butters et al. 1991; Pfefferbaum, Sullivan et al. 1997; Harper 1998; Cardenas, Studholme et al. 2007), that may revert partially in periods of abstinence (Schroth, Naegele et al. 1988; O'Neill, Cardenas et al. 2001; Cardenas, Studholme et al. 2007). These findings suggest brain volume changes likely have a different etiology in MDD and AD. Reductions in glial and neuronal density and size are also reported in the orbitofrontal and dorsolateral prefrontal cortex across both illnesses (Miguel-Hidalgo and Rajkowska 2003). Yet the reduced glial and neuronal cell density seen in MDD is
considered a consequence of exposure to major depressive episodes, as neuronal size is more prominently reduced with longer exposures to MDD, and glial fibrillary acidic protein (GFAP), a specific cytoskeletal marker of astroglia, is more prominently reduced in those with early onset of depression (Si, Miguel-Hidalgo et al. 2004; Rajkowska and Miguel-Hidalgo 2007). In alcohol dependence, the reduced glial and neuronal cell density is considered a consequence of alcohol toxicity since ethanol is known to produce aberrant functional and structural alterations in neurons and glial cells (Snyder 1996; Diamond and Gordon 1997; Fadda and Rossetti 1998). While it is possible that these pathological changes may be facilitated by common underlying vulnerabilities, leading to more rapid progression of both conditions (Miguel-Hidalgo, Wei et al. 2002), they do not specifically account for the very first onset of a major depressive episode in those with alcohol dependence. Hence, to the best of our knowledge, there has not been identification of a pathology in alcohol dependence implicated in the initial onset of MDD.
1.2.5 Monoamine Oxidase A

1.2.5.1 General Properties of MAO-A

Monoamine Oxidase A (MAO-A) is an important enzyme located on the outer mitochondrial membrane of glia and monoamine releasing neurons, particularly norepinephrine releasing neurons, that metabolizes monoamines and participates in the cellular response to mitochondrial toxicity and oxidative stress (Youdim, Edmondson et al. 2006). In human brain, MAO-A density is highest in locus coeruleus; high in the cortex, hippocampus and striatum; lower in cerebellar cortex; and minimal in white matter (Saura, Kettler et al. 1992; Saura, Bleuel et al. 1996; Tong, Meyer et al. 2013). These protein levels of MAO-A in brain tissue typically show a strong, positive correlation with MAO-A activity (Edelstein and Breakefield 1986; Saura, Kettler et al. 1992). MAO-A catalyzes the oxidative deamination of a number of monoamines, including serotonin, norepinephrine, and dopamine, producing hydrogen peroxide, the corresponding aldehyde and either ammonia or a substituted amine. Monoamine oxidase B (MAO-B) is the second isoenzyme of MAO present in most mammalian tissues, and is associated with the outer mitochondrial membrane and found in serotonin releasing neurons and astrocytes (Youdim, Edmondson et al. 2006). The main form of MAO found in the striatum is MAO-B, and MAO-B catalyzes the oxidative deamination of several monoamines (such as phenylethylamine, tyramine, tryptamine, and dopamine) (Youdim, Edmondson et al. 2006). The function of intraneuronal MAO-A and MAO-B is to terminate the action of amine neurotransmitters, regulate intracellular amine stores, and to protect neurons from exogenous amines. Extraneuronal functions include the inactivation of amines taken up by astrocytes and glia cells (Youdim, Edmondson et al. 2006). Substrate specificities and regional densities of both monoamine oxidases are listed in Table I and Figure 1. In a study of binding density of MAO-A using...
autoradiography, it is interesting to note that similar values of $[^3]H$-Ro41-1049 binding (a selective, high affinity ligand for MAO-A) to human brain regions were obtained in the same regions of rat brain ($r=0.89$, $p<0.00001$) (Saura, Bleuel et al. 1996).

**Table I.** Substrate specificities of the monoamine oxidases in cerebral cortex

| Substrate               | **MAO-A** | | **MAO-B** | | |
|------------------------|-----------|-----------|-----------|-----------|
|                        | $K_m$ (μM) | $V_{max}$ (pmol min$^{-1}$ mg protein$^{-1}$) | $V_{max}/K_m$ (μmol M$^{-1}$ min$^{-1}$ mg protein$^{-1}$) | $K_m$ (μM) | $V_{max}$ (pmol min$^{-1}$ mg protein$^{-1}$) | $V_{max}/K_m$ (μmol M$^{-1}$ min$^{-1}$ mg protein$^{-1}$) |
| Adrenaline             | 125 ± 42  | 379 ± 54  | 3.03 ± 1.11 | 266 ± 9  | 465 ± 61  | 1.75 ± 0.23   |
| Dopamine               | 212 ± 33  | 680 ± 123 | 3.21 ± 0.77 | 229 ± 33 | 702 ± 158 | 3.07 ± 0.82   |
| 5-Hydroxy-tryptamine   | 137 ± 24  | 228 ± 31  | 1.66 ± 0.37 | 1093 ± 20| 6.6 ± 1.3 | .006 ± .001  |
| Noradrenaline          | 284 ± 17  | 561 ± 42  | 1.98 ± 0.19 | 238 ± 30 | 321 ± 13  | 1.35 ± 0.18   |
| 2-Phenyl-ethylamine    | 140 ± 22  | 20 ± 8    | 0.14 ± 0.06 | 4 ± 2    | 309 ± 24  | 77.3 ± 39.1   |
| Tryptamine             | 35 ± 6    | 58 ± 5    | 1.66 ± 0.32 | 35 ± 8   | 108 ± 2   | 2.84 ± 0.60   |
| Tyramine               | 127 ± 18  | 182 ± 28  | 1.43 ± 0.30 | 107 ± 21 | 343 ± 48  | 3.21 ± 0.77   |

$K_m$, Michaelis–Menten constant; $V_{max}$, maximum rate. MAO, monoamine oxidase.

Figure 1. Regional distribution of MAO-A and MAO-B protein levels (mean ± SEM) in autopsied human brain (n= 6). Abbreviations: A23, cingulate gyrus posterior; A24, cingulate gyrus anterior; A25, paraolfactory/subgenual gyrus; CCc, corpus callosum caudal; CCr, corpus callosum rostral; cereb, cerebellar cortex; CN, caudate; CNA, hippocampal Ammon’s horn; CSTH, subthalamic nucleus; GD, dentate gyrus; GH, hippocampal gyrus; GPe, globus pallidus external; GPi, globus pallidus internal; GUNC, gyrus of uncus; hypothal, hypothalamus; ICr, internal capsule rostral; LGB, lateral geniculate body; MDT, mediodorsal thalamus; NAM, amygdala; NAV, anterior ventral nucleus of thalamus; N. basalis, nucleus basalis; NL, nucleus lateralis of thalamus; NLV, lateral ventral nucleus of thalamus; NPM, medial pulvinar of thalamus; PUT, putamen; RN, red nucleus; SBI, substantia innominata; SNpc, substantia nigra pars compacta.

MAO-A and MAO-B proteins have approximately 70% homogeneity (Shih, Grimsby et al. 1993), see Table II for amino acid sequences. They are encoded by different genes on the X chromosome, consist of 15 exons with identical exon-intron organization, and have differences in the core promoter regions (Bach, Lan et al. 1988; Lan, Heinzmann et al. 1989; Grimsby, Chen et al. 1991; Shih, Grimsby et al. 1993).

Table II. The amino acid sequence of human MAO

<table>
<thead>
<tr>
<th>Amino Acid Sequence (Bach, Lan et al. 1988)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAO-A</strong></td>
</tr>
<tr>
<td>MENQE KASIA GHMF D VVVGGGIGSGLSAA KL TETY GV</td>
</tr>
<tr>
<td>SVLVLEARDRVGGRTY TiRNHEVDYVDVGGA YVGPT</td>
</tr>
<tr>
<td>QNRI RLSKKG KETYKVNVSELV QYVKGKTYPFGR</td>
</tr>
<tr>
<td>AFPPVWNPIAYLD YNNLWTIDMNMGKEIPTDAPWEAQ</td>
</tr>
<tr>
<td>HADKWDKMTMKELIDK ICWTKTARRFA YLFVINIVVT</td>
</tr>
<tr>
<td>SEPHEV SALWFLW YVKQC GGTTRF STNGQ ERKFV</td>
</tr>
<tr>
<td>GSGQV SERRIMDDL LGDQV KLNHPVTDQ SSDNIIEET</td>
</tr>
<tr>
<td>LNHEHY ECKY VINAIPPTLTATA IHFRPPELPAERNQLIQ</td>
</tr>
<tr>
<td>RLP MGAVIKCM MYYKEAFWKKKDKYCGCMIEEE DAP</td>
</tr>
<tr>
<td>SITLDDTKPDGS LPAIMG F ILARKADRLAKLHKEIRK</td>
</tr>
<tr>
<td>KKICELYAKVLGSQ EALHPVHYE EKNWCEEQYS GGC</td>
</tr>
<tr>
<td>YTA YFPPI GM TQY VRIPVGRIFFAGTETATA KWSG</td>
</tr>
<tr>
<td>YMegaVEA GER AAREVLNLGLK VTEKI DWVQEPESK</td>
</tr>
<tr>
<td>DVP AVEITHTF WERNLP S VSG LKK IIGFSTSVTALGFV</td>
</tr>
<tr>
<td>LKYK LLP R S</td>
</tr>
<tr>
<td><strong>Amino Acid Sequence</strong> (Bach, Lan et al. 1988)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>MAO-B</strong></td>
</tr>
<tr>
<td>MSNKCDVTVGGGSMGMAAKLLHDSGLNVVLEAR</td>
</tr>
<tr>
<td>DRVGGRTYTLRNQKVKVYDGLGGSYVGPTQNRLRLA</td>
</tr>
<tr>
<td>KELGLEYRVENEVERLIHVKGKSYPFGRGFPPVWNPI</td>
</tr>
<tr>
<td>TYLDHNFWRTMDDMGREIPSDAPWKAPLAEEDNMT</td>
</tr>
<tr>
<td>TMKELLDKCLCWTESAKQLATLFLVNLCVTAETHEVSAL</td>
</tr>
<tr>
<td>WFLYVXQCGGTTTRIISTTNGGQERXFVGGSQVSER</td>
</tr>
<tr>
<td>IMDLLGDRVKLERPVIYIDQTRENVLVETLHNHEYEAVY</td>
</tr>
<tr>
<td>KYVISAIPPTLGMKIHFNPPLPMRNRQMITRVPLGVSI</td>
</tr>
<tr>
<td>KCIVYYKEPFWRKKDYCGTMIDGEEAPVAYTLDDTK</td>
</tr>
<tr>
<td>PEGNYAIXGFIHALHKARLALTKEERLKKLCELYA</td>
</tr>
<tr>
<td>KVLGSLEALEPVHYEEKNWCREEQYSGGCYYTFPPGI</td>
</tr>
<tr>
<td>LTQYGRVLRQPVDRIYFAGETETATHWSGMAGAVEA</td>
</tr>
<tr>
<td>GERAARELHAMGXIPEDEIOWOSEPESVDPVPAQPITT</td>
</tr>
<tr>
<td>FLERHLPSVPGLRLRGLITTSATAGFLAHKRGLLVRV</td>
</tr>
</tbody>
</table>

The core promoter of MAO-A consists of two 90 base pair repeats, each of which contains two SP1 elements (Shih, Grimsby et al. 1993). The promoter of MAO-A has bidirectional activation by transcription factor SP1 and upstream repression by transcription factor R1 (by competing with SP1 for binding to SP1 sites) (Zhu, Chen et al. 1994; Chen, Ou et al. 2005; Ou, Chen et al. 2006; Johnson, Stockmeier et al. 2011). The MAO-A promoter also has three consensus glucocorticoid/androgen response elements (GRE/AREs). MAO-A levels are increased by progesterone, testosterone, corticosterone, and glucocorticoids in human fibroblasts and capillary endothelial cells (Edelstein and Breakefield 1986; Youdim, Banerjee et al. 1989). Additionally, the synthetic glucocorticoid, dexamethasone, and androgens increase MAO-A activity in rodent frontal cortex (Luine, Khylchevskaya et al. 1975; Slotkin, Seidler et al. 1998). This interaction was investigated and it was later determined that glucocorticoids enhance MAO-A gene expression through a variety of pathways, including: direct interaction of the glucocorticoid receptor with the third GRE/ARE, indirect interaction of the glucocorticoid receptor with SP1 or R1 on SP1 binding sites, and by regulating R1 translocation (Ou, Chen et al. 2006). Androgens enhance MAO-A gene expression through: direct interaction of the androgen receptor with the third GRE/ARE, and indirect interaction of the androgen receptor with SP1 on SP1 binding sites (Ou, Chen et al. 2006). Furthermore, transforming growth factor-beta-inducible early gene (TIEG2; an SP1 like transcription factor) binds to SP1 sites and upregulates MAO-A promoter activity (Grunewald, Johnson et al. 2012). TIEG2 expression and translocation into the nucleus were promoted by stress both by dexamethasone administration (in vitro) and chronic social defeat in rodents (in vivo), suggesting that both TIEG2 and GR contribute to glucocorticoid induced activation of MAO-A (Grunewald, Johnson et al. 2012).

The core promoter of MAO-B contains two clusters of SP1 sites separated by a CACCC element, all upstream of a TATA box (Shih, Grimsby et al. 1993). Transcription factors SP1, SP3, and
SP4 bind to both the proximal and distal clusters of SP1 sites of the promoter, and overexpression of SP1 and SP4 activates MAO-B promoter activity, while overexpression of SP3 represses this activation (Zhu, Chen et al. 1994; Wong, Chen et al. 2001). SP3 and transforming growth factor-beta-inducible early gene (TIEG2; an SP1 like transcription factor) are repressors at the CACCC element and activators at the proximal SP1 overlapping sites of the promoter, although SP3 has no overall effect on the MAO-B promoter (Ou, Chen et al. 2004). TIEG2 has a higher affinity to the SP1 sites, hence has an overall activating effect on MAO-B promoter activity (Ou, Chen et al. 2004). MAO-B expression is selectively induced by the activation of a mitogen activated protein kinase (MAPK) pathway that includes protein kinase C (PKC), Ras, MEK1, MEK3, MEK7, ERK2, JNK1, and p38/RK (Wong, Ou et al. 2002).

The literature on the protein degradation mechanism of the monoamine oxidases is limited. MAO proteins were shown to be ubiquitinated in cell culture, suggesting a mechanism mediated by the ubiquitin proteasomal system (Jiang, Jiang et al. 2006). Recently, the E3 ubiquitin ligase Rines was shown to bind and ubiquitinate MAO-A and enhance its proteasomal degradation (Kabayama, Sakoori et al. 2013). MAO-A protein and activity levels were also increased in the locus coeruleus of Rines knockout mice (Kabayama, Sakoori et al. 2013).

1.2.5.2 Substrates of MAO-A

Serotonin is a high affinity substrate for MAO-A (Fowler and Oreland 1979; White and Tansik 1979; Schoepp and Azzaro 1981; Kinemuchi, Fowler et al. 1984), and administration of different MAO-A inhibitors (clorgyline, moclobemide, harman, befloxatone) increased extracellular serotonin from 20 to 200 per cent depending on the dose in a variety of brain regions, including the prefrontal cortex, hippocampus, and superior raphe nuclei (Fagervall and Ross 1986; Haefely, Burkard et al. 1992; Celada and Artigas 1993; Bel and Artigas 1995; Adell, Biggs et al.
In these paradigms it was often demonstrated that brain 5-HIAA, the main metabolite of serotonin is reduced (Bel and Artigas 1995; Adell, Biggs et al. 1996; Curet, Damoiseau-Ovens et al. 1998). Extracellular serotonin is also raised substantially (100-200%) in the prefrontal cortex, hippocampus and superior raphe nuclei in the knockout model of MAO-A (Evrard, Malagie et al. 2002). Additionally, administration of selective MAO-A inhibitor moclobemide increased serotonin, and decreased 5-HIAA, in brain homogenates, suggesting similarly increased intracellular serotonin (Da Prada, Kettler et al. 1989).

Dopamine is a high affinity substrate for MAO-A (Fowler and Oreland 1979; Schoepp and Azzaro 1981; Kinemuchi, Fowler et al. 1984) and administration of MAO-A inhibitors increases extracellular dopamine in the striatum under baseline conditions, as well as during precursor loading paradigms (Butcher, Fairbrother et al. 1990; Colzi, d'Agostini et al. 1990; Colzi, d'Agostini et al. 1992; Segal, Kuczenski et al. 1992; Brannan, Prikhojan et al. 1995; Finberg, Wang et al. 1995; Adachi, Watanabe et al. 2001; Wayment, Schenk et al. 2001). Administration of selective MAO-A inhibitor moclobemide also increases dopamine, and decreases 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), metabolites of dopamine, in brain homogenates, suggesting similarly increased intracellular dopamine (Da Prada, Kettler et al. 1989). A few reports detect MAO-A in dopamine synthesizing neurons (Moll, Moll et al. 1990; Luque, Kwan et al. 1995), although it has been postulated that MAO-A outside of dopamine synthesizing neurons is more likely to account for the elevations in extracellular striatal dopamine after MAO-A inhibition (Saura, Bleuel et al. 1996).

Norepinephrine is a high affinity substrate for MAO-A (Houslay and Tipton 1974; White and Tansik 1979) and MAO-A is easily detectable in cells that synthesize norepinephrine (Konradi,
Svoma et al. 1988; Konradi, Kornhuber et al. 1989; Luque, Kwan et al. 1995; Saura, Bleuel et al. 1996). Under conditions of MAO-A inhibition, extracellular norepinephrine is increased in the prefrontal cortex as well as hippocampus (Fagervall and Ross 1986; Finberg, Pacak et al. 1993; Finberg, Pacak et al. 1994), which argues that MAO-A has a substantial role in controlling extracellular norepinephrine in these brain regions. Additionally, administration of selective MAO-A inhibitor moclobemide increased norepinephrine, as well as decreased 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of norepinephrine, in brain homogenates, suggesting similarly increased intracellular norepinephrine (Da Prada, Kettler et al. 1989).

