Monoamine Oxidase A in Relation to Subtypes and Treatment of Major Depressive Disorder

by

Lina Chiuccariello

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Graduate Department of Pharmacology and Toxicology

University of Toronto

© Copyright by Lina Chiuccariello (2015)
Abstract

Identification of central biomarkers related to subtypes of major depressive episodes (MDEs) can contribute to a more personalized treatment approach of major depressive disorder (MDD). Monoamine Oxidase-A (MAO-A) is a replicated central biomarker of MDE and may represent a biomarker of MDE subtypes. The current body of research aimed to identify clinical predictors of elevated MAO-A total distribution volume (V_T), to evaluate the relationship of MAO-A V_T to replicated brain structural changes seen in MDD and to assess the targeting of MAO-A with monoamine oxidase inhibitors (MAOIs). Greater severity and reversed neurovegetative MDE symptoms were associated with elevated MAO-A V_T in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) (Multivariate Analysis of Variance (MANOVA), severity: F(2,38)=5.44, p=0.008; reversed neurovegetative symptoms: F(2,38)=5.13, p=0.01). There was no significant relationship between hippocampal MAO-A V_T and hippocampal volume or PFC and ACC MAO-A V_T to PFC or ACC cortical thickness in MDD. Mean brain MAO-A occupancies ranged from 74-84% for moclobemide (300-1200mg daily dose) and was 87% for phenelzine (45-60mg daily dose). There was significantly greater MAO-A occupancy by phenelzine (45-60mg) and higher dose moclobemide (900-1200mg) compared to lower moclobemide doses (300-600mg) (F(7,16)=3.94, p=0.01). In conclusion, this research suggests elevated MAO-A V_T as a distinct biomarker of MDE
compared to structural brain changes that has the greatest elevation in MDEs with the highest severity or reversed neurovegetative MDE symptoms and this research has identified the optimal occupancy and treatment algorithm of MAOIs available to target MAO-A.
Acknowledgements

I would first like to acknowledge the never ending support of my family in the pursuit of my graduate education. They are my constant source of encouragement and understanding and I could not imagine going through the stressful or the not so stressful times without them. They are always there to listen, even when that means listening to the same thing over and over and over again. I love you.

The completion of this body of research would not have been possible without my supervisor, Dr. Jeffrey H. Meyer. I am grateful to have been a part of his laboratory and learn from his expertise. He is quite passionate about contributing to improving the treatment of mood disorders and increasing the understanding of the neurobiology of these disorders. His enthusiasm was an important driving force for this research and had a significant impact on my motivation to learn, understand and contribute to this field.

I definitely do not feel that my breadth of knowledge surrounding my research topic would have been as extensive if it were not for my co-supervisor, Dr. Stephen J. Kish. He has consistently challenged me, and from my very first committee meeting pushed me to dig deep into my findings and question everything surrounding them, as a true scientist should. Because of him, I always knew that I should never just have one answer to a question, but at least have one or two possible back up answers.

I am always thankful for my committee member, thesis reader and mentor, Dr. Bruna Brands. She is and has always been a fantastic mentor to me throughout not only my
Ph.D. degree but also throughout my M.Sc. degree. The completion of this thesis would not have been possible if it were not for her meticulous and numerous revisions to ensure that the finished copy exemplified my best work. She has a true passion for the students that she mentors and always provides them with support and gives them priority, even at the cost of losing any free time that she might have scheduled. She has somehow found a way, in her indescribably busy work schedule, to balance having a highly established scientific career and being readily available to just have a chat with her students about life. I am forever grateful to have had her on my side throughout the completion of my graduate education.

I am also thankful for the help and expertise of Dr. Isabelle Boileau through the completion of my graduate studies. Having worked with Dr. Boileau through both my M.Sc. and Ph.D., it became increasingly apparent how fantastic of a scientist she is. Her breadth of knowledge always makes me push myself harder. She helped to introduce me to PET research and was always available to help with even the smallest issues and there were definitely times I would not have been able to move forward without her.