1.2.5.3 Radiotracers for Imaging MAO-A

There are three PET radiotracers available that have been applied to measure MAO-A levels in human brain (see Table III). Radiolabelled clorgyline was the first radiotracer developed, because clorgyline is a mechanism based MAO-A inhibitor that binds to MAO-A with high selectivity and demonstrates adequate brain penetration (Fowler, MacGregor et al. 1987). Yet clorgyline irreversibly binds to MAO-A, and does not have reversible time activity curves (Fowler, MacGregor et al. 1987). Hence when $^{11}$C-clorgyline is administered, the enzyme is irreversibly labeled, and the measurement reflects both blood flow (ligand delivery) as well as the measurement of MAO-A, rather than just MAO-A itself. A deuterium labeled version of $^{11}$C-clorgyline was created to attempt to improve reversibility by facilitating the separation of radiotracer delivery from radioligand binding to the enzyme, yet the advance was only a modest improvement such that deuterium labeled $^{11}$C-clorgyline did reduce the binding rate, but also revealed high white matter binding with reduced signal-to-noise ratio (Fowler, Volkow et al. 1996; Fowler, Logan et al. 2001).
More recently, $^{[11]}\text{C}$-befloxatone and $^{[11]}\text{C}$-harmine have been developed. Befloxatone demonstrates qualities of a promising radiotracer including high selectivity and reversibility, and has been modeled in baboons (Curet, Damoiseau-Ovens et al. 1998; Dolle, Valette et al. 2003; Bottlaender, Valette et al. 2010). Yet at this time, it has not been modeled in humans and the test-retest reliability in humans is unknown. $^{[11]}\text{C}$-harmine demonstrates properties of an excellent PET radiotracer for MAO-A including reversible kinetics, substantial brain uptake in humans (Bergstrom, Westerberg et al. 1997; Ginovart, Meyer et al. 2006; Meyer, Ginovart et al. 2006), metabolites which are polar and do not cross the blood brain barrier (Tweedie and Burke 1987; Ginovart, Meyer et al. 2006), and high affinity for the MAO-A enzyme ($K_i= 1-2$ nM (Bergstrom, Westerberg et al. 1997)). $^{[11]}\text{C}$-harmine is also selective for MAO-A, with an affinity three orders of magnitude higher for MAO-A than MAO-B (Bergstrom, Westerberg et al. 1997). The uptake of $^{[11]}\text{C}$-harmine is highest in brain regions with high MAO-A density such as the cortex, and lowest in regions with low MAO-A density such as white matter (Bergstrom, Westerberg et al. 1997). Displacement studies in baboons using MAO-A selective inhibitors show complete displacement of $^{[11]}\text{C}$-harmine in regions with high density of MAO-A (Bergstrom, Westerberg et al. 1997). $^{[11]}\text{C}$-harmine binding is also inhibited by other reversible MAO-A inhibitors such as clorgyline, esuprone, brofaromine, and Ro 41-1049 (Bergstrom, Westerberg et al. 1997; Bergstrom, Westerberg et al. 1997). In humans there are dosing limitations of MAO-A inhibitors based upon tolerability, however, one week of selective MAO-A inhibitor moclobemide administration at 600 mg/day reduces the MAO-A specific binding by 95 per cent during clinically tolerable dosing (Ginovart, Meyer et al. 2006). $^{[11]}\text{C}$-harmine scans also demonstrate high reliability under test-retest conditions, with mean regional absolute difference in MAO-A distribution volume ($V_T$) between 5 and 10 per cent for every region. The average difference (non-absolute) in regional MAO-A $V_T$ after test-retest conditions is approximately zero.
The MAO-A $V_T$ (MAO-A distribution volume) is primarily an index of specifically bound $[^{11}\text{C}]$-harmine at equilibrium, as 80-85% represents specific binding and the free and nonspecific binding levels are similar across individuals (Ginovart, Meyer et al. 2006; Sacher, Houle et al. 2011). MAO-A specific binding is an index of MAO-A density. There is no reference region (i.e. a region with no specific binding) for $[^{11}\text{C}]$-harmine, hence a method involving arterial sampling is required for precise quantification. Among the models assessed, the preferred method was the unconstrained two tissue compartment model. With this model, the MAO-A $V_T$ may be measured with excellent identifiability (Ginovart, Meyer et al. 2006).

Table III. Comparison of PET radiotracers for monoamine oxidase A

<table>
<thead>
<tr>
<th></th>
<th>$[^{11}\text{C}]$-Clorgyline</th>
<th>$[^{11}\text{C}]$-Harmine</th>
<th>$[^{11}\text{C}]$-Befloxatone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selectivity</strong></td>
<td>Excellent (Fowler et al. 1987)</td>
<td>Excellent (Bergstrom et al. 1997b)</td>
<td>Excellent (Dolle et al. 2003)</td>
</tr>
<tr>
<td><strong>Reversibility</strong></td>
<td>Not reversible (Fowler et al. 1987)</td>
<td>Highly reversible (Ginovart et al. 2006)</td>
<td>Highly reversible (Bottlaender et al. 2010)</td>
</tr>
<tr>
<td><strong>Modeling</strong></td>
<td>2-tissue compartment (Fowler et al. 1987)</td>
<td>2-tissue compartment (human) (Ginovart et al. 2006)</td>
<td>2-tissue compartment (baboon) (Bottlaender et al. 2010)</td>
</tr>
<tr>
<td><strong>Reliability</strong></td>
<td>Very good (Fowler et al. 1996)</td>
<td>Excellent (Sacher et al. 2012)</td>
<td>Not reported</td>
</tr>
<tr>
<td><strong>Metabolites crossing the blood brain barrier?</strong></td>
<td>Unlikely</td>
<td>No brain penetrant metabolites (Wilson et al. 2003)</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

Springer and PET and SPECT of Neurobiological Systems, 2014, Monoamine Oxidase A and Serotonin Transporter Imaging with Positron Emission Tomography, Meyer JH, Table 25.1

Comparison of PET radiotracers for monoamine oxidase A, © 2014 is given to the publication in which the material was originally published with kind permission from Springer Science and Business Media.
1.2.6 Role of MAO-A in Mood Disorders

Given the role of MAO-A in metabolizing monoamines, which are involved in mood regulation, MAO-A total volume of distribution ($V_T$), an index of MAO-A density, was measured in patients with major depressive disorder using $[^{11}\text{C}]$-harmine positron emission tomography (PET). Participants had current major depressive disorder and had experienced a major depressive episode in the past month, but were otherwise healthy (no substance abuse or other psychiatric disorders) and were drug free for at least 5 months, although most were antidepressant naive. They were aged 18-50, met DSM-IV diagnosis of current major depressive episode (MDE) and major depressive disorder (MDD), and were non-smoking. Results of the study demonstrated that MAO-A binding was highly significantly elevated ($p<0.001$ each region, average magnitude 34%) in subjects with MDD (Meyer, Ginovart et al. 2006). The data clearly showed that MAO-A elevation was linked to lowered mood because the magnitude was large, the sample was carefully defined, and the method was selective for MAO-A. This finding of elevated MAO-A binding was subsequently replicated in another PET imaging study (Meyer, Wilson et al. 2009), and an increase in catalytic activity and protein levels of MAO-A were shown in post mortem brain of MDD subjects (Johnson, Stockmeier et al. 2011).

There is consistent evidence that MAO-A is also elevated in high risk states for major depression. Shortly prior to the onset of a major depressive episode, in subjects with recovery from major depressive disorder, MAO-A binding was found to be most robustly elevated in the prefrontal and anterior cingulate cortices, as well as other regions (Meyer, Wilson et al. 2009). Greater severity and reversed neurovegetative symptoms of MDD were also shown to be associated with elevated MAO-A $V_T$ (Chiuccariello, Houle et al. 2014). In early postpartum, during the sad mood of postpartum blues, MAO-A binding elevates in affect modulating brain
regions (Sacher, Wilson et al. 2010), and MAO-A was also found to be elevated during post partum depression (Sacher, Rekkas et al. 2015). Additionally, increased MAO-A binding was detected during early cigarette withdrawal in heavy smokers, and this correlated with dysphoric mood (Bacher, Houle et al. 2011).

It is well established that depletion of monoamines is associated with onset of sad mood in humans. Administration of reserpine, which irreversibly blocks the uptake and storage of dopamine and norepinephrine into synaptic vesicles by inhibiting the vesicular monoamine transporter, induces sad mood and depression (Freis 1954). Tryptophan depletion, which depletes serotonin levels in brain, may induce sad mood in healthy individuals as well as those with remitted MDD (Young, Smith et al. 1985; Leyton, Ghadirian et al. 2000; Neumeister, Nugent et al. 2004). Alpha-methylparatyrosine administration, which is a competitive inhibitor of the rate limiting enzyme in catecholamine synthesis, tyrosine hydroxylase, depletes central dopamine and norepinephrine stores and induces depressive mood states (Verhoeff, Kapur et al. 2001; Hasler, Fromm et al. 2008). Additionally, increasing monoamine levels through MAO-A inhibition or inhibition of monoamine transporter function are longstanding therapeutic treatments for depression (Nemeroff and Owens 2002). Hence, an elevation in MAO-A levels represents a single process that could facilitate the loss of the three monoamines, which is associated depressed mood.
1.2.7 Monoamine Oxidase A in Alcohol Dependence in Humans

Four previous investigations examined MAO-A activity in post mortem brain of alcohol dependent individuals (see Table IV). Two of the studies were carried out before MAO subtypes were identified, therefore the effect on MAO-A was inconclusive (Grote, Moses et al. 1974; Gottfries, Oreland et al. 1975). The latter two studies reported no change in the prefrontal cortex of alcohol abusing individuals compared to controls, or a decrease in the hypothalamus and caudate (Oreland, Wiberg et al. 1983; Major, Hawley et al. 1985). Yet these studies were confounded by various factors that associate with MAO-A, including cigarette smoking, other psychiatric comorbidities, and impulsivity.

Table IV. Comparison of post mortem studies of monoamine oxidase in alcohol dependence

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Method</th>
<th>Result</th>
<th>Biases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grote et al. 1974</td>
<td>suicide victims with alcoholism (n= 11)</td>
<td>homogenate, combined MAO-A and MAO-B activity</td>
<td>no effect of group</td>
<td>-MAO-B not separated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-cigarette smoking</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-comorbidities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-impulsivity</td>
</tr>
<tr>
<td>Gottfries et al. 1975</td>
<td>suicide victims with alcoholism (n= 15)</td>
<td>homogenate, combined MAO-A and MAO-B activity</td>
<td>lower in all brain regions including prefrontal cortex and hippocampus</td>
<td>-MAO-B not separated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-cigarette smoking</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-comorbidities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-impulsivity</td>
</tr>
<tr>
<td>Oreland et al. 1983</td>
<td>alcohol abuse (n= 12)</td>
<td>homogenate MAO-A activity</td>
<td>lower in hypothalamus and caudate</td>
<td>-cigarette smoking</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-comorbidities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-impulsivity</td>
</tr>
<tr>
<td>Major et al. 1985</td>
<td>alcohol abuse (n= 10)</td>
<td>homogenate MAO-A activity</td>
<td>no difference in frontal cortex</td>
<td>-cigarette smoking</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-comorbidities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-impulsivity</td>
</tr>
</tbody>
</table>
Cigarette smoking is a confound because it was recently reported that MAO-A binding is reduced during the active smoking state, and increased during withdrawal (Fowler, Volkow et al. 1996; Bacher, Houle et al. 2011). Cigarette smoke also contains beta-carboline compounds such as harman, which inhibit MAO-A and may affect MAO-A measures (discussed in further detail in the Section 1.2.9). Other psychiatric comorbidities are a confound because MAO-A binding was significantly elevated in MDD patients with a current major depressive episode, and well as those with remitted MDD (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009). The final major confound of these early post mortem studies is that impulsive and aggressive personality traits were not controlled for. Aggressive and impulsive personality traits, as defined by the NEO Personality Inventory–Revised questionnaire (NEO-PI-R) (Costa and McCrae 1997; Kurtz, Lee et al. 1999; Reynolds and Clark 2001), are highly associated with alcohol dependence and relapse (Fisher LA 1998; Ruiz MA 2003; Bottlender and Soyka 2005). **Comorbid impulsivity and aggression are powerful biases that must be addressed in studies of MAO-A in AD because:**

i) **Human MAO-A Knockout Mutation is Strongly Associated with Impulsive Aggression:** Males from a large Dutch family carrying a rare point mutation in the eighth exon of the MAO-A gene that results in complete and selective deficiency of MAO-A enzymatic activity display a syndrome of impulsive aggressive behaviours, including arson and rape (Brunner, Nelen et al. 1993).

ii) **Rodent MAO-A Knockout is Associated with Aggression:** MAO-A knockout mice consistently demonstrate aggressive behaviour (Cases, Seif et al. 1995; Chen, Cases et al. 2007; Scott, Bortolato et al. 2008). Consistent with this observation, pharmacological MAO-A inhibition during development is also associated with greater aggression (Mejia,
iii) **Alleles Associated with Reduced MAO-A Level In Vitro are also Associated with Aggression:** A variable number tandem repeat polymorphism in the promoter area of the MAO-A gene that is associated with reduced MAO-A level and activity in vitro is strongly associated with conduct disorder, antisocial personality disorder and aggression in people exposed to childhood maltreatment (Caspi, McClay et al. 2002; Kim-Cohen, Caspi et al. 2006). The link between the alleles of this polymorphism that are associated with reduced MAO-A level in vitro and the link between these alleles and greater risk for aggressive behaviour is strongly suggestive of reduced MAO-A levels in people with aggression. The model that has often been proposed for this relationship is a gene by environment interaction such that particular alleles in combination with exposure to violence lead to aggressive behaviour (Caspi, McClay et al. 2002; Kim-Cohen, Caspi et al. 2006).

iv) **Lower Cerebrospinal Fluid (CSF) Serotonin Metabolite 5-Hydroxyindoleacetic Acid is Associated with Impulsive Aggression:** The association between low concentrations of CSF 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, and impulsive/aggressive behaviour has been described as one of the most robust and consistently replicated findings in biological psychiatry (see Table V).
Table V. Comparison of studies of low concentrations of CSF 5-hydroxyindoleacetic acid (5-HIAA) and impulsive aggressive behaviour

<table>
<thead>
<tr>
<th>Study</th>
<th>Impulsive Aggression</th>
<th>CSF 5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown et al. (Brown, Goodwin et al. 1979)</td>
<td>Personality Disorders (Military)</td>
<td>↓</td>
</tr>
<tr>
<td>Linnoila et al. (Linnoila, Virkkunen et al. 1983)</td>
<td>Personality Disorders (Prisoners)</td>
<td>↓</td>
</tr>
<tr>
<td>Lidberg et al. (Lidberg, Asberg et al. 1984)</td>
<td>Murderers of Own Children</td>
<td>↓</td>
</tr>
<tr>
<td>Lidberg et al. (Lidberg, Tuck et al. 1985)</td>
<td>Murderers of Sexual Partner</td>
<td>↓</td>
</tr>
</tbody>
</table>

Lower CSF 5-HIAA levels also occur when MAO-A is pharmacologically inhibited (Celada and Artigas 1993); therefore, one explanation for reduced 5-HIAA levels in people with aggression is that they have reduced MAO-A level. Low levels of CSF 5-HIAA are also reported in alcohol dependence (Borg, Kvande et al. 1985; Fils-Aime, Eckardt et al. 1996).

v) **Lower Prefrontal Cortex MAO-A V<sub>T</sub> (and MAO-A Binding) is Associated with Impulsivity and Aggression in Healthy Subjects:** A recent PET study reported that brain MAO-A binding was inversely correlated with trait measures of aggression in healthy humans (Alia-Klein, Goldstein et al. 2008). This finding has been replicated using [11C]-harmine PET, where orbitofrontal and prefrontal cortex MAO-A V<sub>T</sub> was significantly lower among healthy participants reporting higher levels of the angry-hostility facet of the NEO-PI-R (r = -0.52; p < .001) (Soliman, Bagby et al. 2011).

vi) **The MAO-A gene is considered a candidate gene for susceptibility to alcohol dependence:** Low activity variants of the MAO-A gene have been associated with
antisocial alcoholism (Samochowiec, Lesch et al. 1999; Schmidt, Sander et al. 2000; Guindalini, Scivoletto et al. 2005), while other studies report no association (Lu, Lee et al. 2002; Saito, Lachman et al. 2002; Koller, Bondy et al. 2003).

Given these associations between low MAO-A levels and aggression/impulsivity, and the association between alcohol dependence and aggressive/impulsive personality traits, the only way to remove this bias from studies of MAO-A in AD would be to match participants for these personality facets as measured by the NEO-PI-R. These associations were not discovered at the time of the early post mortem studies, hence were not addressed. In the context of alcohol dependence, this evidence can be interpreted as a neurodevelopmental model where low MAO-A may be inherited, leading to the development of personality traits such as aggression/anger and impulsivity in childhood, that may increase the risk for AD later in life. The role of serotonin in adulthood may be different; for example, studies of serotonin depletion in adulthood have been associated with increased aggression (Young and Leyton 2002). The current series of studies aim to investigate whether, after the development of AD, there may be shifts toward elevated MAO-A (which are associated with dysphoric mood/suicidality and are elevated during major depressive episodes), as recent prospective studies suggest AD increases the risk for MDD, and low mood occurs during withdrawal and may persist into abstinence (Wang and Patten 2002; Ramsey, Kahler et al. 2004; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009).
1.2.8 Rationale for Investigating MAO-A in Alcohol Dependence

1.2.8.1 Alcohol Dependence and Dysphoric Mood

As outlined in Section 1.2.3, alcohol dependence is associated with dysphoric mood during early withdrawal and this increases the risk of developing a major depressive disorder (Glenn and Parsons 1991; Ramsey, Kahler et al. 2004). Currently, there is no clear neurochemical explanation for the high rate of comorbidity between alcohol dependence and major depression. Two studies of MAO-A binding in human brain demonstrate that MAO-A is elevated during major depressive episodes (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009). MAO-A expression is also elevated in postmortem brain of MDD subjects (Johnson, Stockmeier et al. 2011). Additionally, elevations in MAO-A binding are seen in many high risk states for major depressive disorder (Meyer, Wilson et al. 2009; Sacher, Wilson et al. 2010; Bacher, Houle et al. 2011). These links between MAO-A and low mood states, as well as the high rate of comorbid MDD and dysphoric mood in AD, suggest that MAO-A may elevate in alcohol dependence.

1.2.8.2 Monoamine Oxidase A and Models of Alcohol Dependence in Rodents

The perspective that elevated MAO-A level may be acquired through alcohol exposure is suggested by two investigations of alcohol exposure in rodents. Rimondini et al. reported a 2.5 fold increase in MAO-A mRNA using a microarray analysis in the medial prefrontal cortex after chronic alcohol vapor exposure that yielded blood alcohol concentrations similar to human intoxication (150-250 mg/dl) (Rimondini, Arlinde et al. 2002). Zimatkin et al. reported that ethanol preferring rats had greater MAO-A activity in neurons of the raphe pontis and dorsal raphe nucleus after chronic ethanol consumption (6 g/kg/day) (Zimatkin, Tsydik et al. 1997). The
body of literature on the effect of alcohol exposure on MAO-A activity in rodents is inconsistent, with a variety of early studies showing no effect (Wiberg, Wahlstrom et al. 1977; Morinan 1987; Wahlstrom, Magnusson et al. 1991; Sherif, Wahlstrom et al. 1993). Yet these studies employed methods (i.e. intraperitoneal injection, free choice or forced ethanol solution) that did not result in blood alcohol concentrations comparable to humans, nor did the levels induce intoxication or dependence in rodents. It is currently unknown whether alcohol administration at levels comparable to human addiction affect MAO-A levels in rodent brain.

1.2.8.3 Alcohol Dependence and Predisposition to Apoptosis

As outlined in Section 1.2.2.3 and 1.2.2.4, there are many reports of markers associated with predisposition to apoptosis (Casey, Nanji et al. 2001; Ikegami, Goodenough et al. 2003; Ou, Johnson et al. 2011) and oxidative stress (Wu and Cederbaum 2003; Wu, Zhai et al. 2006; Das and Vasudevan 2007) present in brain and other organs during AD. There is emerging literature that oxidative stress and predisposition to apoptosis lead to elevated MAO-A activity and protein levels in neuroblastoma and glioblastoma cell lines (Ou, Chen et al. 2006; Youdim, Edmondson et al. 2006; Fitzgerald, Ufer et al. 2007; Fitzgerald, Ufer et al. 2007). Induction of apoptosis through depletion of neurotrophic factor in rodent PC12 cells paralleled an increase in MAO-A expression (De Zutter and Davis 2001). In human melanoma m14 cells, clorgyline, a MAO-A inhibitor, was able to protect cells from apoptosis induced by serum starvation (Malorni, Giammarioli et al. 1998). MAO-A was also shown to be a target of the dopaminergic neurotoxin, N-methyl-(R)-salsolinol, which leads to apoptosis in human neuroblastoma cell lines (Yi, Akao et al. 2006). In a serum starvation paradigm, cell proliferation was significantly decreased, while MAO-A mRNA and activity increased 4.3 and 1.5 fold, respectively, in human neuroblastoma cells (Ou, Chen et al. 2006). Another study corroborated this result, demonstrating a 2 fold
increase in MAO-A mRNA and activity after serum starvation, and also showed that clorgyline reduced the number of cells with apoptotic cell morphology (Fitzgerald, Ufer et al. 2007). Another method of induced apoptosis, staurosporine, similarly led to an increase in MAO-A mRNA and protein levels (Fitzgerald, Ufer et al. 2007). These findings collectively suggest that MAO-A plays an important role in the modulation of apoptotic signaling in response to biological stressors, and hence may be involved in the predisposition to apoptosis observed in alcohol dependence.
1.2.9 Role of Monoamine Oxidase A Inhibitor Harman in Addiction

The beta-carboline alkaloids, including harman, were first discovered to produce a pharmacological effect through their use as a sedative and hallucinogenic in South American traditional medicine (Hashimoto 1988). Derived from the *Peganum Harmala* plant, harman has since been shown to bind to many receptor systems in the brain, with moderate affinity for monoamine oxidase (May, Rommelspacher et al. 1991; Kim, Sablin et al. 1997) and the imidazoline receptor (Husbands, Glennon et al. 2001). Harman is selective for MAO-A and acts as a reversible, competitive inhibitor at this enzyme (Ki: 220 nM for MAO-A, Ki: 57,000 nM for MAO-B) (May, Rommelspacher et al. 1991; Kim, Sablin et al. 1997). Harman also demonstrates modest affinity for the benzodiazepine receptor (Rommelspacher, Nanz et al. 1980), 5-hydroxytryptamine and dopamine receptor subtypes (Glennon, Dukat et al. 2000), and influences neurotransmitter levels (Adell and Myers 1995; Baum, Hill et al. 1995; Baum, Hill et al. 1996; Iurlo, Leone et al. 2001). See Table VI. Beta-carbolines are found endogenously in human and rodent tissues, including the brain (Airaksinen and Kari 1981; Melchior and Collins 1982; Rommelspacher, May et al. 1994), and are also found in many food and beverage products, including various types of alcohol (wine, beer, whiskey, sake) and cigarette smoke (Pfau and Skog 2004). The approximate plasma half life of harman is 50 minutes in humans and 30 minutes in rats (Guan, Louis et al. 2001; Rommelspacher, Meier-Henco et al. 2002). Reported harman levels are highest in cigarette smoke (range 0.36- 4 ug/g) and vary across different types of alcoholic beverages (7.3-140 ng/ml) (Pfau and Skog 2004). The existence of beta-carbolines in addictive substances was the first indication that they may be involved in the neurobiology of drug addiction.
Table VI. Relative inhibitory concentrations of harman

<table>
<thead>
<tr>
<th>Harman Binds to</th>
<th>IC$_{50}$ (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazoline 1 Receptor (Husbands, Glennon et al. 2001)</td>
<td>0.031</td>
</tr>
<tr>
<td>MAO-A (Glover, Liebowitz et al. 1982)</td>
<td>0.500</td>
</tr>
<tr>
<td>MAO-B (Glover, Liebowitz et al. 1982)</td>
<td>5.000</td>
</tr>
<tr>
<td>5-HT$_2$ (serotonin) Receptor (Glennon, Dukat et al. 2000)</td>
<td>6.750</td>
</tr>
<tr>
<td>Benzodiazepine Receptor (Rommelspacher, Nanz et al. 1980)</td>
<td>7.000</td>
</tr>
<tr>
<td>D$_2$ (dopamine) Receptor (Glennon, Dukat et al. 2000)</td>
<td>163.0</td>
</tr>
</tbody>
</table>

IC$_{50}$, half maximal inhibitory concentration

The body of literature linking beta-carbolines to alcohol and tobacco dependence has yielded equivocal results. In alcohol dependent (Rommelspacher, Schmidt et al. 1991; Rommelspacher, Dufeu et al. 1996; Spies 1998) and heavy smoking individuals (Breyer-Pfaff, Wiatr et al. 1996; Spijkerman, van den Eijnden et al. 2002; Bacher, Houle et al. 2011), plasma levels of harman and norharman are elevated. Yet these studies were confounded by co-morbid alcohol and tobacco use, as well as whether the individual was abstinent at the time of the study. Some evidence suggests the elevation is due to cigarette smoking, as increased norharman appears in heavy smokers regardless of their drinking profile (Breyer-Pfaff, Wiatr et al. 1996; Spijkerman, van den Eijnden et al. 2002). Levels of urine harman and norharman are shown to be elevated in alcohol dependent patients (Wodarz, Wiesbeck et al. 1996), and levels of brain and urine harman are elevated after ethanol administration in rodents (Rommelspacher, Damm et al. 1984). Yet other studies have demonstrated no effect of ethanol on harman levels in humans or rodents (Bosin, Borg et al. 1988; Rommelspacher, Schmidt et al. 1991). Harman has also been implicated in alcohol addiction because its infusion produces a robust increase in voluntary
drinking in rats (Myers and Oblinger 1977; Airaksinen, Mahonen et al. 1983; Rommelspacher, Buchau et al. 1987; Myers 1989; Adell and Myers 1994), with alcohol intake increasing by 100% in one study (Tuomisto, Airaksinen et al. 1982). These effects have been interpreted to be related to the non-specificity of harman binding in the brain; although harman acts as a MAO-A inhibitor, it is also an inverse agonist at the benzodiazepine receptor (Rommelspacher, Nanz et al. 1980; Adell and Myers 1995; Baum, Hill et al. 1996; Arib, Rat et al. 2010). Beta-carbolines have also been identified in marijuana smoke condensate (Kettenes Van den Bosch 1977), and are shown to be elevated in the plasma of heroin users (Stohler, Rommelspacher et al. 1995), demonstrating an association to the reinforcing properties of a variety of addictive substances.

Harman is found in substantial concentrations in alcoholic beverages, but it is also proposed that the condensation products of acetaldehyde, the main metabolite of ethanol, with biogenic amines may play a role in alcohol addiction. Beta-carbolines and tetrahydroisoquinolines (TIQs) are formed through the Pictet-Spangler condensation reaction of acetaldehyde with indolamines (i.e. serotonin, tryptophan, tryptamine) and catecholamines (i.e. dopamine, norepinephrine), respectively. Specifically, harman is formed from the condensation reaction of acetaldehyde with tryptamine or tryptophan (see Figure 2). It is hypothesized that this formation of harman then leads to MAO-A inhibition, which increases monoamine levels, particularly dopamine, that act at the mesolimbic reward pathway and reinforce addictive behaviour (Quertemont and Tambour 2004; Talhout, Opperhuizen et al. 2007). Therefore beta-carbolines are proposed to be involved in alcohol addiction by means of the products of ethanol metabolism, as well as through direct consumption of alcoholic beverages.
Figure 2. Condensation products of acetaldehyde with several biogenic amines (and tryptophan).

A challenging issue when investigating addictive substances is that they may contain multiple compounds implicated in the reinforcing behaviour of recurrent intake. One approach to address this issue is to study the effects of individual constituents rather than all of them concurrently. As previously mentioned, harman is found in common addictive substances such as cigarette smoke and alcohol containing beverages (Pfau and Skog 2004). Harman is a reversible inhibitor of MAO-A with moderately high affinity (May, Rommelspacher et al. 1991; Kim, Sablin et al. 1997), and is sufficiently brain penetrant to affect levels of monoamines metabolized by MAO-A (Adell and Myers 1995; Baum, Hill et al. 1996). The effect of chronic harman exposure on its primary target of MAO-A is currently unknown.

An important question in the literature pertains to whether harman is harmful, since it is present in cigarette smoke and many alcohol containing beverages, and is a product of alcohol metabolism (Rommelspacher, Damm et al. 1984; Bosin and Faull 1988; Pfau and Skog 2004). During early withdrawal from cigarette smoking, MAO-A $V_T$, an index of unoccupied MAO-A binding sites, is increased by 25% in the prefrontal and anterior cingulate cortices of heavy smoking individuals (Bacher, Houle et al. 2011). A subsequent study found increased MAO-A activity and mRNA in the lung of cigarette smoke exposed rodents (Lau, Li et al. 2012). During alcohol withdrawal in rodents, a two fold increase in MAO-A mRNA was reported in the prefrontal cortex (Rimondini, Arlinde et al. 2002). This upregulation of MAO-A may be viewed as harmful, as greater MAO-A $V_T$ during withdrawal from heavy smoking was correlated with more depressed affect (Bacher, Houle et al. 2011), and depressed mood in early withdrawal is associated with greater risk for relapse (Caster and Parsons 1977; Parsons, Schaeffer et al. 1990; Glenn and Parsons 1991). Furthermore, elevated MAO-A and/or MAO-B activity is implicated as pathophysiologically relevant to several diseases including the PFC and ACC of major depressive disorder and postpartum depression (Meyer, Ginovart et al. 2006; Meyer, Wilson et
al. 2009; Johnson, Stockmeier et al. 2011; Sacher, Rekkas et al. 2015), the striatum of Huntington’s disease (Richards, Messer et al. 2011), and predisposition to Alzheimer’s disease (Wu, Fischer et al. 2007; Pennington, Wei et al. 2011). Beta-carboline administration is being investigated for antidepressant and anxiolytic effects, and the ability to reduce craving, hence there is great importance in determining whether harman influences MAO-A level.
1.3 Study Objectives and Research Hypotheses

The current studies aim to answer three specific questions with regard to the influence of alcohol dependence upon MAO-A. The first is whether MAO-A is elevated during human alcohol withdrawal, the second is whether harman, a MAO-A inhibitor implicated in addiction, upregulates MAO-A in rodent brain, and the third is whether alcohol exposure upregulates MAO-A in rodent brain.

Study Specific Objectives:

1) To investigate MAO-A levels in AD in humans and avoid confounding biases that influence MAO-A levels such as cigarette smoking, major depressive disorder, and impulsivity. We chose an in vivo approach by applying $[^{11}\text{C}]$-harmine positron emission tomography (PET). $[^{11}\text{C}]$-harmine PET measures brain MAO-A $V_T$, an index of MAO-A density.

2) To investigate MAO-A levels in rodent brain after chronic harman exposure. We chose 21 day harman administration via osmotic minipump and two experimental timepoints, where rodents were sacrificed immediately after 21 day exposure, or after 8 hours of withdrawal from harman. The 8 hour withdrawal timepoint was chosen because it exceeds 5 half lives of harman and is a point proximal to harman withdrawal that was used in previous PET studies associated with elevated MAO-A level (Guan, Louis et al. 2001; Bacher, Houle et al. 2011; Matthews, Kish et al. 2013).

3) To investigate MAO-A levels in rodent brain after chronic alcohol exposure. We chose 8 week alcohol vapor administration and four experimental timepoints, where rodents were
sacrificed immediately after 8 week exposure, or after 24 hours, 4 days, or 21 days of withdrawal from alcohol vapor. These timepoints were chosen based on the behavioural characterization of withdrawal using the alcohol vapour exposure paradigm (Rimondini, Arlinde et al. 2002; Sommer, Rimondini et al. 2008; Vendruscolo, Barbier et al. 2012).

**Study Specific Hypotheses and Rationale:**

1) **Hypothesis:** MAO-A V<sub>T</sub> would be increased in the prefrontal cortex in alcohol dependence in humans. Our second main hypothesis was that greater years of exposure to heavy alcohol use would be associated with the elevation in MAO-A level.

**Rationale:** First, greater oxidative stress and markers associated with apoptosis occur in liver and brain during alcohol dependence (Casey, Nanji et al. 2001; Ikegami, Goodenough et al. 2003; Ou, Johnson et al. 2011), and MAO-A levels may elevate under such conditions (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007; Fitzgerald, Ufer et al. 2007). Second, alcohol dependence is associated with elevated risk for major depressive disorder and depressed mood (Hasin and Grant 2002; Wang and Patten 2002; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009), and MAO-A levels and/or MAO-A V<sub>T</sub> are elevated most robustly in the prefrontal and anterior cingulate cortex (and in other regions) in the midst of major depressive episodes, as well as during high risk states for MDD (Meyer, Wilson et al. 2009; Sacher, Wilson et al. 2010; Bacher, Houle et al. 2011). The PFC was chosen as the primary region of interest because it has a functional role related to mood. For example, the PFC and ACC are activated during sad mood induction paradigms using fMRI in health, remitted MDD, and MDD (Liotti, Mayberg et al. 2002; Price and Drevets 2010), and also demonstrate abnormalities of regional cerebral blood flow in MDD, which normalize in recovery (Bench, Friston et al.
The PFC and ACC also display the highest sensitivity to environmental and disease stimuli, and consistently demonstrate the greatest effect size of elevated MAO-A in MDD, cigarette withdrawal, post partum depression, and prior to recurrence to MDD (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Bacher, Houle et al. 2011; Sacher, Rekkas et al. 2015). The PFC was prioritized because a microarray analysis evaluating the effects of chronic alcohol vapor exposure in rodents reported a 2.5 fold elevation in MAO-A mRNA in this brain region (Rimondini, Arlinde et al. 2002). This study did not sample other regions; hence additional brain regions may demonstrate similar effects.

2) **Hypothesis:** MAO-A level and activity would be upregulated in the prefrontal and anterior cingulate cortex following harman administration.

**Rationale:** Harman inhibits MAO-A and increases levels of monoamines (Baum, Hill et al. 1996; Kim, Sablin et al. 1997). Cigarette smoke also contains harman, and MAO-A binding was found to be elevated in cigarette withdrawal in heavy smokers (Bacher, Houle et al. 2011). The PFC and ACC were prioritized because they have a functional role related to mood and display the highest sensitivity to environmental and disease stimuli (see (1) Rationale).

3) **Hypothesis:** MAO-A level and activity would be upregulated in the prefrontal and anterior cingulate cortex following ethanol administration.

**Rationale:** A microarray analysis evaluating the effects of chronic alcohol vapor exposure in rodents reported a 2.5 fold elevation in MAO-A mRNA in the prefrontal cortex (Rimondini, Arlinde et al. 2002). The PFC and ACC were prioritized because they
have a functional role related to mood and display the highest sensitivity to environmental and disease stimuli (see (1) Rationale).
2 Methods

2.1 Human PET Imaging Study of MAO-A in Alcohol Dependence


2.1.1 Study Participants and Protocol for PET Imaging Scan

Sixteen individuals with alcohol dependence (mean age, 35, standard deviation [SD] [8]) were compared to 16 healthy controls (mean age, 34 [9]). Some of the healthy participants (n= 14) participated in earlier studies (Meyer, Wilson et al. 2009; Bacher, Houle et al. 2011). Participants were within the age range of 18 to 50 years and in good physical health.

The key inclusion criteria for the alcohol dependent group was an AD diagnosis using the Structured Clinical Interview for DSM-IV(First, Spitzer et al. 1995). Severity of AD was assessed using the Alcohol Dependence Scale (Skinner 1984). Our intent was to recruit subjects with AD who had not acquired additional psychiatric or medical illnesses, and we chose a minimum drinking cutoff commonly associated with AD that required consuming at least 4 (for women) or 5 (for men) drinks per day at least 5 days of the week (Mann 2004; Saha, Stinson et al. 2007). This cutoff is a well accepted level for hazardous drinking, as consumption beyond this level across several years has an extremely high probability of leading to AD (Dawson, Grant et al. 2005). In a previous study, moderate to heavy cigarette smoking was associated with greater MAO-A $V_T$ in early withdrawal and reduced MAO-A $V_T$ during active smoking (Bacher, Houle
et al. 2011); hence cigarette smoking was an exclusion criterion in the current study. Although 50% of people with AD smoke cigarettes (Sarsour, Johnston et al. 2012), this cutoff of alcohol consumption ensures a representative sampling of alcohol intake behaviour in AD subjects, regardless of smoking status (Dawson, Grant et al. 2005). Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urine ethyl glucuronide were also measured.

To avoid factors that could bias MAO-A levels in each group, exclusion criteria included any current or past Axis 1/Axis 2 disorder (First, Spitzer et al. 1995) (apart from alcohol dependence in the AD group), cigarette smoking, herbal, drug or medication use within eight weeks of scanning, history of psychiatric or medical illness, or any other substance abuse/dependence. Lifetime history of comorbid axis I disorders, including past major depressive episodes and anxiety disorders, were exclusionary. Screening included exhaled carbon monoxide level (MicroSmokerlyzer; Bedfont Scientific Ltd, Kent, England), plasma cotinine levels, and a urine drug test at screening and on the PET scan day. Those with positive results for other substances were excluded. For women, phase of menstrual cycle was recorded by self report. In a previously collected sample, there was no relationship between phase of menstrual cycle and MAO-A V_T (Meyer, Wilson et al. 2009). To avoid confound from variations in plasma estrogen levels, women in early postpartum, perimenopause or menopause were excluded. Participants were required not to drink tea or coffee on the day of scanning. All subjects reported no prior head injury, nor had neurological disorders associated with alcohol dependence (Korsakoff syndrome, Wernicke’s encephalography). For each participant, written consent was obtained after the procedures were fully explained. The study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto.
All study participants underwent a single $^{11}$C-harmine PET scan. Alcohol metabolism may lead to the formation of a beta-carboline compound called harman (Rommelspacher, Damm et al. 1984; Rommelspacher, Schmidt et al. 1991), and alcoholic beverages frequently contain harman (Pfau and Skog 2004), which has moderate affinity for MAO-A. To avoid temporary occupancy effects of harman (Bacher, Houle et al. 2011), $^{11}$C-harmine PET scanning was timed at a point when harman levels were negligible (Breyer-Pfaff, Wiatr et al. 1996), which was verified by plasma sampling. Subjects were instructed to maintain their usual alcohol intake behaviour and to stop drinking at 12 am the evening before the $^{11}$C-harmine PET scan. A breathalyzer screen was taken and results were consistent with recent cessation of alcohol intake. Also, prior to scanning, 12-cm visual analog scales (VASs) for mood (i.e., happy-depressed), energy (i.e., most-least), and anxiety (i.e., relaxed- tense), and the Clinical Institute Withdrawal Assessment (CIWA-Ar) for measurement of severity of withdrawal symptoms, were completed. For the VAS, participants were instructed to draw a vertical line crossing the 12-cm linear scale at the point corresponding to the strength of their experience of the given dimension of the mood state.