I would also like to thank all of the members of the Meyer laboratory and the Research Imaging Centre. I would especially like to thank Dr. Pablo Rusjan. I cannot count the number of times Dr. Rusjan helped me in overcoming analyses problems or the amount of hours he spent with me explaining how to overcome issues. He was always readily available to help and was always willing to take the time to explain everything. He really helped me to gain an in-depth knowledge of image analysis that I do not think would
have been otherwise possible. I would also like to thank Alvina Ng and Laura Nyguen. They were always very accommodating in helping me schedule time sensitive scans and help me with anything I was unsure of and of course, provided great control room entertainment.

I would also like to acknowledge all of the collaborators and funding sources that helped toward the completion of this body of research:

Drs. Cooke and Levitan, who treated a number of individuals that participated in the MAO-A occupancy study.

The laboratory of Dr. Glen Baker for completing the plasma sample analyses for the MAO-A occupancy study.

Dr. Grazyna Rajkowska, for helping to develop the subregions of the prefrontal cortex template.

The laboratory of Dr. Mallar Chakravarty for helping with the cortical thickness and hippocampal volume analyses.

The funding sources that enabled completion of this body of research including, Canadian Institutes of Health Research, Canadian Foundation for Innovation, Ontario
Ministry for Research and Innovation and Ontario Mental Health Foundation Studentship.

Finally, I would like to thank all of the individuals that dedicated their time to participate in these studies, since otherwise these research studies would just be interesting ideas. I am hopeful that direct participation in these studies was beneficial and that the information gained from this body of research will contribute to better treatment of individuals in the future.
# Table of Contents

Abstract ii

Acknowledgements iv

List of Tables xv

List of Figures xvi

List of Abbreviations xvii

Section 1.0 INTRODUCTION 1

1.1 Statement of the Problem 1

1.2. Purpose of the study, objectives and hypotheses 5

1.2.1 Overall Thesis Purpose, Objective and Hypothesis 5

1.2.2 Specific Research Objectives and Hypotheses 6

1.3. Review of the Literature 8

1.3.1 Major Depressive Disorder 8

1.3.1.1. Treatment Resistance in MDD 9

1.3.1.2 Pathologies of MDD 12

1.3.2 Monoamine Oxidase (MAO): A Player in MDD 14

1.3.2.1 Functional Role of MAO-A 17

1.3.2.2 Genetics of MAO-A 19

1.3.2.3 The Importance of MAO-A in MDD Pathology 20

1.3.2.4 Potential Mechanism of Elevated MAO-A Levels 22

1.3.3 Structural Brain Changes in MDD Pathology 23

1.3.3.1 Decreased Grey Matter and Neurogenesis in MDD Pathology 23
1.3.3.2 Changes in Cortical Thickness in MDD

1.3.4 Pharmacological Treatments of MDD

1.3.4.1 Monoamine Oxidase Inhibitors

1.3.4.2 The Tyramine Reaction of MAOIs Leading to the Underutilization

1.3.4.3 Efficacy of Phenelzine and Moclobemide Compared to Other

Antidepressants in Treating MDD

1.3.4.4 Efficacy of Phenelzine and Moclobemide in Treating Subtypes of MDD

1.3.4.5 Pharmacokinetics of Phenelzine and Moclobemide

1.3.4.6 Pharmacodynamics of Phenelzine and Moclobemide

1.3.5 Target Imaging Through Positron Emission Tomography (PET)

1.3.5.1 Imaging MAO-A using PET

1.3.5.2 Utilizing Neuroimaging to Identify Central Biomarkers of MDD:

Matching Treatment to Pathology

1.4. Restatement of purpose, objectives and hypotheses

1.4.1 Overall Thesis Purpose, Objective and Hypothesis

1.4.2 Specific Research Objectives and Hypotheses

Section 2.0. MATERIALS AND METHODS

2.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive

Episodes is associated with Greater Symptom Severity and Reversed Neurovegetative