2.1.2 PET Image Acquisition and Analysis

The PET images were obtained using a High Resolution Research Tomograph PET camera (in-plane resolution; full width at half maximum, 3.1 mm; 207 axial sections of 1.2 mm; Siemens Molecular Imaging, Knoxville, Tennessee) in a manner described previously (Meyer, Wilson et al. 2009). A dose of 370 MBq of intravenous $^{11}$C-harmine was administered as a bolus. The $^{11}$C-harmine was of high radiochemical purity (99.16 ± 1.12%) and high specific activity (94.27 ±33.71 GBq/µmol) at the time of injection. The emission scan was reconstructed in 15 frames of 1 minute, followed by 15 frames of 5 minutes. An automatic blood sampling system was used to measure arterial blood radioactivity continuously for the first 10 minutes after
injection. Manual samples were obtained at 2.5, 7.5, 15.0, 20.0, 30.0, 45.0, 60.0, and 90.0 minutes. The radioactivity in whole blood and plasma were measured as described previously (Ginovart, Meyer et al. 2006).

Each participant also underwent magnetic resonance imaging (GE Sigma 1.5-Tesla scanner; fast spoiled gradient echo, T₁-weighted image; x, y, z voxel dimensions, 0.78, 0.78, and 1.5 mm; GE Medical Systems, Milwaukee, Wisconsin). Regions of interest (ROIs) were delineated on these magnetic resonance images using a semiautomated method based on linear and non-linear transformations of an ROI template in standard space to the individual MRI, followed by a refinement process based upon the gray matter probability (Ashburner and Friston 1997; Rusjan, Mamo et al. 2006). The MRI was coregistered to the summated [¹¹C]-harmine PET image using a mutual information algorithm (Studholme C 1999), and the resulting transformation was applied to sample the ROIs from the PET image. The location of the ROI was verified by visual assessment on the summated [¹¹C]-harmine PET image.

The primary ROI was the whole prefrontal cortex but additional regions with high MAO-A density were sampled, including the anterior cingulate cortex, dorsal putamen, ventral striatum, thalamus, hippocampus, and midbrain. The definitions of the regions of interest were similar to our previous investigations (Meyer, Wilson et al. 2001; Meyer, McMain et al. 2003; Meyer, Ginovart et al. 2006) and are based upon a neuroanatomy atlas of structural MRI and post-mortem tissue (Duvernoy 1999), with the exception of the divisions of dorsal putamen and ventral striatum, which are described by Malawi (Mawlawi, Martinez et al. 2001). The prefrontal cortex (PFC) is defined as the entirety of the frontal cortex anterior to precentral gyrus (Duvernoy 1999). The superior to inferior extent of the anterior cingulate cortex is defined as those transverse MRI slices in MNI orientation (as found in MNI space) in which the caudate,
putamen and globus pallidus are all visualized. The anterior cingulate cortex is bounded medially by the interhemispheric fissure, anteriorly by the cingulate sulcus, laterally by white matter, and posteriorly by the anterior genu of the corpus callosum. The superior extent of the caudate and putamen is defined as those transverse MRI slices in MNI orientation in which these structures are visualized. The putamen is bounded medially by the globus pallidus, anteriorly by the internal capsule, and laterally by the external capsule. The border between the ventral striatum and dorsal caudate and putamen is defined (from a coronal view) by a boundary joining the intersection between the outer edge of the putamen (with a vertical line going through the most superior and lateral point of the internal capsule) and the center of the portion of the anterior commissure transaxial plane overlying the striatum, extending to the internal edge of the caudate (Mawlawi, Martinez et al. 2001). The superior to inferior extent of the thalamus is defined as those transverse MRI slices superior to the anterior commissure in which the caudate, putamen and globus pallidus are visualized. The entirety of the hippocampus is sampled, and its boundaries are defined primarily from the coronal view using the neuroanatomy atlas described (Duvernoy 1999). The superior to inferior extent of the midbrain is identified by those transverse MRI slices in which the superior colliculus and crux cerebri are visualized.

Subregions of the prefrontal cortex, including the dorsolateral, medial, orbitofrontal and ventrolateral prefrontal cortices, were sampled to evaluate whether the effects observed in the main analyses of the prefrontal cortex were consistent within these subregions. The subregions were defined based upon their cytoarchitectural differentiation from surrounding tissue, which was mapped onto the external morphology of the cortex (Rajkowska and Goldman-Rakic 1995; Rajkowska and Goldman-Rakic 1995; Uylings, Sanz-Arigita et al. 2010).
MAO-A total distribution volume ($V_T$) was measured via $^{11}$C-harmine PET. MAO-A $V_T$ equals the ratio of tissue to plasma concentration of $^{11}$C-harmine at equilibrium, of which 85% represents radioligand specifically bound to MAO-A (Ginovart, Meyer et al. 2006; Sacher, Houle et al. 2011). Hence, changes in MAO-A $V_T$ may be interpreted as representing changes in harmine binding to MAO-A. The $V_T$ can be expressed in terms of kinetic rate parameters as follows: $V_T = (K_1/k_2) \times (k_3/k_4) + (K_1/k_2)$, where $K_1$ and $k_2$ are influx and efflux rate parameters, respectively, for radiotracer passage across the blood-brain barrier, and $k_3$ and $k_4$ describe the radioligand transfer between the free and nonspecific compartment and the specific binding compartment. Among different groups, $K_1/k_2$ is similar (for further details, see Ginovart et al (Ginovart, Meyer et al. 2006)). MAO-A $V_T$ can be measured validly and reliably with an unconstrained 2-tissue compartment model or the Logan model with arterial sampling; the latter technique was applied in the current study (Ginovart, Meyer et al. 2006).

2.1.3 Statistical Analysis

The primary analysis was an independent samples t-test to assess the group effect (i.e. alcohol dependence versus health) upon MAO-A $V_T$ in the prefrontal cortex. In addition, to further characterize the comparison between the alcohol dependent group and healthy controls, a multivariate analysis of variance (MANOVA) was used to assess the group effect upon MAO-A $V_T$ in all brain regions assayed. Independent samples t-tests were then applied at each region of interest to compare groups.

The main secondary analysis was a Pearson correlation coefficient to assess the relationship between years of heavy alcohol use and MAO-A $V_T$ within the prefrontal cortex. In addition, to further characterize the relationship between years of heavy alcohol use and MAO-A $V_T$, Pearson correlation coefficients were determined for each region of interest.
2.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents

2.2.1 Animals

Male Sprague-Dawley rats (Charles River, Quebec), initially weighing 225–250g, were maintained at 22-24 °C room temperature on a 12h light–dark cycle with free access to food and water. Upon arrival, a week of acclimatization to the facility was allowed and rats were paired housed. All procedures were approved by the Animal Care Committee at the Centre for Addiction and Mental Health and complied with Canadian Council on Animal Care standards and guidelines.

2.2.2 Harman Osmotic Minipump Exposure

Rats were randomly assigned to receive 0, 2, 5, or 15 mg/kg/day of harman (Sigma Aldrich, Canada) for 21 days via Alzet minipumps (Alzet model 2ML4, Durect Corp., Cupertino, CA). Rats were divided into two experimental conditions, one group scheduled for sacrifice on day 21, and the other group scheduled for sacrifice after 8 hour removal of the minipump on day 21. This resulted in a total of 8 groups across four different doses and two different durations of harman cessation (n= 10 in each group). The dosing regimen of harman was consistent with the literature examining the behavioural effects of beta-carboline administration, including antidepression (Fortunato, Reus et al. 2010) and anti-nociception effects (Aricioglu, Korcegez et al. 2003). Harman was first dissolved in glacial acetic acid, then titrated with 5 N NaOH to bring the pH to 6-7 and diluted to expected concentrations for minipumps, while vehicle was sterile saline.
Osmotic minipumps had the capacity of 2 mL and were primed to infuse 31.25 μg/μL over the 21 day interval.

On day 7, rats underwent minipump implantation and were singly housed for the following 21 days. Each rat was anesthetized briefly using the inhalant anesthetic isoflurane. Once anesthetized, a section of the animal's back was shaved and sterilized with isopropyl alcohol and betadine solution. An incision was made within the shaved area followed by dissection of connective tissue with blunt-tipped forceps. The minipumps were sterilized with isopropyl alcohol and then inserted subcutaneously slightly posterior to the scapulae according to manufacturer's specifications. The incision was closed using 9 mm surgical staples, and analgesia included topical bupivacaine (0.12 %) and intramuscular ketoprofen (5 mg/kg). Post-operative animals recovered from the anesthetic in a heated Plexiglas cage. Blood samples were taken from the trunk and plasma was assayed for harman levels using mass spectrometry and high performance liquid chromatography (MS/HPLC) (Bacher, Houle et al. 2011).

2.2.3 Ethanol Vapor Exposure

In order to obtain plasma levels consistent with human exposure, the current study employed chronic intermittent alcohol vapor exposure. Vapor exposure has many unique advantages, including: the ability to achieve stable blood alcohol concentrations that can be maintained over a long period of time without detrimental health effects (i.e. weight loss or loss of ingestive behaviour), that it is not limited to the genetic predisposition of an animal to consume alcohol, and that it leads to physical dependence to alcohol, as seen by the development of tolerance and withdrawal symptoms (Gilpin, Richardson et al. 2008). Alcohol vapor exposed rats also display increased alcohol intake (i.e. in fixed ratio tests), and compulsive behaviour toward alcohol (i.e. in progressive ratio and adulteration tests) in
comparison to non-exposed rats, and these behaviours continue into withdrawal (Vendruscolo and Roberts 2014).

Rats were exposed to ethanol vapor or control condition for 17 hours per day for 8 weeks, and were divided into four experimental withdrawal groups and were sacrificed immediately after 8 week exposure (0h), acute withdrawal (24h), protracted withdrawal (4 day), and protracted abstinence (3 week) (n= 8/group).

Ethanol exposure was in specially-designed chambers (LJari Inc., La Jolla, California). HPLC pumps delivered ethanol into electrically heated stainless steel coils (60°C) connected to an airflow of 18 l/min. Ethanol concentration was adjusted by changing pump flow and monitored via a spectrometer. Exposure was 17 h during each 24 h period (on 10.00; off 03.00). Rats were allowed to habituate to the chambers for 1 week to minimize stress, then exposed to escalating ethanol concentrations for 1 week, and finally exposed for 7 weeks to ethanol vapor levels yielding blood alcohol concentrations (BACs) between 150 and 300 mg/dl. The duration of exposure was based on previous reports indicating that shorter periods of exposure increased the proportion of high drinking rodents, while 6 weeks of exposure was necessary to induce robust high drinking in most rats (Rimondini, Arlinde et al. 2002). Controls were kept in identical chambers with normal air flow. Rat blood alcohol concentrations were determined once per week, and blood samples were taken from the saphenous vein. Blood alcohol concentrations were analyzed by an NAD/NADPH enzyme/spectrophotometric assay kit (Sigma Aldrich) according to the manufacturer’s instructions.
2.2.4 Measurement of MAO Protein

Quantitative immunoblotting assays of protein levels of MAO-A and MAO-B followed published procedures (Tong, Meyer et al. 2013). Rat brain tissue was homogenized in 2x HEPES buffer saline (100 mM HEPES- NaOH, pH 7.4, 235 mM NaCl, 10 mM KCl, 4 mM CaCl$_2$, 2.8 mM MgCl$_2$) with the protease inhibitor leupeptin at 20 µM, aliquoted, and stored at -80°C for future analysis. It was confirmed that leupeptin does not affect the assay of the protein levels of MAO-A or MAO-B. The antibodies used for MAO-A were a rabbit polyclonal antibody from Santa Cruz Biotechnology (Dallas, TX, USA) (sc-20156, H-70, raised against C-terminal amino acids 458 to 527 of human MAO-A, used at 1:5,000) and a rabbit monoclonal antibody from Abcam (Cambridge, MA, USA) (ab126751, used at 1:10,000). Unlike the monoclonal ab126751 (EPR7101), the polyclonal sc-20156 (H-70) is detected in rat tissue samples at the expected 65 kDa position for MAO-A, with an additional protein band with slightly larger molecular weight of ~70 kDa, which was determined to be enriched in the soluble tissue fraction and thus non-specific (Morishima, Harada et al. 2006). The lack of effect on MAO-A protein by harman treatment was observed with both MAO-A antibodies. After probing for MAO-A, the polyvinylidene difluoride membranes were stripped and reprobed for MAO-B. The antibody used for MAO-B was a rabbit polyclonal antibody from Abcam (Cambridge, MA, USA) (ab67297, raised against amino acids 448 to 466 of human MAO-B, used at 1:50,000). The secondary antibody used was a goat anti-rabbit immunoglobulin G (IgG) (heavy chain and light chain, H&L) horseradish peroxidase (used at 1:10,000) from Southern Biotech (cat. no. 4050-05, Birmingham, AL, USA). Five concentrations of tissue standard (3-15 µg) consisting of a pooled rat cerebral cortex crude membrane fraction were run together with the samples (10 µg protein). Levels of MAO were expressed as µg tissue standard protein per µg sample protein.
For the effect of harman treatment on MAO (Section 3.2.1), MAO-B was also measured for comparison, as norharman is a metabolite of harman, and demonstrates a modest affinity for MAO-B (Ki: 2200 nM) (May, Rommelspacher et al. 1991).

For the effect of ethanol treatment on MAO (Section 3.2.2), MAO-B expression was examined because elevations in MAO-B level have been reported in neuronal cells exposed to alcohol, in the prefrontal cortex of rodents fed an alcohol diet, and post mortem prefrontal cortex of alcohol dependent humans (Carlsson, Adolfsson et al. 1980; Ou, Stockmeier et al. 2010).

### 2.2.5 Measurement of MAO Activity

Monoamine oxidase activities were determined by a spectrophotometric peroxidase-linked assay using amplex red as the chromogen. This assay was adapted from the fluorometric method of Zhou and Panchuk-Voloshina (1997) and is based on detection of $H_2O_2$ produced by MAO action in a horseradish peroxidase-coupled reaction with amplex red, which is colorless itself but becomes red upon being oxidized to resorufin (Zhou and Panchuk-Voloshina 1997). Rat brain tissue was homogenized in 2x HEPES buffer saline (100 mM HEPES- NaOH, pH 7.4, 235 mM NaCl, 10 mM KCl, 4 mM CaCl$_2$, 2.8 mM MgCl$_2$) with the protease inhibitor leupeptin at 20 µM, aliquoted, and stored at -80°C for future analysis. It was confirmed that leupeptin does not affect the assay of the activities of MAO-A or MAO-B. Protein concentration was determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) with bovine plasma albumin as the standard. MAO activities in 1x HEPES buffer saline were determined in tissue homogenates (final protein concentration at ~30 µg/ml) at 37°C for 1 hour with tyramine (0.75 mM) as the substrate, amplex red (100 µM) plus horseradish peroxidase (1 unit/ml) as the chromogen, and in the presence of 100 nM of deprenyl (MAO-B inhibitor) for MAO-A activity or in the presence of 50 nM of clorgyline (MAO-A inhibitor) for MAO-B activity. The appropriate concentrations of
MAO inhibitors used were determined by titrations. Adsorption at 571 nm was obtained, subtracting the background determined with 10 μM clorgyline to inhibit both MAOs, and converted to activities in nmol/hour/mg protein by using the extinction coefficient of resorufin (54,000 cm⁻¹ M⁻¹) (Zhou and Panchuk-Voloshina 1997). The assay was linear for at least 2 hours and up to a protein concentration of at least 100 μg/ml. The within and between assay coefficient of variance was determined to be 9.2% (n= 5 determinations) and 9.4% (n= 4 determinations), respectively.

2.2.6 Measurement of GFAP

For the effect of ethanol treatment on MAO (Section 3.2.2), GFAP expression was examined because glial pathology is observed in AD (outlined in Section 1.2.2.2), and ethanol directly affects the function of glial cells, reducing motility of astrocytic processes, glial cell proliferation, and GFAP expression in vitro (Kane, Berry et al. 1996; Guerri and Renau-Piqueras 1997; Crews, Collins et al. 2004).

Rat brain tissue was homogenized in 2x HEPES buffer saline (100 mM HEPES- NaOH, pH 7.4, 235 mM NaCl, 10 mM KCl, 4 mM CaCl₂, 2.8 mM MgCl₂) with the protease inhibitor leupeptin at 20 μM, aliquoted, and stored at -80°C for future analysis. It was confirmed that leupeptin does not affect the assay of the activities or protein levels of MAO-A or MAO-B and prevents the breakdown of the intermediate filament protein GFAP upon brain tissue homogenization. The antibody used for quantitative immunoblotting assay of GFAP levels was a mice monoclonal antibody from Millipore (Billerica, MA, USA) (cat. no. MAB360, clone GA5, isotype IgG1, used at 1:80,000). This antibody detected in rat brain tissue a single major protein band at the expected molecular weight of 50 kDa and has been employed in previous studies of biopsied and autopsied human brains (Tong, Furukawa et al. 2011; Tong, Fitzmaurice et al. 2014). The
secondary antibody used was a goat anti-mouse IgG1 horseradish peroxidase (used at 1:20,000) from Southern Biotech (cat. no. 1070-05, Birmingham, AL, USA). Five concentrations of tissue standard (3-15 µg) consisting of a pooled rat cerebral cortex crude membrane fraction were run together with the samples (7.5 µg protein). Levels of GFAP were expressed as µg tissue standard protein per µg sample protein.

2.2.7 Statistical Analysis

Results are expressed as mean ± SEM and statistical significance for all analyses was set at P < 0.05.

To assess the effect of harman treatment upon MAO protein and activity levels, a separate multivariate analysis of variance (MANOVA) was applied and the main analysis included PFC and ACC level as the dependent variables and dose (0, 2, 5, 15 mg/kg/day) as a predictive factor. MAO protein and activity levels were also assessed in the caudate-putamen and hippocampus.

To assess the effect of ethanol vapor exposure upon MAO protein and activity levels, a separate MANOVA was applied and included PFC and ACC level as the dependent variables and group (control versus alcohol exposure) as a predictive factor. MAO protein and activity levels were also assessed in the caudate-putamen and hippocampus. To assess the effect of ethanol vapor exposure upon GFAP levels, a separate analysis of variance (ANOVA) was applied for each brain region and included group (control versus alcohol exposure) as the predictive factor.
3 Results

3.1 Human PET Imaging Study of MAO-A in Alcohol Dependence

Results (Section 3.1) have been published in: Matthews BA, Kish SJ, Xu X, Boileau I, Rusjan PM, Wilson AA, DiGiacomo D, Houle S, Meyer JH, Greater Monoamine Oxidase A Binding in Alcohol Dependence, Biological Psychiatry, Vol. 75, Issue 10, pp. 756-764. © 2014, with permission from Elsevier.

3.1.1 Difference in MAO-A $V_T$ between the Alcohol Dependent Group and Healthy Controls

Demographics of study participants are listed in Table VII. The primary finding was a significant elevation in MAO-A $V_T$, an index of MAO-A density, in the prefrontal cortex of alcohol dependent subjects compared to healthy controls (magnitude 37%, independent samples t-test, $t_{30}= 3.93$, $p<0.001$), with an effect size of 1.4. There was also a significant elevation in all of the brain regions analyzed (multivariate analysis of variance, MANOVA, $F_{7,24}= 3.67$, $p= 0.008$). The mean difference across all regions was 32%, range 21% to 40%, see Figure 3. In addition, subregions of the prefrontal cortex (dorsolateral, medial, orbitofrontal, ventrolateral) were assessed and the results were similar (multivariate analysis of variance, MANOVA, $F_{4,27}= 2.97$, $p= 0.038$; mean difference 27%, range 21 to 31%). Post hoc tests reveal the group difference was significant in each prefrontal region (independent samples t-tests, $p= 0.004$ to 0.02).
Table VII. Demographic and Clinical Characteristics of Study Participants\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects (n= 16)</th>
<th>Alcohol Dependent Subjects (n= 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>34 (8.8)</td>
<td>35 (7.6)</td>
</tr>
<tr>
<td>Sex, Male/Female</td>
<td>14/2</td>
<td>14/2</td>
</tr>
<tr>
<td>SCID diagnosis of major depressive disorder</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HAM-D</td>
<td>0.8 (1.2)</td>
<td>3.7 (2.0)</td>
</tr>
<tr>
<td>VAS Depressed Mood\textsuperscript{b}</td>
<td>2.5 (1.5)</td>
<td>4.2 (2.0)</td>
</tr>
<tr>
<td>Angry-Hostility\textsuperscript{c}</td>
<td>12.1 (5.1)</td>
<td>12.1 (5.4)</td>
</tr>
<tr>
<td>Deliberation\textsuperscript{c}</td>
<td>16.4 (3.7)</td>
<td>16.3 (6.0)</td>
</tr>
</tbody>
</table>

**Alcohol Intake Behaviours**

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects (n= 16)</th>
<th>Alcohol Dependent Subjects (n= 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Drinks per Day</td>
<td>0.14 (0.25)</td>
<td>8.0 (3.5)</td>
</tr>
<tr>
<td>No. Drinking Days per Week</td>
<td>0 (0)</td>
<td>6.3 (0.9)</td>
</tr>
<tr>
<td>Duration of Alcohol Dependence, y</td>
<td>NA</td>
<td>6.3 (4.1)</td>
</tr>
<tr>
<td>Alcohol Dependence Scale</td>
<td>NA</td>
<td>12.7 (6.9)</td>
</tr>
<tr>
<td>CIWA- Ar at scan</td>
<td>NA</td>
<td>9.8 (1.4)</td>
</tr>
</tbody>
</table>

Abbreviations: SCID, Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders; HAM-D, Hamilton Rating Scale for Depression; VAS, Visual Analogue Scale; NA, not applicable; CIWA-Ar, Clinical Institute Withdrawal Assessment for Alcohol, revised.

\textsuperscript{a} Values are expressed as mean (SD) except where indicated.

\textsuperscript{b} Endorsement of depressed mood on the Visual Analogue Scale at the time of the scan.

\textsuperscript{c} Personality facet within the NEO Personality Inventory–Revised questionnaire.

None of the healthy controls drank alcohol on a regular basis, nor had a diagnosis of alcohol dependence or withdrawal. Independent samples t-tests showed no significant difference for age (t\textsubscript{30}= 0.56, P= 0.58), angry-hostility (t\textsubscript{30}= 0.002, P= 0.998), deliberation (t\textsubscript{30}= 0.049, P= 0.962). There was a significant difference for HAM-D and VAS scores (t\textsubscript{30}= 4.95, P< 0.001, t\textsubscript{30}= 2.79, P= 0.009, respectively), yet all participants were below the HAM-D threshold for a major depressive episode.
Figure 3. Greater Monoamine Oxidase-A V\textsubscript{T} Level in Alcohol Dependent Subjects Compared to Healthy Controls

Monoamine oxidase A (MAO-A) V\textsubscript{T} was significantly greater in the prefrontal cortex (independent samples t-test, t\textsubscript{30}= 3.93, p<0.001). There was also a significant elevation in all of the brain regions analyzed (multivariate analysis of variance, MANOVA, F\textsubscript{7,24}= 3.67, p= 0.008). Independent-samples t-tests were also applied at each region of interest to compare groups.

\begin{itemize}
  \item[a] p ≤ 0.001
  \item[b] p ≤ 0.005
  \item[c] p ≤ 0.05
\end{itemize}
3.1.2 Relationship between MAO-A $V_T$ and Duration of Heavy Alcohol Use

A positive correlation between MAO-A $V_T$ and the duration of heavy alcohol use was found in the prefrontal cortex (Pearson correlation coefficient, $R=0.67$, $p=0.005$). There was also a significant positive correlation found in all other brain regions analyzed ($R=0.73$ to $0.57$, $p=0.001$ to $0.02$), see Figure 4.
Figure 4. Greater Monoamine Oxidase-A Vₜ Level in Alcohol Dependent Subjects

Correlates with Years of Heavy Alcohol Use

Monoamine oxidase A (MAO-A) Vₜ was significantly correlated with years of heavy alcohol use in the prefrontal cortex (Pearson correlation coefficient, R = 0.67, p = 0.005). There was also a significant positive correlation in all other brain regions analyzed (R = 0.73 to 0.57, p = 0.001 to 0.02). Years of heavy alcohol use was defined as the number of years in which participants drank more than five drinks per day, at least five days per week, and for which symptoms of AD were continuously present.
3.1.3 Relationship between MAO-A $V_T$ and Other Clinical Features

For this analysis, we focused upon depressed mood measured with the visual analogue scale (VAS) on the PET scan day and MAO-A $V_T$ in the prefrontal and anterior cingulate cortices. The VAS was used in this correlation because it is a measure of mood at the time of the scan when alcohol dependent subjects were experiencing withdrawal. The HAM-D was not used because these scores reflect traits other than low mood (i.e. appetite, sleep disturbance, etc) and were normative in both the alcohol dependent and control group, as major depressive disorder was an exclusion criterion for enrollment in the study. A stepwise regression was applied that included three predictor variables: the visual analogue score for depressed mood, group (alcohol dependence versus health, as it is well established that depressed mood is associated with alcohol dependence (Locke and Newcomb 2001; Caldwell, Rodgers et al. 2002)), and angry-hostility, a personality facet (from the NEO Personality Inventory Score-Revised) previously associated with MAO-A $V_T$ (Alia-Klein, Goldstein et al. 2008; Soliman, Bagby et al. 2011). All factors were significant (multivariate analysis of covariance (MANCOVA), effect of depressed mood, $F_{2,26}= 4.88$, $p= 0.02$; effect of group, $F_{2,26}= 5.38$, $p= 0.01$; effect of angry-hostility, $F_{2,26}= 6.02$, $p= 0.007$) and were related to the dependent variables in a manner consistent with greater MAO-A $V_T$ when depressed mood is elevated and lower MAO-A $V_T$ when angry/hostility is present, see Figure 5. See Table VIII for an overview of all statistical analyses of MAO-A $V_T$. 

A multivariate analysis of covariance with factors of depressed mood from the visual analogue scale, angry-hostility, and group were predictive of MAO-A $V_T$ in the prefrontal and anterior cingulate cortex (MANCOVA, effect of depressed mood, $F_{2,26}= 4.88$, $p= 0.02$; group, $F_{2,26}= 5.38$, $p= 0.01$; angry-hostility, $F_{2,26}= 6.02$, $p= 0.007$). The residual MAO-A $V_T$ values are shown after regressing for angry-hostility to demonstrate the relationship between depressed mood and MAO-A $V_T$ for controls (●) and alcohol dependence (▲). When the highest residual value is removed, Pearson correlation coefficients remain significant (PFC: $R= 0.5$, $p= 0.007$, ACC: $R= 0.5$, $p= 0.003$).
Table VIII. Overview of Analyses of MAO-A $V_T$

<table>
<thead>
<tr>
<th>Analysis Ranking</th>
<th>Comparison of MAO-A $V_T$ Values</th>
<th>Region(s)</th>
<th>Statistic</th>
<th>*$p$-value</th>
<th>Mean % Change</th>
<th>Direction of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Difference in PFC between AD and health</td>
<td>PFC</td>
<td>independent samples t-test, $t_{30} = 3.93$</td>
<td>0.000</td>
<td>37%</td>
<td>Increase</td>
</tr>
<tr>
<td>Sub-analyses</td>
<td>Difference in all regions between AD and health</td>
<td>PFC, ACC, Dorsal Putamen, Ventral Striatum, Thalamus, Ventral Striatum, Thalamus, Hippocampus, Midbrain</td>
<td>multivariate analysis of variance, MANOVA, $F_{7,24} = 3.67$</td>
<td>0.008</td>
<td>32%</td>
<td>Increase</td>
</tr>
<tr>
<td>Related to</td>
<td>Individual regional comparisons</td>
<td>PFC, ACC, Dorsal Putamen, Ventral Striatum, Thalamus, Hippocampus, Midbrain</td>
<td>$t_{30} = 3.93$, $t_{30} = 4.02$, $t_{30} = 3.20$, $t_{30} = 2.32$, $t_{30} = 3.15$, $t_{30} = 2.53$, $t_{30} = 3.51$</td>
<td>0.000, 0.000, 0.003, 0.027, 0.004, 0.017, 0.001</td>
<td>37%, 40%, 33%, 24%, 34%, 21%, 33%</td>
<td>Increase, Increase, Increase, Increase, Increase, Increase, Increase</td>
</tr>
<tr>
<td>Primary Analysis</td>
<td>Difference in subregions of the PFC between AD and health</td>
<td>dlPFC, OFC, mPFC, vIPFC</td>
<td>MANOVA, $F_{4,27} = 2.97$</td>
<td>0.038</td>
<td>27%</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>Individual PFC subregion comparisons</td>
<td>dlPFC, OFC, mPFC, vIPFC</td>
<td>$t_{30} = 2.80$, $t_{30} = 2.61$, $t_{30} = 2.47$, $t_{30} = 3.15$</td>
<td>0.009, 0.014, 0.020, 0.004</td>
<td>27%, 26%, 22%, 31%</td>
<td>Increase, Increase, Increase, Increase</td>
</tr>
<tr>
<td>Secondary</td>
<td>Correlation with Years of Heavy Drinking in PFC</td>
<td>PFC</td>
<td>Pearson correlation, $R = 0.67$</td>
<td>0.005</td>
<td>N/A</td>
<td>Positive</td>
</tr>
<tr>
<td>Analysis Ranking</td>
<td>Comparison of MAO-A VT Values</td>
<td>Region(s)</td>
<td>Statistic</td>
<td><em>p</em>-value</td>
<td>Mean % Change</td>
<td>Direction of Change</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Sub-analyses Related to Secondary Analysis</td>
<td>Correlation with Years of Heavy Drinking in all other regions</td>
<td>ACC, Dorsal Putamen, Ventral Striatum, Thalamus, Hippocampus, Midbrain</td>
<td>R= 0.67, R= 0.71, R= 0.73, R= 0.70, R= 0.69, R= 0.57</td>
<td>0.005, 0.002, 0.001, 0.002, 0.003, 0.020</td>
<td>N/A</td>
<td>Positive</td>
</tr>
<tr>
<td>Additional Relationship to Depressed Mood in PFC and ACC</td>
<td>Relationship to Depressed Mood in PFC and ACC</td>
<td>PFC, ACC</td>
<td>MANCOVA, $F_{2,26} = 4.88$, (effect of depressed mood) $F_{2,26} = 5.38$, (effect of group) $F_{2,26} = 6.02$, (effect of angry-hostility)</td>
<td>0.02, 0.01, 0.007</td>
<td>N/A</td>
<td>Increased with greater depressed mood, AD group Decreased with greater angry-hostility</td>
</tr>
</tbody>
</table>

*Uncorrected P-values shown. Values across the regions analyzed were highly correlated in the AD and healthy groups ($r = 0.99$ to 0.70). For correction of multiple comparisons, there were three main independent sets of analyses of MAO-A VT: AD versus health, relationship of MAO-A VT with years of heavy alcohol use, and relationship between MAO-A VT in the PFC and ACC with depressed mood.

Abbreviations: PFC, prefrontal cortex; AD, alcohol dependence; ACC, anterior cingulate cortex; dLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; mPFC, medial prefrontal cortex; vLPFC, ventrolateral prefrontal cortex; MANCOVA, multivariate analysis of covariance.
3.1.4 Additional Characteristics of the Alcohol Dependent Group

There were no significant differences in radioligand binding to plasma protein between groups ($t_{28}= 0.92, p= 0.36$). As expected, given the 12 hour period since previous drinking of alcoholic beverages, the plasma level of beta-carboline harman was negligible for all subjects on the day of the scan (<5 pg/ml). There were several post-hoc analyses not specifically hypothesized, but were of interest to understand the relationship between indices of alcohol dependence and prefrontal cortex MAO-A $V_T$. The correlations between PFC MAO-A$V_T$ and number of drinks per day in the week prior to scanning, as well as the CIWA-Ar score prior to scanning, were not significant (Pearson correlation coefficient, $R= 0.30, p= 0.20$, $R= 0.17, p= 0.54$, respectively). Additionally, angry-hostility did not correlate with depression ($R= 0.17, p= 0.53$), nor with AD duration ($R= 0.26, p= 0.33$). Depression scores did not correlate with AD duration ($R= 0.19, p= 0.47$).
3.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents

3.2.1 Effect of Harman Exposure on MAO

Plasma concentrations of harman (ng/ml) obtained at the end of 21 day harman exposure and following 8 hours of withdrawal are displayed in Figure 6. Osmotic minipump administration dose dependently increased plasma levels of harman and withdrawal significantly decreased plasma levels of harman. There was a strong correlation between the harman infusion dose via osmotic minipump (0, 2, 5, 15 mg/kg/day) and the harman plasma concentrations after 21 day administration (R= 0.84 p<0.0001). A similar relationship was present following 8 hours of harman cessation after 21 day administration (R= 0.66, p<0.0001).

Figure 6. Effect of 21 day osmotic minipump administration of harman, and 8 hour withdrawal of harman following 21 day administration, on plasma levels of harman (mean ± SEM, n= 10/group).
There was no effect of chronic harman administration on MAO-A activity or protein levels in the PFC and ACC, our primary hypothesized regions (multivariate analysis of variance, MANOVA, $F_{6,70} = 1.12$, $p= 0.36$, $F_{6,68} = 0.62$, $p= 0.71$, respectively), nor was there an effect in the caudate-putamen (ANOVA, $F_{3,32} = 0.60$, $p= 0.62$, $F_{3,30} = 1.66$, $p= 0.20$, respectively) or hippocampus (ANOVA, $F_{3,36} = 0.37$, $p= 0.78$, $F_{3,36} = 0.17$, $p= 0.92$, respectively). The mean value (± standard deviation) of MAO-A activity in the control group after chronic harman administration was 89.83 (± 6.52) nmol/hour/mg protein in the PFC, 90.65 (± 10.83) nmol/hour/mg protein in the ACC, 94.79 (± 11.90) nmol/hour/mg protein in the caudate-putamen, and 92.53 (± 6.06) nmol/hour/mg protein in the hippocampus.

Similarly, there was no effect of 8 hour withdrawal after chronic harman administration on MAO-A activity or protein levels in the PFC and ACC (MANOVA, $F_{6,62} = 0.54$, $p= 0.78$; $F_{6,68} = 0.20$, $p= 0.98$, respectively), nor was there an effect of withdrawal in the caudate-putamen (ANOVA, $F_{3,30} = 0.53$, $p= 0.67$, $F_{3,28} = 0.81$, $p= 0.50$, respectively) or hippocampus (ANOVA, $F_{3,36} = 0.55$, $p= 0.65$, $F_{3,36} = 0.99$, $p= 0.41$, respectively). The mean value (± standard deviation) of MAO-A activity in the control group after 8 hours of withdrawal following chronic harman administration was 90.61 (± 8.96) nmol/hour/mg protein in the PFC, 89.41 (± 5.27) nmol/hour/mg protein in the ACC, 92.98 (± 10.90) nmol/hour/mg protein in the caudate-putamen, and 94.99 (± 5.24) nmol/hour/mg protein in the hippocampus. See Figure 7.
Figure 7. Effect of 21 day harman administration on MAO-A activity level (a), 8 hour harman cessation on MAO-A activity level (b), 21 day harman administration on MAO-A protein level (c), and 8 hour harman cessation on MAO-A protein level (d). Analysis indicated no significant effect of treatment. The results are expressed as percent control (mean ± SEM, n= 7-10/group). PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPu, caudate-putamen; Hippo, hippocampus.
There was no effect of chronic harman administration on MAO-B activity or protein levels in the PFC and ACC (MANOVA, $F_{6,70} = 1.50$, $p = 0.20$, $F_{6,68} = 0.91$, $p = 0.49$, respectively), nor was there an effect in the caudate-putamen (ANOVA, $F_{3,30} = 0.29$, $p = 0.84$, $F_{3,30} = 0.45$, $p = 0.72$, respectively) or hippocampus (ANOVA, $F_{3,36} = 0.08$, $p = 0.97$, $F_{3,36} = 0.27$, $p = 0.85$, respectively).

The mean value (± standard deviation) of MAO-B activity in the control group after chronic harman administration was 68.10 (± 6.49) nmol/hour/mg protein in the PFC, 74.52 (± 5.20) nmol/hour/mg protein in the ACC, 62.05 (± 6.18) nmol/hour/mg protein in the caudate-putamen, and 73.37 (± 4.16) nmol/hour/mg protein in the hippocampus.

Similarly, there was no effect of 8 hour withdrawal after chronic harman administration on MAO-B activity or protein levels in the PFC and ACC (MANOVA, $F_{6,64} = .027$, $p = 0.95$, $F_{6,68} = 0.74$, $p = 0.62$, respectively), nor was there an effect of withdrawal in the caudate-putamen (ANOVA, $F_{3,29} = 1.61$, $p = 0.21$, $F_{3,28} = 0.61$, $p = 0.61$, respectively) or hippocampus (ANOVA, $F_{3,36} = 0.31$, $p = 0.82$, $F_{3,36} = 1.88$, $p = 0.15$, respectively). The mean value (± standard deviation) of MAO-B activity in the control group after 8 hours of withdrawal following chronic harman administration was 72.14 (± 9.55) nmol/hour/mg protein in the PFC, 77.42 (± 9.07) nmol/hour/mg protein in the ACC, 56.33 (± 4.59) nmol/hour/mg protein in the caudate-putamen, and 74.97 (± 7.97) nmol/hour/mg protein in the hippocampus. See Figure 8.
Figure 8. Effect of 21 day harman administration on MAO-B activity level (a), 8 hour harman cessation on MAO-B activity level (b), 21 day harman administration on MAO-B protein level (c), and 8 hour harman cessation on MAO-B protein level (d). Analysis indicated no significant effect of treatment. For (b), Tukey post hoc tests revealed no significant difference between 0 mg/kg/day and 2mg/kg/day (p= 0.43) or 5 mg/kg/day (p= 0.27) in the CPu. The results are expressed as percent control (mean + SEM, n= 7-10/group). PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPu, caudate-putamen; Hippo, hippocampus.
3.2.2 Effect of Ethanol Exposure on MAO

Blood alcohol concentrations (mg/dl) obtained at the end of the 17 hour exposure period once per week across the 8 week ethanol vapor exposure are displayed in Figure 9.