Symptoms

2.1.1 Study Design

2.1.2 Subject Selection

2.1.3 Subject Recruitment
2.1.4 Sample Size Justification 50
2.1.5 Assessment Day Procedures 50
2.1.6 Study Day Procedures 51
2.1.7 PET Image Acquisition 51
2.1.8 MR Image Acquisition 52
2.1.9 Primary Behavioural Measure Outcome 53
2.1.10 Ethical Considerations 53
2.1.11 Data Analysis 54
  2.1.11.1 PET Image Analysis 54
  2.1.11.2 Statistical Analyses 56

2.2 Study 2. The Relationship between MAO-A V_T, Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness 56
  2.2.1 Study Design 56
  2.2.2 Subject Selection 57
  2.2.3 Sample Size Justification 58
  2.2.3 MR Image Acquisition 59
  2.2.4 Image Analysis 59
    2.2.4.1 Total Hippocampus and Subfield Segmentation 59
    2.2.4.2 Derivation of Cortical Thickness 60
  2.2.5 Statistical Analyses 61
    2.2.5.1 Relationship between Hippocampal MAO-A V_T, Total Hippocampal Volume and Hippocampal Subfield Volume in MDD and Healthy Controls 61
2.2.5.2 Relationship between MAO-A $V_T$ and Prefrontal and Anterior Cingulate Cortical Thickness in MDD and Healthy Controls

2.3. Study 3. Dose-occupancy Relationship of Phenelzine and Moclobemide: Potential Implications for Novel Antidepressant Development and Optimal Dosing of Existing Monoamine Oxidase Inhibitors

2.3.1 Study Design

2.3.2 Subject Selection

2.3.3 Subject Recruitment

2.3.4 Sample Size Justification

2.3.5 Assessment Day Procedures

2.3.6 Study Day Procedures

2.3.7 PET Image Acquisition

2.3.8 MR Image acquisition

2.3.9 Primary Behavioural Measure Outcome

2.3.10 Ethical Considerations

2.3.11 Data Analysis

2.3.11.1 PET Image Analysis

2.3.11.2 Occupancy Calculation and Relationship to Dose

2.3.11.3 Moclobemide and Phenelzine Plasma Assays

2.3.11.4 Statistical Analyses

Section 3.0. RESULTS
3.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive Episodes is associated with Greater Severity and Reversed Neurovegetative Symptoms

3.1.1 Demographic and Clinical Characteristics in Subgroups of MDE 71
3.1.2 Effect of MDE Severity upon MAO-A V_T 74
3.1.3 Effect of Reversed Neurovegetative MDE Symptoms upon MAO-A V_T 77
3.1.4 Relationship of MAO-A V_T with Severity as a Continuous Variable 80
3.1.5 Comparison of MAO-A V_T between Subgroups with Healthy Controls 82
3.1.6 Plasma Free Fraction 82

3.2. Study 2. The Relationship between MAO-A V_T, Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness 83

3.2.1 Comparison of Demographic Variables between MDD and Healthy Controls 83
3.2.2 The Relationship of Hippocampal MAO-A V_T to Hippocampal Volume 84
   3.2.2.1 Hippocampal MAO-A V_T and Total Hippocampal Volume in the Combined Sample of MDD and Healthy Controls 84
   3.2.2.2 Hippocampal MAO-A V_T and Total Hippocampal Volume in Healthy Controls Alone 86
   3.2.2.3 Hippocampal MAO-A V_T and Total Hippocampal Volume in MDD Alone 86
3.2.3 Hippocampal MAO-A V_T and Hippocampal Subfield Volume 86
   3.2.3.1 Hippocampal MAO-A V_T and Hippocampal Subfield Volume in the Combined Sample of MDD and Healthy Controls 86
3.2.3.2 Hippocampal MAO-Vₜ and Hippocampal Subfield Volume in Healthy Controls Alone 87

3.2.3.3 Hippocampal MAO-A Vₜ and Hippocampal Subfield Volume in MDD Alone 87

3.2.3 The Relationship of MAO-A Vₜ to Cortical Thickness in the PFC and ACC 90

3.2.3.1 MAO-A Vₜ and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in the Combined Sample of MDD and Healthy Controls 90