Figure 9. Blood alcohol concentrations (BAC) obtained at the end of 17h exposure across the 8 week exposure period (mean ± SEM, n= 32). Values for week 8 refer to rodents that were sacrificed immediately after exposure. For 24 h, 4 day, and 21 day withdrawal groups, BACs were at 0 mg/dl at the time of sacrifice.
There was no effect of 8 week ethanol vapor exposure after immediate sacrifice (no withdrawal) on MAO-A activity or protein levels in the PFC and ACC, our primary hypothesized regions (multivariate analysis of variance, MANOVA, \( F_{2,13} = 1.26, p=0.32, F_{2,13} = 0.55, p=0.59 \), respectively), nor was there an effect in the caudate-putamen (ANOVA, \( F_{1,14} = 0.53, p=0.48, F_{1,14} = 3.19, p=0.10 \), respectively) or hippocampus (ANOVA, \( F_{1,14} = 2.06, p=0.17, F_{1,14} = 1.64, p=0.22 \), respectively). The mean value (± standard deviation, SD) of MAO-A activity in the control group after 8 week ethanol vapor exposure was 102.7 (± 7.5) nmol/hour/mg protein in the PFC, 99.9 (± 12.2) nmol/hour/mg protein in the ACC, 108.2 (± 6.7) nmol/hour/mg protein in the caudate-putamen, and 87.0 (± 7.6) nmol/hour/mg protein in the hippocampus.

Similarly, there was no effect of 8 week ethanol vapor exposure after immediate sacrifice (no withdrawal) on MAO-B activity or protein levels in the PFC and ACC (MANOVA, \( F_{2,8} = 1.77, p=0.23; F_{2,13} = 0.04, p=0.97 \), respectively), nor was there an effect in the caudate-putamen (ANOVA, \( F_{1,14} = 0.35, p=0.57, F_{1,14} = 0.26, p=0.62 \), respectively) or hippocampus (ANOVA, \( F_{1,14} = 0.02, p=0.89, F_{1,14} = 2.37, p=0.15 \), respectively). The mean value (± SD) of MAO-B activity in the control group after 8 week ethanol vapor exposure was 90.7 (± 8.3) nmol/hour/mg protein in the PFC, 102.2 (± 10.7) nmol/hour/mg protein in the ACC, 73.4 (± 7.5) nmol/hour/mg protein in the caudate-putamen, and 83.0 (± 8.7) nmol/hour/mg protein in the hippocampus. See Figure 10.
Figure 10. Effect of 8 week ethanol vapor exposure (no withdrawal) on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d). The results are expressed as percent control (mean ± SEM, n= 8/group). Analysis indicated no significant effect of treatment. PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPu, caudate-putamen; Hippo, hippocampus.
There was a significant elevation in MAO-A activity and protein levels at 24 hour withdrawal following 8 week ethanol vapor exposure in the PFC and ACC, our primary hypothesized regions (multivariate analysis of variance, MANOVA, $F_{2,13} = 3.82$, $p = 0.05$, $F_{2,13} = 5.13$, $p = 0.02$, respectively). There was no effect in the caudate-putamen (ANOVA, $F_{1,14} = 0.13$, $p = 0.73$, $F_{1,14} = 0.01$, $p = 0.93$, respectively) or hippocampus (ANOVA, $F_{1,14} = 0.67$, $p = 0.43$, $F_{1,14} = 0.60$, $p = 0.46$, respectively). The mean value ($\pm$ SD) of MAO-A activity in the control group after 24 hour withdrawal following 8 week ethanol vapor exposure was 81.7 ($\pm$ 10.8) nmol/hour/mg protein in the PFC, 76.8 ($\pm$ 7.9) nmol/hour/mg protein in the ACC, 93.6 ($\pm$ 4.9) nmol/hour/mg protein in the caudate-putamen, and 92.6 ($\pm$ 4.4) nmol/hour/mg protein in the hippocampus. See Figure 11.

Similarly, there was a significant elevation in MAO-B activity levels at 24 hour withdrawal following 8 week ethanol vapor exposure in the PFC and ACC (MANOVA, $F_{2,10} = 5.73$, $p = 0.02$). There was a trend for an elevation in MAO-B protein levels found in the PFC and ACC (MANOVA, $F_{2,13} = 2.4$, $p = 0.13$). There was no effect on MAO-B activity or protein levels in the caudate-putamen (ANOVA, $F_{1,14} = 0.66$, $p = 0.43$, $F_{1,14} = 0.55$, $p = 0.47$, respectively) or hippocampus (ANOVA, $F_{1,13} = 0.34$, $p = 0.57$, $F_{1,14} = 2.11$, $p = 0.17$, respectively). The mean value ($\pm$ SD) of MAO-B activity in the control group after 24 hour withdrawal following 8 week ethanol vapor exposure was 72.5 ($\pm$ 7.0) nmol/hour/mg protein in the PFC, 72.9 ($\pm$ 12.7) nmol/hour/mg protein in the ACC, 61.6 ($\pm$ 12.2) nmol/hour/mg protein in the caudate-putamen, and 77.9 ($\pm$ 10.9) nmol/hour/mg protein in the hippocampus. See Figure 11.

There were no significant differences in our loading control, neuron specific enolase (NSE), at 24 hour withdrawal following 8 week ethanol vapor exposure in the PFC and ACC (MANOVA, $F_{2,13} = 1.17$, $p = 0.34$), or in the caudate-putamen (ANOVA, $F_{1,14} = 0.76$, $p = 0.40$) or hippocampus (ANOVA, $F_{1,14} = 0.002$, $p = 0.97$).
Figure 11. Effect of 24 hour withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d). The results are expressed as percent control (mean + SEM, n= 8/group). *p< 0.05, **p< 0.01, significant difference between alcohol exposed group and control. PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPu, caudate-putamen; Hippo, hippocampus.
There was no effect of 4 day withdrawal following 8 week ethanol vapor exposure on MAO-A activity or protein levels in the PFC and ACC, our primary hypothesized regions (multivariate analysis of variance, MANOVA, $F_{2,13} = 0.13$, $p = 0.88$, $F_{2,13} = 0.04$, $p = 0.96$, respectively). There was a significant elevation in MAO-A activity and protein levels in the caudate-putamen (ANOVA, $F_{1,14} = 4.84$, $p = 0.05$, $F_{1,14} = 8.73$, $p = 0.01$, respectively), that was no longer significant after correcting for multiple comparisons of additional regions. There was no effect on MAO-A activity or protein levels in the hippocampus (ANOVA, $F_{1,14} = 0.26$, $p = 0.62$, $F_{1,14} = 1.66$, $p = 0.22$, respectively). The mean value (± SD) of MAO-A activity in the control group after 4 day withdrawal following 8 week ethanol vapor exposure was 88.6 (± 4.2) nmol/hour/mg protein in the PFC, 82.4 (± 8.3) nmol/hour/mg protein in the ACC, 88.2 (± 6.4) nmol/hour/mg protein in the caudate-putamen, and 85.0 (± 3.6) nmol/hour/mg protein in the hippocampus.

Similarly, there was no effect of 4 day withdrawal following 8 week ethanol vapor exposure on MAO-B activity or protein levels in the PFC and ACC (MANOVA, $F_{2,13} = 1.27$, $p = 0.31$; $F_{2,13} = 1.54$, $p = 0.25$, respectively). There was a significant elevation in MAO-B activity levels in the caudate-putamen (ANOVA, $F_{1,14} = 7.61$, $p = 0.02$), that was no longer significant after correcting for multiple comparisons of additional regions. There was no significant difference in MAO-B protein levels in the caudate-putamen (ANOVA, $F_{1,14} = 1.84$, $p = 0.20$). There was no effect on MAO-B activity or protein levels or hippocampus (ANOVA, $F_{1,14} = 1.48$, $p = 0.24$, $F_{1,14} = 1.84$, $p = 0.20$, respectively). The mean value (± SD) of MAO-B activity in the control group after 4 day withdrawal following 8 week ethanol vapor exposure was 75.7 (± 4.3) nmol/hour/mg protein in the PFC, 75.7 (± 9.3) nmol/hour/mg protein in the ACC, 66.2 (± 5.3) nmol/hour/mg protein in the caudate-putamen, and 70.7 (± 4.7) nmol/hour/mg protein in the hippocampus. See Figure 12.
Figure 12. Effect of 4 day withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d). The results are expressed as percent control (mean + SEM, n= 8/group). *p< 0.05, **p< 0.01, significant difference between alcohol exposed group and control. PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPU, caudate-putamen; Hippo, hippocampus.
There was no effect of 3 week withdrawal following 8 week ethanol vapor exposure on MAO-A activity or protein levels in the PFC and ACC, our primary hypothesized regions (multivariate analysis of variance, MANOVA, $F_{2,12} = 1.19$, $p = 0.34$, $F_{2,12} = 0.56$, $p = 0.59$, respectively), nor was there an effect in the caudate-putamen (ANOVA, $F_{1,13} = 1.07$, $p = 0.32$, $F_{1,13} = 1.96$, $p = 0.19$, respectively) or hippocampus (ANOVA, $F_{1,13} = 0.002$, $p = 0.97$, $F_{1,13} = 1.65$, $p = 0.22$, respectively). The mean value ($\pm$ SD) of MAO-A activity in the control group after 3 week withdrawal following 8 week ethanol vapor exposure was 90.4 ($\pm$ 2.1) nmol/hour/mg protein in the PFC, 89.4 ($\pm$ 7.7) nmol/hour/mg protein in the ACC, 89.6 ($\pm$ 3.8) nmol/hour/mg protein in the caudate-putamen, and 89.2 ($\pm$ 5.8) nmol/hour/mg protein in the hippocampus.

Similarly, there was no effect of 3 week withdrawal following 8 week ethanol vapor exposure on MAO-B activity or protein levels in the PFC and ACC (MANOVA, $F_{2,12} = 1.04$, $p = 0.38$; $F_{2,12} = 3.80$, $p = 0.053$, respectively), nor was there an effect in the caudate-putamen (ANOVA, $F_{1,13} = 0.28$, $p = 0.60$, $F_{1,13} = 0.04$, $p = 0.85$, respectively) or hippocampus (ANOVA, $F_{1,13} = 0.59$, $p = 0.46$, $F_{1,13} = 4.36$, $p = 0.06$, respectively). The mean value ($\pm$ SD) of MAO-B activity in the control group after 3 week withdrawal following 8 week ethanol vapor exposure was 85.3 ($\pm$ 3.8) nmol/hour/mg protein in the PFC, 89.3 ($\pm$ 12.5) nmol/hour/mg protein in the ACC, 70.9 ($\pm$ 4.9) nmol/hour/mg protein in the caudate-putamen, and 80.2 ($\pm$ 6.6) nmol/hour/mg protein in the hippocampus. See Figure 13.
Figure 13. Effect of 3 week withdrawal following 8 week ethanol vapor exposure on MAO-A activity level (a), MAO-A protein level (b), MAO-B activity level (c) and MAO-B protein level (d). The results are expressed as percent control (mean + SEM, n= 7-8/group). Analysis indicated no significant effect of treatment. PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPU, caudate-putamen; Hippo, hippocampus.
There was no effect of 8 week ethanol vapor exposure after immediate sacrifice (no withdrawal) on GFAP levels in the PFC (ANOVA, $F_{1,14}=0.06$, $p=0.82$), ACC ($F_{1,14}=0.40$, $p=0.54$), caudate-putamen ($F_{1,14}=0.56$, $p=0.47$), or hippocampus ($F_{1,14}=0.44$, $p=0.52$).

There was no effect of 24 hour withdrawal following 8 week ethanol vapor exposure on GFAP levels in the PFC (ANOVA, $F_{1,14}=0.69$, $p=0.42$), ACC ($F_{1,14}=1.19$, $p=0.30$), caudate-putamen ($F_{1,14}=0.01$, $p=0.92$), or hippocampus ($F_{1,14}=0.07$, $p=0.80$).

There was no effect of 4 day withdrawal following 8 week ethanol vapor exposure on GFAP levels in the PFC (ANOVA, $F_{1,14}=0.09$, $p=0.77$), ACC ($F_{1,14}=0.02$, $p=0.89$), caudate-putamen ($F_{1,14}=0.01$, $p=0.92$), or hippocampus ($F_{1,14}=0.55$, $p=0.47$).

There was no effect of 3 week withdrawal following 8 week ethanol vapor exposure on GFAP levels in the PFC (ANOVA, $F_{1,13}=1.63$, $p=0.22$), ACC ($F_{1,13}=0.17$, $p=0.69$), caudate-putamen ($F_{1,13}=1.65$, $p=0.22$), or hippocampus ($F_{1,13}=0.08$, $p=0.78$). See Figure 14.
**Figure 14.** Protein levels of GFAP (glial fibrillary acidic protein) following 8 week ethanol vapor exposure (no withdrawal) (a), 24 hour withdrawal (b), 4 day withdrawal (c), and 3 week withdrawal (d). The results are expressed as percent control (mean ± SEM, n= 7-8/group). Analysis indicated no significant effect of treatment. PFC, prefrontal cortex; ACC, anterior cingulate cortex; CPu, caudate-putamen; Hippo, hippocampus.
4 Discussion

4.1 Human PET Imaging Study of MAO-A in Alcohol Dependence


This was the first in vivo investigation of brain MAO-A during alcohol dependence in which the biases of cigarette smoking, comorbid psychiatric illness, personality traits of impulsivity/aggression, and medication use were collectively addressed. The primary finding was a robust elevation of MAO-A $V_T$, an index of MAO-A density, in the prefrontal cortex, as well as all other brain regions analyzed, in AD. In addition, there was a strong positive correlation between MAO-A $V_T$ in all regions and duration of heavy alcohol use, as well as an association between prefrontal and anterior cingulate cortex MAO-A $V_T$ and severity of depressed mood. These results have important implications for elucidating the pathophysiology of AD, developing new therapeutics for alcohol dependence, and understanding the relationship of AD to depressed mood.

This finding represents an important direction for understanding the underlying pathology of AD, as MAO-A levels influence key processes related to many of the brain pathologies typically observed later in AD, such as abnormal mitochondrial function, reduced glial cell density, and thinned cortex (Miguel-Hidalgo, Wei et al. 2002; Albano 2006; Harper 2009). For example, reduced mitochondrial respiration, an index of reduced mitochondrial functioning, occurs when
MAO-A activity is increased (Cohen and Kesler 1999), and alcohol dependence is associated with reduced N-acetylaspartate levels, an index of reduced mitochondrial function (Meyerhoff and Durazzo 2008; Licata and Renshaw 2010). Also, in contrast to many other brain proteins, MAO-A is present homogenously throughout the cortex, including glial cells (Saura, Kettler et al. 1992), and when MAO-A activity is greater, glial cell lines are predisposed towards apoptosis (Ou, Chen et al. 2006). MAO-A activity itself creates oxidative stress (Youdim, Edmondson et al. 2006), and markers of oxidative damage to mitochondrial DNA have been reported in AD (Hoek, Cahill et al. 2002; Albano 2006). Additionally, inherited vulnerability to oxidative stress has been associated with reduced cortical thickness in AD (Srivastava, Buzas et al. 2010). Given the diverse pathological implications of chronically elevated MAO-A level, intervening upon this target has intriguing implications for treating AD.

New therapeutic targets for AD are necessary, as cessation rates after common clinical treatments such as naltrexone and acamprosate are 20 to 50% (Friedmann 2013). Preclinical models demonstrate promise for MAO-A inhibitors to reduce ethanol consumption (Cohen, Curet et al. 1999; Mega, Sheppard et al. 2002), and MAO-A inhibitors also have the potential to protect mitochondria against ethanol induced toxicity in multiple organs (Youdim, Edmondson et al. 2006), as well as treat mood symptoms which are often observed in AD (Locke and Newcomb 2001; Caldwell, Rodgers et al. 2002). However, there have been no investigations of MAO-A inhibitors as a pharmacotherapy for AD in humans. Historically, MAO-A inhibitors were not used to treat AD because the original non-selective, irreversible MAO-A/B inhibitor compounds were associated with greater risk for hypertensive crisis from inadequate peripheral metabolism of tyramine, which is ingested through many different alcoholic beverages. This issue has been overcome with modern therapeutic development of selective reversible MAO-A inhibitors, and MAO inhibitors with high brain to periphery concentration ratios, which do not
require dietary restriction of tyramine (Youdim, Edmondson et al. 2006). Thus MAO-A inhibitors are available, and have the potential to resolve both toxicity and mood related disturbances that result from excessive alcohol use.

The correlation between years of heavy alcohol intake and greater MAO-A $V_T$ suggests that elevated MAO-A $V_T$ was acquired through chronic alcohol exposure, rather than by other mechanisms. This perspective is consistent with the effects of chronic alcohol exposure in rodent models, which includes greater MAO-A mRNA in the prefrontal cortex and greater MAO-A activity in the dorsal raphe nucleus (Zimatkin, Tsydik et al. 1997; Rimondini, Arlinde et al. 2002). The specific mechanism through which alcohol exposure might lead to increased MAO-A level is unknown, but it is possible that pro-apoptotic pathways contribute since TIEG2, a transcription factor that initiates a cell death cascade and promotes expression of MAO-A, is elevated in prefrontal cortex of rodents after chronic ethanol exposure (Ou, Johnson et al. 2011). The prefrontal cortex was the only region examined in this study; hence it is possible that the same effect may be occurring in other regions. Future investigations in this direction have important implications for developing novel treatment strategies that could reverse elevated MAO-A level through mechanisms other than direct MAO-A inhibition. Another mechanism to consider is whether elevated MAO-A $V_T$ is a pre-existing trait. AD is associated with greater levels of aggression and impulsivity (Ruiz MA 2003; Bottlender and Soyka 2005), and such traits are associated with measures of low MAO-A binding in health (Alia-Klein, Goldstein et al. 2008; Soliman, Bagby et al. 2011), suggesting a pre-existing elevation in MAO-A level is unlikely. Future investigations might also consider gene by environment interactions in regards to the effect of AD upon MAO-A levels.
The biological mechanism of dysphoria in AD has traditionally been unclear, but the present finding of greater MAO-A V_T in prefrontal and anterior cingulate cortices provides new insight into the etiology of dysphoric mood and high risk for major depressive episodes in AD (Ramsey, Kahler et al. 2004; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009), as subregions of these structures are activated in mood induction studies and implicated in generation of depressed mood (Sharot, Riccardi et al. 2007). As previously mentioned, MAO-A has a role in apoptosis, and abnormal expression of genes related to apoptosis in the prefrontal cortex have been reported in depressed mood (Shelton, Claiborne et al. 2011). Also, when MAO-A density is elevated, monoamine metabolism is increased (Youdim, Edmondson et al. 2006), and monoamine loss via acute monoamine depletions or chronic removal with reserpine is associated with depressed mood (Freis 1954; Young, Smith et al. 1985; Laruelle, D'Souza et al. 1997; Leyton, Young et al. 1997; Verhoeff, Hussey et al. 2002; Neumeister, Nugent et al. 2004). The association of depressed mood with MAO-A V_T in the present study is supportive of a relationship between MAO-A level and depressed mood in AD, possibly through the aforementioned mechanisms, particularly in AD with long periods of heavy alcohol intake. Given the link between greater MAO-A V_T with high risk states for MDE, and the correlation between duration of years of alcohol dependence with greater MAO-A V_T seen in the current study, one way to understand why alcohol dependence may predispose to MDD is that years of heavy alcohol intake may lead to greater MAO-A level, which may then predispose to MDD. This model provides a mechanism to explain the most common pathway of comorbidity, AD leading to MDD, prospectively studied in several investigations (Wang and Patten 2002; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009). The question could be raised as to whether dysphoria predisposes to greater MAO-A V_T. Available evidence does not support this direction as a recent study by Soliman et al. found that induction of acute stress,
which also created dysphoria, was not associated with elevated MAO-A VT, thereby demonstrating that sad mood need not induce a rise in MAO-A level (Soliman, Udemgba et al. 2012).