3.2.3.2 MAO-A Vₜ and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in Healthy Controls Alone 91

3.2.3.3 MAO-A Vₜ and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in MDD Alone 92

3.3 Study 3. Dose-occupancy Relationship of Phenelzine and Moclobemide: Potential Implications for Novel Antidepressant Development and Optimal Dosing of Existing Monoamine Oxidase Inhibitors 95

3.3.1 Demographics and Clinical Characteristics 95

3.3.2 Dose-Occupancy Relationship of Moclobemide 97

3.3.3 MAO-A Occupancy of Three Groupings (Total Daily Dose of 300mg to 600mg Moclobemide, Total Daily Dose of 900mg to 1200mg Moclobemide, Total Daily Phenelzine Dose of 45 to 60mg) 99

3.3.4 Post-hoc Analysis of the Relationship between MAO-A Occupancy in the Prefrontal and Anterior Cingulate Cortex and Remission 101

3.3.5 Post-hoc Analysis Comparison of MAO-A Vₜ Targets across Treatment Groups as Determined from the Lassen Plot 101
4.0. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive Episodes is associated with Greater Severity and Reversed Neurovegetative Symptoms

4.2 Study 2. Monoamine Oxidase A Total Distribution Volume (V_T) in Relation to Hippocampal Volume and Cortical Thickness in Major Depressive Disorder

4.3. Study 3. Monoamine Oxidase-A Occupancy by Moclobemide and Phenelzine: Implications for the Development of Monoamine Oxidase Inhibitors

4.4 Overall Conclusions

4.5 Future Directions

4.6 Final Comment

5.0 REFERENCES
List of Tables

Table 1.1. Stages of Treatment Resistance in Major Depressive Disorder 11
Table 1.2. Summary of Literature on Cortical Thickness in Major Depressive Disorder 27
Table 1.3. Studies Investigating the Efficacy of Phenelzine or Moclobemide in MDE with Severe Symptoms or Atypical Symptoms 34
Table 1.4: Comparison of PET Radiotracers for Monoamine Oxidase A 41
Table 2.1. Moclobemide and Phenelzine Target Dose Selection 65
Table 3.1. Demographic and Clinical Characteristics of Individuals with Moderate to Severe and Mild to Moderate MDE 72
Table 3.2. Demographic and Clinical Characteristics of Individuals with and without Reversed Neurovegetative MDE Symptoms 73
Table 3.3. Demographic and Clinical Characteristics of Individuals with MDD and Healthy Controls 83
Table 3.4. Relationship of Hippocampal MAO-A $V_T$ and Hippocampal Volume in MDD and Healthy Controls 89
Table 3.5. Effect of Group on Hippocampal Volume 89
Table 3.6. The Relationship between MAO-A $V_T$ and Cortical Thickness in Prefrontal Cortex Subregions and Cingulate Gyrus 94
Table 3.7. The Effect of Group on Cortical Thickness in the Prefrontal Cortex and Cingulate Gyrus 94
Table 3.8. Demographics, Clinical History and Treatment Response for Treatment Groups 96
List of Figures