Excessive monoamine metabolism may also be influential upon other circuits implicated in AD. For example, decreased ventral striatal dopamine release and insensitivity to this release has been implicated in AD as a mechanism that impairs the ability to develop an adaptive behaviour to abstain from drinking (Volkow, Wang et al. 1996; Martinez, Gil et al. 2005; Volkow, Wang et al. 2007). Dopamine is a substrate of MAO-A, and in the present study elevated MAO-A VT was found in the ventral striatum. Interestingly, greater 5-HT1B binding was also found in this region in AD using [11C]-P943 PET (Hu, Henry et al. 2010), and depletion of 5-HT through p-chlorophenylalanine has been associated with elevated expression of 5-HT1B receptors (Compan, Segu et al. 1998). 5-HT1A receptor binding was reported as normal in AD in humans (Martinez, Slifstein et al. 2009), whereas studies of SERT are mixed, with some reports of decreased SERT level and/or binding in anterior cingulate cortex, dorsal striatum, and midbrain (Chen, Casanova et al. 1991; Heinz, Ragan et al. 1998; Mantere, Tupala et al. 2002; Storvik, Tiihonen et al. 2006), and other reports of no change (Brown, George et al. 2007; Martinez, Slifstein et al. 2009). Future work should consider how a monoamine lowering process, such as elevated MAO-A level, might interact with other pathologies observed in AD.

One of the limitations of the present study is that there was no PET scan during the intoxication state. This is because alcohol intake has two mechanisms that lead to elevated plasma levels of a beta-carboline compound called harman, which has moderate affinity for MAO-A and may influence available MAO-A binding in humans (Fowler, Volkow et al. 1996; Bacher, Houle et al. 2011). To avoid temporary occupancy effects of harman, [11C]-harmine PET scanning was timed
when plasma harman levels were negligible. Also, arterial sampling of the radiotracer necessitates a radial arterial line, and participants could not be safely scanned while intoxicated. Another limitation is that the main outcome measure of $[^{11}\text{C}]-\text{harmine PET, MAO-A } V_T$, is an index of MAO-A density and represents total $[^{11}\text{C}]-\text{harmine in tissue relative to arterial plasma at equilibrium. It is computationally efficient, highly stable, and the least variable measure of }[^{11}\text{C}]-\text{harmine binding, however, approximately 15% of this measure reflects free and nonspecific binding (Ginovart, Meyer et al. 2006; Sacher, Houle et al. 2011). This free and nonspecific component is consistent across individuals, and while tremendous changes in free and nonspecific binding (i.e., of 250%) could lead to the changes in MAO-A } V_T \text{ observed, this is very unlikely (Ginovart, Meyer et al. 2006). The elevation in MAO-A } V_T \text{ may also reflect greater affinity of MAO-A, yet our overall interpretation of the results would not change because greater affinity of MAO-A would be expected to have similar functional effects as compared to a rise in MAO-A density.}

In summary, this was the first study of brain MAO-A in alcohol dependence in which key biases that affect MAO-A level and activity were addressed. Hence, the difference in MAO-A $ V_T$ between groups represents an effect of alcohol dependence, rather than a comorbid condition. The magnitude of elevation in MAO-A $ V_T$ in alcohol dependence was substantial, being 37% in the prefrontal cortex and 32% across all brain regions. Given the role of MAO-A in oxidative stress, apoptosis, and monoamine metabolism, this abnormality represents a new, therapeutically targetable marker present in AD. The best explanation for the elevated MAO-A $ V_T$ in AD is chronic alcohol exposure, based upon our data of a strong positive correlation between duration of heavy alcohol use and elevated MAO-A $ V_T$, as well as previous reports of greater MAO-A mRNA and TIEG2, a transcription factor that promotes MAO-A synthesis, in the prefrontal cortex after alcohol exposure in rodents. Also, elevated MAO-A level in regions that participate
in depressed mood, such as the prefrontal and anterior cingulate cortices, represents a novel mechanism to explain dysphoria observed in AD. Overall, greater MAO-A V_T represents the first biological marker present in AD that can account for both the neurotoxicity and symptoms of depression that develop with chronic alcohol use, and represents a new opportunity for therapeutic development, as it is targetable by the newest generation of MAO inhibitors.
4.2 Studies of the effect of Harman and Alcohol Exposure on MAO-A in Rodents

4.2.1 Effect of Harman Exposure on MAO

This was the first study to investigate the effect of chronic harman administration on MAO-A levels in rodent brain. The primary finding was no significant effect of harman on protein or activity levels of MAO-A at physiologically relevant doses in any of the brain regions assayed (prefrontal cortex, anterior cingulate cortex, hippocampus, caudate-putamen). After 8 hours of withdrawal from harman, MAO-A levels and activity were also unaffected. Collectively, these findings suggest that chronic inhibition of MAO-A by harman does not lead to the subsequent upregulation of this enzyme. The results of this study have important implications for the involvement of beta-carbolines in the regulation of MAO-A, the neurobiology of addiction to substances containing harman, and the safety profile of addictive substances containing beta-carbolines.

Our findings indicate that chronic harman exposure is unlikely to account for the elevations in MAO-A level in the prefrontal and anterior cingulate cortices during withdrawal from cigarette smoking and alcohol dependence (Bacher, Houle et al. 2011; Matthews, Kish et al. 2013). This is an important issue because elevated MAO-A level in the PFC and ACC is implicated in depressive behaviour (Meyer 2012). Depressive behaviour has several important roles in cigarette smoking and alcohol dependence: it is increased during withdrawal and is associated with greater risk for recurrence (Caster and Parsons 1977; Parsons, Schaeffer et al. 1990; Glenn and Parsons 1991), it is associated with higher risk of developing major depressive disorder in prospective studies (Klungsoyr, Nygard et al. 2006; Fergusson, Boden et al. 2009), and it is
associated with greater risk of suicide (Hughes 2008; Flensborg-Madsen, Knop et al. 2009; Li, Yang et al. 2012). As a strategy for harm reduction, there are regulations that aim to remove compounds from addictive substances that are implicated in the reinforcing behaviour of compulsive intake or are associated with specific toxicities, in order to increase their safety profile (Nutt, Phillips et al. 2014). For example, a variety of different techniques have been applied to reduce the harm from cigarettes, including filters that target different toxicants (i.e. volatile phenols/acids, hydrogen cyanide), smaller cigarette circumference, increased carbon filter length, and the development of electronic cigarettes (Dittrich, Fieblekorn et al. 2014; Gualano, Passi et al. 2014). In this context, given that chronic harman exposure does not increase MAO-A levels, it seems unlikely that incorporating strategies to remove harman from alcohol containing beverages or cigarettes would have any utility in preventing the associated harm resulting from elevated MAO-A level seen in human withdrawal.

Beta-carbolines are currently being investigated for their therapeutic potential in several directions, including the treatment of depressive, anxious and craving behaviours. Across several rodent models of depression (chronic mild stress and forced swim test) and anxiety (elevated plus maze), harman and harmine consistently induce dose dependent antidepressant and anxiolytic effects (Arıcıoglu and Altunbas 2003; Farzin and Mansouri 2006; Fortunato, Reus et al. 2010; Fortunato, Reus et al. 2010). To date, there have been no clinical trials examining the therapeutic efficacy of beta-carbolines in humans. Our results remove an important barrier for development of harm in humans, since they suggest there is little risk of developing the adverse effects consequent to elevated MAO-A levels.

With a negative study, it is important to consider whether the power was adequate. In this study, we anticipated a 30% effect on MAO-A levels and/or activity based on the change in MAO-A
binding observed in the sample of heavy cigarette smoking subjects exposed to elevated plasma harman levels (Bacher, Houle et al. 2011). With an alpha coefficient of 0.05, and an estimated standard deviation of 15% and 8% respectively for the Western blot and activity assays, the power was greater than 80% and 90% respectively with 10 rodents in each group. To optimize sensitivity, in the current study we adapted a novel colorimetric MAO activity assay using amplex red as the chromogen. This method has the advantage of high sensitivity, being 10 fold more sensitive than the aminoantipyrine/vanillic acid-based colorimetric assay used to measure monoamine oxidases (Holt and Palcic 2006). Our assay requires much less tissue (as low as 5 µg protein/ml or 3.5 µg protein/assay in our setting of rat brain tissue homogenate), which is essential for measurement of MAO activities in small brain regions such as the anterior cingulate cortex (< 10 mg wet weight tissue available). The spectrophotometric assay also has the advantage of wider accessibility than the original fluorometric assay (Zhou and Panchuk-Voloshina 1997) and without the possible confound of fluorescence quenching by some MAO substrates (Holt and Palcic 2006).

The plasma levels of harman reported in the present study correspond to those found in alcohol and tobacco dependent individuals (Rommelspacher, Schmidt et al. 1991; Breyer-Pfaff, Wiatr et al. 1996; Rommelspacher, Dufeu et al. 1996; Spies 1998; Bacher, Houle et al. 2011), with the lowest dose (2 mg/kg/day) being the most similar to typical human plasma levels. Additionally, the doses of harman used in the present study correspond to those that led to increased release of dopamine and 5-hydroxytryptamine in the nucleus accumbens (doses greater than 2 mg/kg harman) (Baum, Hill et al. 1996), up to four times greater levels of serotonin in brain (doses of 5-20 mg/kg harman) (Adell, Biggs et al. 1996), and increased firing of dopamine neurons (2 mg/kg harman) (Arib, Rat et al. 2010) in rodents. At these doses, harman has also been shown to facilitate dopaminergic transmission by enhancing levodopa induced stereotypy in mice.
(Pimpinella and Palmery 1995). Since the harman dosing regimen employed corresponds to plasma levels of harman associated with an increase in monoamine oxidase A substrates, and are comparable to plasma harman levels in human addiction, it is unlikely that the lack of change in MAO-A level can be attributed to inadequate plasma levels. On the other hand, one limitation of the current study related to dose is the 21 day administration period, as human addiction occurs across several years. The standard length of treatment for chronic harman administration in the animal literature is 14 days (Adell and Myers 1994; Fortunato, Reus et al. 2010; Reus, Stringari et al. 2010), hence the 21 day paradigm represents prolonged administration in rodents. It is possible that longer treatment could have led to an effect, yet this is unlikely, at least through influencing monoamine levels, as a study of two year serotonin depletion did not change MAO-A activity in rodents (Timiras, Hudson et al. 1984). Some evidence suggests that an accumulation of substrate may lead to MAO-A upregulation acutely, as increased MAO-A protein and mRNA levels were seen after 48 hour incubation with dopamine in rodents (Pizzinat, Marchal-Victorion et al. 2003), and in a PET study, decreased levels of MAO-A in the PFC were seen 4 hours after tryptophan depletion, and increased levels of MAO-A were observed in the striatum 2.5 hours after levodopa-carbidopa administration (Sacher, Rabiner et al. 2012). Future investigations could include examination of the acute affects of monoamine administration on MAO-A levels in rodent brain.

In summary, we report the first observation that chronic harman administration, a beta-carboline that acts as a moderately high affinity MAO-A inhibitor, does not lead to upregulation of MAO-A protein or activity levels. After 8 hours of withdrawal from harman, levels of MAO-A remain unchanged. Our findings provide important insight into the mechanism of elevated MAO-A levels seen during withdrawal from heavy alcohol and tobacco use, suggesting harman is unlikely to contribute. Since chronic beta-carboline administration has no effect on MAO-A
level, this also suggests a favorable side effect profile at the level of influencing MAO-A, which is important as these compounds are being investigated for antidepressant and anxiolytic effects, and the ability to reduce craving.
4.2.2 Effect of Ethanol Exposure on MAO

The present study was the first to investigate the effect of chronic alcohol vapor exposure, which maintained blood alcohol concentrations comparable to humans at 150-300 mg/dl per week, on MAO-A and MAO-B protein and activity levels, and GFAP levels in rodent brain. The primary finding was significantly elevated MAO-A and MAO-B activity and MAO-A protein levels in the PFC and ACC at 24 hours of withdrawal after 8 week ethanol vapor exposure. There were no changes in MAO-A and MAO-B activity or protein levels at immediate sacrifice, 4 day, or 21 day withdrawal. Additionally, GFAP was unaltered for all groups subsequent to ethanol exposure. Collectively, these findings suggest that chronic ethanol exposure leads to the subsequent upregulation of MAO-A and MAO-B activity in the PFC and ACC during acute withdrawal. The results of this study have important implications for the involvement of MAO-A in the dysphoric mood state of withdrawal and the development of mood disorders that emerge in alcohol dependence, as well as treatment for these conditions.

Alcohol dependence and major depressive disorder are highly comorbid disorders (Kessler, Crum et al. 1997), with proposed pathways including shared vulnerability, greater likelihood of alcohol consumption during MDD, or AD leading to increased risk of MDD. Recent prospective studies suggest the high comorbidity may be due to prolonged, heavy alcohol intake leading to the development of depressive symptoms (Hasin and Grant 2002; Wang and Patten 2002; Fergusson, Boden et al. 2009; Flensborg-Madsen, Mortensen et al. 2009). Depressed mood occurs during withdrawal, and increases the likelihood of a subsequent major depressive episode and the risk of relapse (Brown and Schuckit 1988; Hartka, Johnstone et al. 1991; Hasin, Tsai et al. 1996; Greenfield, Weiss et al. 1998; Ramsey, Kahler et al. 2004). Despite these links between AD and MDD, there are very few investigations examining common neuropathologies between
these two disorders (outlined in Section 1.2.4). MAO-A is known to be elevated in major depressive disorder (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Johnson, Stockmeier et al. 2011), hence to the best of our knowledge, our finding of elevated MAO-A protein and activity levels is the first identification of a pathology following alcohol exposure that is implicated in the initial onset of MDD. These results suggest that developing strategies to avoid raising MAO-A as a consequence of alcohol exposure may prevent the risk for subsequent major depressive episodes, and that MAO-A inhibition may have therapeutic potential for the treatment of AD.

Two questions arise from the time course results of the current study; the first being why elevated MAO-A was seen at 24 hours of withdrawal but not at immediate sacrifice (no withdrawal), and the second being why the elevation at 24 hours does not persist into protracted withdrawal. The lack of persistence of elevated MAO-A activity and protein levels in the PFC and ACC into 4 and 21 day withdrawal may be due to the magnitude of change, because human investigations of MAO-A in mood disorders suggest that magnitudes of elevation of 40% or greater are associated with persistence (Meyer, Wilson et al. 2009; Sacher, Rekkas et al. 2015). For example, individuals with MDD in recovery who went on to have a recurrence had a 42% increase in MAO-A $V_T$ in the PFC and ACC, and there was a 43% elevation in MAO-A level during the early post partum period, and a subset of individuals go on to have elevated MAO-A $V_T$ during post partum depression (Meyer, Wilson et al. 2009). The elevation in MAO-A seen at 24 hours of withdrawal (and not at immediate sacrifice) may be a consequence of the early withdrawal period and related to the stress of acute withdrawal. A recent study reported an increase in MAO-A and TIEG2, a transcription factor that upregulates catalytic activity and protein levels of MAO-A, after dexamethasone (a synthetic glucocorticoid that induces cellular stress) exposure in vitro, as well as following chronic social stress in rodents (Grunewald,
Additionally, many studies demonstrate elevations in MAO-A and MAO-B activity after exposure to chronic unpredictable mild stress (Lin, Liu et al. 2005; Hu, Liu et al. 2011; Zhong, Wu et al. 2012; Yu, Wang et al. 2013). The chronic mild stress model relates to the ethanol vapor exposure model used in the present study because rodents were exposed to ethanol for 17 hours per day, and were likely experiencing the stress of withdrawal at subthreshold levels throughout the 8 week exposure period. Further investigations are required to determine the mechanism of the lack of effect when animals are not withdrawn from alcohol, and for the lack of persistence of elevated MAO-A into withdrawal.

In addition to MAO-A, MAO-B also demonstrated a significant elevation in catalytic activity in the PFC and ACC at 24 hours of withdrawal, but not at any other time points. This finding is consistent with prior reports of elevated MAO-B level in the PFC of rodents fed an ethanol diet, and postmortem tissue of alcohol dependent individuals (Ou, Stockmeier et al. 2010; Ou, Johnson et al. 2011). MAO-B inhibition was shown to counteract this effect in cell lines, providing additional support for the utility of MAO inhibition to treat alcohol use disorders (Ou, Lu et al. 2009).

The lack of effect on GFAP in all brain regions in the current study is consistent with human investigations, where no significant changes in GFAP expression were seen in postmortem dorsolateral prefrontal cortex and orbitofrontal cortex of individuals with uncomplicated alcohol dependence (Miguel-Hidalgo, Wei et al. 2002; Lewohl, Wixey et al. 2005; Miguel-Hidalgo, Waltzer et al. 2010). Decreases in glial size and density in the dorsolateral PFC in postmortem alcohol dependence were more severe in AD with depressive symptoms, suggesting a stronger relationship to MDD, yet alterations in GFAP immunoreactivity were not seen in this group (Miguel-Hidalgo and Rajkowska 2002; Lewohl, Wixey et al. 2005). The lack of effect may also
be due to the dual actions of ethanol upon GFAP in vivo, with high doses producing neuronal damage and inducing gliosis and increases in GFAP expression within the first few weeks, while prolonged exposure down regulates the synthesis of GFAP (Franke 1995; Franke, Kittner et al. 1997). For example, in the rat hippocampus, 10% (v/v) ethanol over 4 and 12 weeks led to an increase in GFAP immunoreactivity, while 10% (v/v) ethanol over 36 weeks decreased total GFAP immunoreactivity (Franke 1995; Franke, Kittner et al. 1997). Depending on the time of measurement, these two competing mechanisms may cancel out any pronounced effects of ethanol exposure on GFAP levels. It is important to note that the average daily blood ethanol concentration in these studies was 58 mg/dl, which is considerably lower than what was maintained in the present study of ethanol vapor exposure and what is comparable to human intoxication (150-300 mg/dl). Another report demonstrated significant elevations in GFAP immunoreactivity after 21 days, and 3 and 6 months of ethanol exposure at an average blood alcohol concentration of 148 mg/dl in the hippocampus, cingulate cortex, and cerebral cortex (Vongvatcharanon, Mukem et al. 2010; Udomuksorn, Mukem et al. 2011). It could be that our higher average blood alcohol levels may have led to an earlier compensatory down regulation of GFAP, cancelling out this effect (Franke 1995; Franke, Kittner et al. 1997). Hence our study was the first to examine GFAP levels at clinically relevant blood alcohol concentrations, and demonstrated that GFAP is not significantly altered during the intoxication state after 8 weeks of ethanol exposure, or after 24 hour, 4 day, or 21 day withdrawal periods.