Figure 1.1. The mechanism of neurotoxicity induced by iron and hydrogen peroxide, via the Fenton reaction 18
Figure 1.2. MAO-A specific distribution volume (DVs) in the prefrontal cortex in individuals with a major depressive episode. 36
Figure 2.1. Study 3 study design. 63
Figure 3.1. The effect of severe MDE symptoms (HRSD ≥ 20) compared to mild to moderate MDE symptoms (HRSD ≤ 19) on MAO-A VT in regions of interest. 75
Figure 3.2. The effect of severe MDE symptoms (HRSD ≥ 20) compared to mild to moderate MDE symptoms (HRSD ≤ 19) on MAO-A VT in subregions of the prefrontal cortex. 76
Figure 3.3. The effect of reversed neurovegetative MDE symptoms compared to no reversed neurovegetative MDE symptoms on MAO-A VT in regions of interest. 78
Figure 3.4. The effect of reversed neurovegetative MDE symptoms compared to no reversed neurovegetative MDE symptoms on MAO-A VT in subregions of the prefrontal cortex. 79
Figure 3.5. The relationship of MAO-A VT with symptom severity as a continuous variable in the prefrontal and anterior cingulate cortices. 81
Figure 3.6. Hippocampal volume in those with a major depressive episode and healthy controls. 85
Figure 3.7. The relationship between monoamine oxidase-A occupancy and dose of moclobemide. 98
Figure 3.8. Monoamine oxidase-A occupancy is higher with high doses of moclobemide (900-1200mg) and phenelzine (45-60mg) compared to low doses of moclobemide (300-600mg). 100
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>Radiolabeled Carbon-11</td>
</tr>
<tr>
<td>Cmax</td>
<td>Maximum Concentration</td>
</tr>
<tr>
<td>CREB</td>
<td>cyclicAMP Response Element Binding Protein</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsal Lateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>DV$_s$</td>
<td>Specific Distribution Volume</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin Adenine Dinucleotide</td>
</tr>
<tr>
<td>GPO</td>
<td>Glutathione Peroxidase</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>HRSD</td>
<td>Hamilton Rating Scale for Depression</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibitory Concentration 50%, concentration of a substance to produce 50% inhibition of a marker biological process</td>
</tr>
<tr>
<td>MANOVA</td>
<td>Multivariate Analysis of Variance</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine Oxidase</td>
</tr>
<tr>
<td>MAO-A(B)</td>
<td>Monoamine Oxidase A(B)</td>
</tr>
<tr>
<td>MAOI</td>
<td>Monoamine Oxidase Inhibitors</td>
</tr>
<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
</tr>
<tr>
<td>MDE</td>
<td>Major Depressive Episode</td>
</tr>
<tr>
<td>mPFC</td>
<td>Medial Prefrontal Cortex</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbital frontal Cortex</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
</tr>
<tr>
<td>R1</td>
<td>Repressor 1</td>
</tr>
<tr>
<td>RIMA</td>
<td>Reversible Inhibitor of Monoamine Oxidase-A</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>TAC</td>
<td>Time Activity Curve</td>
</tr>
<tr>
<td>TIEG-2</td>
<td>TGF Beta Inducible Early Response Gene 2</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>VT</td>
<td>Total Distribution Volume</td>
</tr>
<tr>
<td>VLPFC</td>
<td>Ventral Lateral Prefrontal Cortex</td>
</tr>
</tbody>
</table>
Section 1.0 INTRODUCTION

1.1 Statement of the Problem

Major depressive disorder (MDD) is the leading cause of death and disability in mid to high income countries (WHO 2008). It is thought that by the year 2030 it will be the leading cause of death worldwide (WHO 2008). It has a high lifetime prevalence of approximately 20% and a large proportion (up to 40%) of individuals with MDD do not respond to first-line antidepressant treatment (Trivedi, Rush et al. 2006; Patten 2009). There are many potential pathologies that may lead to the presentation of MDD symptoms. An important method by which to improve the treatment of MDD is to identify targetable central biomarkers of underlying pathologies.

Monoamine Oxidase A (MAO-A) is a logical target to study as a contributor to the pathology of MDD since it plays a role in oxidative stress, apoptosis and metabolizing monoamines involved in mood, including serotonin, dopamine and norepinephrine (Jones, Pare et al. 1972; Ou, Chen et al. 2006). It has long been know that monoamines are involved in mood since depletion of monoamines can lead to low mood in otherwise healthy individuals and reoccurrence of depressive symptoms in those in remission (Young, Smith et al. 1985; Verhoeff, Christensen et al. 2003). Of note, all currently available antidepressants cause an increase in monoamines. MAO-A is also an interesting enzyme to study in the pathology of MDD since medications are available to directly target MAO-A (i.e. monoamine oxidase inhibitors (MAOIs)).
Elevated MAO-A levels in the brain in MDD is a robustly replicated finding (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Johnson, Stockmeier et al. 2011). In 2006, a $^{[11]}$C]harmine Positron Emission Tomography (PET) study found that patients experiencing a major depressive episode (MDE) had a 34% elevation in MAO-A specific distribution volume, an index of MAO-A density, compared to healthy controls (Meyer, Ginovart et al. 2006). This finding was further replicated in another PET study in 2009 (Meyer, Wilson et al. 2009) and has more recently been replicated in post mortem brain showing a 40% increase in MAO-A levels in the orbitofrontal cortex of individuals with MDD (Johnson, Stockmeier et al. 2011). To date, no studies have been conducted to determine whether there are certain subtypes of MDD that might have the greatest elevation in MAO-A levels.