There are some discrepancies between the PET imaging study of alcohol dependence, where a 37 to 40% increase in MAO-A level was observed in the PFC and ACC in human participants (Matthews, Kish et al. 2013), and the current study, where a 25 to 31% increase in MAO-A protein level, and a 12 to 15% increase in MAO-A activity, were observed in the PFC and ACC of rodents. One explanation for this discrepancy could be the amount of time study subjects were
exposed to alcohol. The average duration of alcohol dependence in the human PET study was 6 years, and the years of heavy alcohol use demonstrated a strong, positive correlation with the rise in MAO-A (Matthews, Kish et al. 2013). Hence the 8 week ethanol vapor exposure may not have been a long enough period to lead to similar magnitudes of elevated MAO-A. The difference in magnitude of effect may also relate to the selection of participants, as the rodents used were not predisposed to alcohol intake, whereas the human sample was already vulnerable to heavy drinking at the time of study enrollment. Future investigations could employ alcohol preferring rodents or longer exposure periods to examine whether there may be a greater magnitude of effect on MAO-A levels.

In summary, we report the first observation that chronic ethanol vapor exposure leads to the upregulation of MAO-A and MAO-B catalytic activity and MAO-A protein levels in the PFC and ACC during acute (24 hour) withdrawal. After 8 weeks of exposure, 4 days of withdrawal, and 3 weeks of withdrawal, MAO-A and MAO-B levels were not significantly different from controls. GFAP levels were unaltered for all groups. Our findings provide important insight into the mechanism of elevated MAO-A level seen during alcohol withdrawal in humans, suggesting ethanol exposure is involved in the upregulation of this enzyme. Greater MAO-A levels are implicated in the development of mood disorders, raising the possibility of MAO-A inhibition for therapeutic development in order to avoid the adverse sequelae of major depressive episodes that may emerge during withdrawal from prolonged, heavy alcohol use.
4.3 Implications for Treatment of Depressed Mood and Alcohol Dependence

The PET imaging study of the present thesis demonstrated significantly elevated MAO-A binding in the prefrontal and anterior cingulate cortices, as well as a number of other brain regions, in alcohol dependence (Matthews, Kish et al. 2013). These participants had an average duration of AD of 6 years, moderate levels of alcohol dependence (according to the alcohol dependence scale), and reported dysphoric mood during early withdrawal (Matthews, Kish et al. 2013). The results of this study argue for clinical trials to examine the efficacy of MAO-A inhibition to treat mood symptoms of withdrawal. Traditionally, the treatment of co-occurring depression in alcohol dependence was discouraged among psychiatrists. It was proposed that depressive symptoms would resolve after substance abuse problems were managed, and that treatment for mood symptoms would deter focus from treatment of the addiction (Schuckit 1994). Yet each of these disorders increase the risk of development of the other (Lynskey 1998; Abraham and Fava 1999; Kuo, Gardner et al. 2006), and a strong association exists between drinking severity and level of depression (Bonin, McCreary et al. 2000; Wang and Patten 2002; Graham, Massak et al. 2007), suggesting each illness exacerbates the symptoms of the other. Additionally, although depressive symptoms that occur during alcohol intoxication or withdrawal may be transient in nature (Brown and Schuckit 1988; Schuckit 1994), many persist into abstinence and contribute to relapse (Glenn and Parsons 1991; Mueller, Lavori et al. 1994; Hasin, Tsai et al. 1996; Kranzler, Del Boca et al. 1996; Greenfield, Weiss et al. 1998; Hasin and Grant 2002) and the conversion to a major depressive episode (Ramsey, Kahler et al. 2004). Depressive symptoms in withdrawal also affect a patient’s willingness to remain in treatment (Glenn and Parsons 1991; Schuckit, Tipp et al. 1997; Hoencamp, Haffmans et al. 1998; Rae,
Joyce et al. 2002; Lin, Chen et al. 2007). Collectively, this body of evidence supports the notion that alcohol dependence should not be a barrier to the treatment of depression; that each disorder should be identified and treated concurrently, and that MAO-A inhibition may be a promising new therapeutic target for mood symptoms in AD.

There are some clinical data to support the perspective that pharmacotherapy designed to treat each syndrome present in AD (i.e. depressed mood and addiction to alcohol) may result in optimal treatment. For example, early case studies demonstrated additional efficacy when medication for both depressive symptoms and alcohol consumption were prescribed to comorbid patients concurrently (Nunes, McGrath et al. 1993; Farren and O'Malley 1999). More recently, a large scale, double blind, randomized controlled trial confirmed this theory. Combined naltrexone, an opioid receptor antagonist commonly prescribed for AD, and sertraline, a selective serotonin reuptake inhibitor antidepressant, led to significant improvements in abstinence rates as well as depressed mood (Pettinati, Oslin et al. 2010). The results of our PET imaging study support the described syndrome approach to pharmacotherapy, and suggest MAO-A inhibition may be more efficacious than SSRI treatment, given that we discovered MAO-A levels are upregulated in AD (Matthews, Kish et al. 2013), and SSRI’s were shown not to influence MAO-A levels (Meyer, Wilson et al. 2009). MAO-A inhibitors were not initially investigated in AD because alcoholic beverages contain tyramine, and the original non-selective, irreversible MAO-A/B inhibitors were associated with greater risk for hypertensive crisis from inadequate peripheral metabolism of tyramine. Yet this is no longer an issue due to the development of selective reversible MAO-A inhibitors, and MAO inhibitors with high brain to periphery concentration ratios, which do not require dietary restriction of tyramine (Youdim, Edmondson et al. 2006).
Early clinical trials of tricyclic antidepressants (TCA’s) for AD with depressive symptoms suggested positive results on both mood symptoms and alcohol consumption (Nunes, McGrath et al. 1993; Mason, Kocsis et al. 1996; McGrath, Nunes et al. 1996). These studies were followed up by several investigations of selective serotonin reuptake inhibitors, which were initially examined due to better tolerability and safety compared to TCA’s. A recently published meta-analysis of randomized controlled trials of SSRI and TCA pharmacotherapy in comorbid AD and MDD found that treatment with an antidepressant reduces depressive symptoms, but has little impact on alcohol consumption (Pettinati 2004). Another meta-analysis that included two additional studies similarly concluded a benefit of antidepressants for mood symptoms only (Torrens, Fonseca et al. 2005). Any improvements on alcohol outcomes were related to decreases in depressed mood, and there were no effects on alcohol intake in patients without comorbid depression. Additionally, no class of antidepressant was superior to another. Conclusions from these reviews suggest that antidepressant treatment with TCA’s or SSRI’s can only resolve the depressive symptoms of comorbid patients. This is still clinically relevant because better drinking outcomes are associated with fewer and less severe comorbid psychiatric disorders, particularly depression, high self confidence about outcome, and the capacity to remain in intensive treatment (Schuckit 2009), each of which can be improved with antidepressant treatment. The limited efficacy of TCA’s or SSRI’s on alcohol consumption suggests a need for further investigation of other classes of antidepressants.

Preclinical models demonstrate promise for MAO-A inhibitors to reduce ethanol consumption. One study demonstrated reduced alcohol self administration (in rats trained to self-administer ethanol, 10 % v/v, orally in a free-choice two lever operant task) after administration of reversible MAO-A inhibitor, befloxatone (0.3–3 mg/kg), and irreversible MAO-A inhibitor, clorgyline (10–30 mg/kg) (Cohen, Curet et al. 1999). There was a significant correlation between
the ED$_{50}$ (median effective dose) for inhibiting MAO-A activity (but not MAO-B) and the potency for decreasing rates of ethanol self-administration (Cohen, Curet et al. 1999). Both clorgyline (2 mg/kg) and reversible MAO-A inhibitor BW A616U (50-75 mg/kg) were also shown to reduce the proportion of ethanol consumption to total fluids by over 50% in genetic drinking Myers high ethanol preferring rats (Mega, Sheppard et al. 2002). Clorgyline (5 mg/kg) was also shown to reduce voluntary ethanol consumption (with no change in water or saccharin intake) in mice following a drinking in the dark paradigm (Ledesma, Escrig et al. 2014). These studies suggest that in addition to the treatment of mood symptoms in withdrawal, MAO-A inhibition may additionally suppress alcohol consumption.

The impact pharmacotherapy for alcohol cessation has had on depressive symptoms in a comorbid population is largely unknown. Alcohol cessation aids were predicted to be efficacious in co-occurring alcohol dependence and depression because negative affect induced by alcohol use should resolve as alcohol consumption decreases. Some studies of naltrexone suggest greater efficacy for reducing alcohol intake among those with comorbid depression compared to those without, and some ability to reduce depressive symptoms (Kiefer, Helwig et al. 2005; Krystal, Gueorguieva et al. 2008). Additional randomized controlled trials are required to validate these conclusions. Combined naltrexone and disulfiram, one of the initial medications prescribed for AD that acts as an aldehyde dehydrogenase inhibitor, provided no additional benefit to either treatment alone, and led to modest improvements on depressive symptoms and alcohol outcomes (Petrakis, Poling et al. 2005; Petrakis, Ralevski et al. 2007). A meta-analysis of depressed patients from several clinical trials suggested that acamprosate, a medication commonly prescribed in AD which inhibits glutamatergic neurotransmission, produced similar efficacy in those with or without comorbid depression, and led to a modest reduction in alcohol consumption and depressive symptoms (Lejoyeux and Lehert 2011). The effectiveness of alcohol
cessation pharmacotherapy in comorbid patients is difficult to ascertain, as there are large inconsistencies across studies in terms of baseline measurements of mood symptoms and alcohol use, and trials are often confounded by concomitant use of antidepressants or other psychotropic medication. Taken together, alcohol cessation aids have shown efficacy in reducing alcohol consumption in a comorbid sample, but firm conclusions cannot be made about their benefit on mood symptomatology.

Optimal treatment of alcohol dependent patients with comorbid depressive symptoms remains a significant clinical challenge. It is clear that pharmacotherapy that focuses solely on mood symptoms, or reducing alcohol consumption, does not lead to major clinical improvement. We propose that adjunct therapy of a traditional medication for AD with an MAO-A inhibitor may be the most beneficial avenue of treatment for AD with depressive symptoms. Naltrexone is well tolerated, has high compliance rates in six to eight week clinical trials (Thase, Salloum et al. 2001), and does not appear to have any adverse reactions when taken with antidepressants or alcohol (Croop, Faulkner et al. 1997). Acamprosate may be recommended in the case of non-response to naltrexone, as it does not affect hepatic metabolism and is not expected to interact with antidepressants (Thase, Salloum et al. 2001). Disulfiram is contraindicated in cases of comorbidity, as it has a number of interactions with other medications and generally low compliance rates (Thase, Salloum et al. 2001). Hence, combination therapy of an alcohol cessation aid and a MAO-A inhibitor will likely be well tolerated and may improve patient outcomes for both alcohol intake and mood symptoms.

In summary, alcohol dependence and affective disorders frequently coexist and impact the clinical course and treatment of each illness. Over the past 20 years, support has developed for the utility of antidepressants to treat the mood symptoms of comorbid cases, as depression in
alcohol dependence contributes to poorer prognosis. Novel approaches include targeting both of the key factors that comprise the illness, by means of treating patients with an antidepressant as well as an alcohol cessation aid. More research is required to optimize certain combinations of therapy for individual cases, and the results of the present thesis argue for clinical trials with MAO-A inhibitors. This integrated outlook represents the future of treatment for comorbid alcoholism and depression, and has the potential to greatly improve traditional therapy, thereby decreasing the economic burden and personal harm caused by inadequate treatment success.
4.4 Recommendation for Future Investigations

The main findings of the current thesis were at the discovery level, hence there are a variety of avenues by which this work can be further investigated. The first avenue would be to determine potential mechanisms of elevated MAO-A in AD (Matthews, Kish et al. 2013). Using the rodent model of chronic ethanol vapor exposure, which leads to similar blood alcohol levels seen in human intoxication, one could examine markers associated with oxidative stress or apoptosis. For oxidative stress, these markers could include lipid peroxidation (i.e. through the measurement of thiobarbituric acid reactive species), protein degradation (i.e. through the measurement of protein carbonyls), or levels of antioxidants (i.e. through the measurement of glutathione or superoxide dismutase). For apoptosis, these markers could include levels of apoptotic protein caspase 3, anti-apoptotic Bcl-2, DNA fragmentation (i.e. through measurement of the number of TUNEL positive cells), or TIEG2. Each of these described markers were shown to elevate in models of AD (Casey, Nanji et al. 2001; Agar, Demir et al. 2003; Ikegami, Goodenough et al. 2003; Matsuda-Matsumoto, Iwazaki et al. 2007; Ou, Johnson et al. 2011), yet the relationship of MAO-A with apoptosis was only recently discovered and investigated in vitro (Ou, Chen et al. 2006; Youdim, Edmondson et al. 2006; Fitzgerald, Ufer et al. 2007; Fitzgerald, Ufer et al. 2007). Should these markers elevate along with MAO-A protein and activity levels in the rodent model of AD, this would suggest oxidative stress and/or predisposition to apoptosis may be involved in the upregulation of MAO-A. In addition, a TIEG2-knockout mouse has been generated, which may be useful to determine whether TIEG2 is involved in producing increases in MAO-A and/or contributing to the cell death pathway induced by ethanol in rodents (Song, Gavrilidis et al. 2005). The use of recently developed CRISPR-Cas9 knockin mice may also allow for the specific targeting of certain genes involved in the vulnerability or resistance to
oxidative stress and/or apoptosis, and could be used to further characterize the mechanism of elevated MAO-A in AD (Platt, Chen et al. 2014).

As outlined in Section 4.2.1, there are regulations that aim to add or remove compounds from addictive substances that may reduce specific toxicities as a strategy for harm reduction (Nutt, Phillips et al. 2014). Should oxidative stress markers be present in rodent models of AD that also induce elevations in MAO-A, it can then be determined whether antioxidant treatment may reverse the oxidative stress and/or elevated MAO-A. There are some rodent studies that report reduced markers of alcohol induced oxidative stress in brain after antioxidant treatment (El-Sokkary, Reiter et al. 1999; Mansouri, Demeilliers et al. 2001; Agar, Demir et al. 2003). Whether this has an effect on MAO-A is currently unknown. The addition of an antioxidant compound to an alcoholic beverage may demonstrate the potential to reduce or reverse elevated MAO-A in AD.

A number of directions can be taken to further explore the main findings of the human PET study of alcohol dependence. One direction would be to examine a time course of alcohol dependence. For example, it would be clinically relevant to determine whether MAO-A level remains elevated into early or protracted abstinence in humans, whether MAO-A level may elevate to a greater extent in those who relapse in the future, or whether those who have the greatest elevation in MAO-A level may go on to develop a major depressive episode in the future. Results from these studies would then be able to guide treatment regimens, and may suggest a time course of adjunct pharmacotherapy for AD with an antidepressant. From a therapeutic standpoint, clinical trials with MAO-A inhibitors would be a next step, in order to determine whether there can be reversal of the adverse sequelae of elevated MAO-A (i.e. depressed mood) in early withdrawal, and whether MAO-A inhibitor treatment in early
withdrawal may be able to prevent later development of a major depressive episode. It would also be useful to test the effectiveness of an MAO-A inhibitor in combination with an alcohol cessation aid (i.e. naltrexone or acamprosate). Findings from these studies could greatly improve clinical outcomes for AD and comorbid AD and MDD.
4.5 Conclusion

The results of the current thesis identify elevated MAO-A level as a new pathological marker present in AD. MAO-A $V_T$, a measure of MAO-A density, was elevated during acute withdrawal in alcohol dependent individuals. The highest magnitude of elevation was in the prefrontal and anterior cingulate cortices, two regions with a functional role related to mood, at 37% and 40% elevation, respectively. There were two hypothesized pathways by which MAO-A may elevate in alcohol dependence. The first pathway being that alcoholic beverages contain the beta-carboline alkaloid harman, which is a MAO-A inhibitor and may lead to upregulation of MAO-A after chronic intake. Cigarette smoke also contains harman, and MAO-A was found to be elevated in the dysphoric mood state of cigarette withdrawal in heavy smokers (Bacher, Houle et al. 2011). The second pathway may be that chronic alcohol exposure upregulates MAO-A, as elevated MAO-A mRNA was shown in rodents exposed to ethanol (Zimatkin, Tsydik et al. 1997; Rimondini, Arlinde et al. 2002). Additionally, chronic alcohol exposure induces markers associated with apoptosis (Ikegami, Goodenough et al. 2003; Wu, Zhai et al. 2006; Ou, Johnson et al. 2011), and MAO-A is elevated during pro-apoptotic conditions in cell lines (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007). We found that MAO-A protein and activity levels were not changed by chronic administration of harman, suggesting prolonged MAO-A inhibition does not alter MAO-A level. We replicated our human PET findings in rodents chronically exposed to alcohol vapor, resulting in blood alcohol concentrations similar to human intoxication, with a 25-31% elevation in MAO-A protein level and 12-15% elevation in MAO-A activity in the prefrontal and anterior cingulate cortices during acute (24 hour) withdrawal. Based on the correlation with years of heavy alcohol use and elevated MAO-A $V_T$ in our study of human alcohol dependence, and the replication study in rodents, it appears that alcohol exposure likely
led to elevated MAO-A in AD. Because elevated MAO-A is implicated in major depressive disorder, and high risk states for major depression, we propose that greater MAO-A levels in brain may contribute to the development of depressive mood and MDD during AD that is seen in prospective studies. This is further supported by the correlation between depressed mood and elevated MAO-A V₇ seen in our study of alcohol dependence. The results from this series of studies suggest that MAO-A inhibition may be useful for the treatment of mood symptoms in AD. Also, given the association between AD and markers associated with apoptosis (Ikegami, Goodenough et al. 2003; Wu, Zhai et al. 2006; Ou, Johnson et al. 2011), and that MAO-A may elevate under such conditions (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007), MAO-A inhibition may have an additional role in resolving the neurotoxic effects of chronic alcohol use.
References


Yi, H., Y. Akao, et al. (2006). "Type A monoamine oxidase is the target of an endogenous dopaminergic neurotoxin, N-methyl(R)salsolinol, leading to apoptosis in SH-SY5Y cells." J Neurochem 96(2): 541-549.