Those with the greatest severity and reversed neurovegetative MDE symptoms have been historically shown to be more responsive to treatment with MAOIs (McGrath, Stewart et al. 1987; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993; Lonnqvist, Sihvo et al. 1994). Therefore, it might be expected that those with the greatest severity of depressive symptoms and those with MDE with reversed neurovegetative MDE symptoms might represent two subgroups with significantly elevated MAO-A levels in the brain. A retrospective data analysis using an existing data set in the Meyer et al., laboratory demonstrated in MDE subjects that a score of greater or equal to 20 on the 17-item Hamilton Rating Scale for Depression (HRSD) corresponded to a 15% elevation in the MAO-A $V_T$ in the prefrontal cortex relative to those MDE with a HRSD of less than
20 (specific subanalysis not published). If those that are historically most responsive to treatment with MAOIs do have the greatest increase in MAO-A $V_T$, this may suggest a better match of treatment to specific pathology.

Another highly replicated finding (demonstrated through both neuroimaging and postmortem techniques) in studying the pathology of MDD is decreased hippocampal volume (Bremner, Narayan et al. 2000; Campbell, Marriott et al. 2004; Stockmeier, Mahajan et al. 2004; Videbech and Ravnkilde 2004). For example, meta-analyses examining hippocampal volume in MDE patients and healthy controls show reductions in volume of approximately 4-9% of individuals with MDE (Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009). More recently, decreases in cortical thickness in MDD have been shown in the prefrontal and anterior cingulate cortices (Jarnum, Eskildsen et al. 2011; van Eijndhoven, van Wingen et al. 2013; Papmeyer, Giles et al. 2014). One of the most proposed mechanisms for decreases in volume in MDD is hypercortisolemia leading to a toxic environment. It has been shown that MAO-A expression is regulated by glucocorticoids and that a dexamethasone challenge can increase MAO-A levels (Slotkin, Seidler et al. 1998; Ou, Chen et al. 2006). Therefore, it is of interest to see whether there is a relationship between elevated MAO-A levels and common structural changes in MDD in order to better understand the pathology.

MAOIs are available to target MAO-A. They are currently underutilized medications although they have benefit in the treatment of MDD as well as neurodegenerative disorders, such as Parkinsons Disease (Youdim, Edmondson et al. 2006; Riederer and
MAOIs are associated with a great benefit in treatment response for those individuals with MDD that are more resistant to other medications (Thase, Frank et al. 1992; Quitkin, Stewart et al. 1993). Although in the past, these medications were associated with adverse events, mainly the tyramine reaction (where the ingestion of tyramine can lead to increases in blood pressure and hypertensive crisis), there is an initiative to develop novel forms of these medications, mainly prodrugs and medications that are highly selective for MAO-A and therefore avoid these side effects. To date, the MAO-A occupancy of these medications at a range of therapeutic doses has yet to be investigated. The dose-occupancy relationship of MAOIs may provide useful information as these medications may be a tool to aid in inhibiting multiple MDD pathologies (i.e. decreased monoamine availability and increased oxidative stress). There is important information that can be gained through the investigation of existing forms of these medications to inform the treatment of MDD with MAOIs as well as the development of novel MAOIs.

It may be that a number of different pathologies may lead to similar depressive symptoms in different individuals with MDD. Identification of potential central biomarkers and the relationship between them can lead to a better understanding of the heterogeneity of MDD pathology as well improved treatment of the disorder. Elevated MAO-A level may represent one example of a specific central biomarker of MDD by which depressive symptoms of particular subgroups of individuals is marked by this brain change. Structural brain changes may also represent a central biomarker of MDD and may be related to elevated MAO-A levels in the brain due to the role of MAO-A in apoptosis and
oxidative stress. Furthermore, identification of specific subgroups of individuals with the greatest elevation in MAO-A levels in the brain may suggest that these individuals may be best treated with MAOIs, thereby better matching the medication to the disease pathology.

1.2. Purpose of the study, objectives and hypotheses

1.2.1 Overall Thesis Purpose, Objective and Hypothesis

Purpose: To better understand the role of MAO-A in MDD by determining if MAO-A total distribution volume ($V_T$) is significantly elevated in MDE with specific symptoms, the relationship between MAO-A $V_T$ and structural changes in MDD, and assessing the targeting of MAO-A by MAOIs.

Objectives:

1. To identify potential subtypes of MDE with elevated MAO-A $V_T$.

2. To identify a relationship between elevated MAO-A $V_T$ and structural changes in MDD.

3. To assess the targeting of MAO-A by MAOIs.
Overall Hypothesis: MAO-A $V_T$ is significantly elevated in MDE with a high severity and reversed neurovegetative MDE symptoms, elevated MAO-A $V_T$ is correlated to decreases in hippocampal volume and prefrontal and anterior cingulate cortical thickness and currently available MAOIs at therapeutic doses target an occupancy of MAO-A of at least 80%.

1.2.2 Specific Research Objectives and Hypotheses

Study 1. MAO-A Elevation in MDEs with Severe or Reversed Neurovegetative Symptoms.

Specific Objective: To determine if those individuals with a high severity or reversed neurovegetative MDE symptoms have significantly greater elevation in MAO-A $V_T$.

Primary hypothesis: MAO-A $V_T$, an index of MAO-A density, will be elevated in the prefrontal and anterior cingulate cortices of individuals with severe and/or reversed neurovegetative MDE symptoms relative to those without these symptoms.

Secondary Hypothesis: MAO-A $V_T$ will be elevated in all regions sampled (including the thalamus, midbrain, hippocampus, putamen and ventral striatum, as well as subregions of the prefrontal cortex) in individuals with severe and/or reversed neurovegetative MDE symptoms compared to those without these symptoms.
Study 2. The Relationship Between MAO-A Total Distribution Volume, Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness

Specific Objective: To determine the relationship between elevated MAO-A $V_T$ and decreased hippocampal volume and prefrontal and anterior cingulate cortical thickness in those with MDD.

Primary Hypothesis: MAO-A $V_T$ will be inversely correlated to hippocampal volume and prefrontal and anterior cingulate cortical thickness in MDD.

Secondary Hypothesis: Hippocampal volume and prefrontal and anterior cingulate cortical thickness will be decreased in MDD relative to healthy controls.

Study 3. Dose-occupancy Relationship of Currently Available MAOIs

Specific Objective: To determine the dose-occupancy relationship of a selective, reversible inhibitor of MAO-A (moclobemide) and a non-selective, irreversible inhibitor of MAO (phenelzine).

Primary Hypothesis: There will be a dose-dependent decrease in MAO-A $V_T$ after 6 weeks of treatment with moclobemide and phenelzine across all brain regions sampled.
Secondary Hypothesis: Phenelzine and doses of moclobemide that require tyramine restrictions will have a higher MAO-A occupancy relative to average clinical doses of moclobemide.

1.3. Review of the Literature

1.3.1 Major Depressive Disorder

Major Depressive Disorder (MDD) is primarily defined by DSM-IV as the occurrence of low or sad mood and the loss of interest in activities normally enjoyed. A current major depressive episode (MDE) is defined as having the previously listed two symptoms in the past month and persisting for at least two weeks as well at least three of the following seven symptoms:

Changes in appetite resulting in weight loss or weight gain
Sleeping difficulties
Motor agitation or retardation
Low energy
Feelings of worthlessness
Problems with concentration, memory or decision making
Thoughts of death or a suicide plan

In order for the episode to meet diagnostic criteria as a MDE, it should not be due to bereavement and should impair normal social functioning (APA 2000). Recently, the DSM 5 has been released. Although most criterion for diagnosis of MDD have remained
the same, the criterion that the symptoms should not be due to bereavement has been excluded as to not overlook MDD at the time of bereavement (APA 2013).

MDD is recognized as the leading cause of death and disability in mid to high income countries and it is thought that within the next two decades it will be the leading cause of death and disability worldwide (WHO 2008). It is a very debilitating disorder associated with a prevalence of up to 20% worldwide, with a higher prevalence in females relative to males (Stephens and Joubert 2001; Patten 2009; Olchanski, McInnis Myers et al. 2013). It is also a very heterogeneous disorder, associated with a high rate of treatment resistance, where up to 40% of individuals do not respond to first-line monotherapy (Trivedi, Rush et al. 2006). The economic burden of MDD is very high, with those that are classified as treatment-resistant incurring approximately 29.3% higher medical costs (Olchanski, McInnis Myers et al. 2013).

1.3.1.1. Treatment Resistance in MDD

MDEs are considered treatment-resistant when individuals have inadequate response to a treatment trial and fail to achieve remission (Fava 2003). The mechanism by which people become treatment resistant is likely heterogeneous. There are thought to be a number of pathologies associated with MDD, ranging from decreases in the availability of monoamines associated with mood, to increased cytokines and decreased grey matter (Maes, Bosmans et al. 1997; Stockmeier, Mahajan et al. 2004; Neumeister, Wood et al. 2005; Ruhe, Mason et al. 2007; Schmidt, Shelton et al. 2011; Fagundes, Glaser et al.)
Treatment resistance may be influenced by a number of factors including but not limited to an inadequate treatment course (which may be more accurately termed pseudo resistance), non-adherence (up to 20%) (Souery, Amsterdam et al. 1999), family history of psychiatric illness (Milne, Caspi et al. 2009), co-morbidity with other psychiatric disorders (Souery, Oswald et al. 2007), number of previous MDEs (Souery, Oswald et al. 2007) or severity of MDE symptoms (Thase 2000; Souery, Oswald et al. 2007). Thase and Rush (Thase and Rush 1997) have proposed a stage level of treatment resistance as can be seen in table 1.1 (adapted from (Fava 2003)). Here, an adequate trial of an antidepressant is considered to be 12 weeks. Perhaps the best approach to overcoming the high level of treatment resistance associated with MDD is to identify potential biomarkers, either centrally or peripherally, of MDD subgroups in order to better match pathology on a subgroup level to the treatment. In this way, individuals could be stratified to the treatment course that has the greatest efficacy in that subpopulation of MDD.
### Table 1.1. Stages of Treatment Resistance in Major Depressive Disorder (adapted from (Thase and Rush 1997; Fava 2003))*

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Failure of at least one adequate trial of one class of antidepressant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td>Failure of 2 adequate trials of antidepressants from different classes</td>
</tr>
<tr>
<td></td>
<td>Failure of 2 adequate trials of antidepressants of different classes plus a</td>
</tr>
<tr>
<td></td>
<td>tricyclic antidepressant</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Failure of 2 adequate trials of antidepressants of different classes and a</td>
</tr>
<tr>
<td></td>
<td>tricyclic antidepressant plus a monoamine oxidase inhibitor</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Failure of an adequate trials of all classes of antidepressants plus</td>
</tr>
<tr>
<td></td>
<td>electroconvulsive therapy</td>
</tr>
</tbody>
</table>

*Reprinted from Biological Psychiatry, 53 (8) Fava et al., “Diagnosis and definition of treatment-resistant depression”, pp. 649-659, Copyright (2003), with permission from Elsevier.*
1.3.1.2 Pathologies of MDD

Although there are a number of neural mechanisms thought to be involved in the etiology of MDD, decreased monoamine availability in the brain was the first recognized theory (Jones, Pare et al. 1972). Early research showed that iproniazid, which inhibited the metabolism of monoamines, was capable of causing euphoric mood in individuals treated for tuberculosis (West and Dally 1959). The mechanism underlying this increase in euphoric mood was later found to be related to an increase in serotonin and norepinephrine levels in the brain. The role of monoamines in regulating mood has also been shown by monoamine depletion. Reserpine, which depletes monoamines by blocking the transport mechanism, produces depressive symptoms in otherwise healthy individuals (Freis 1954). Furthermore, tryptophan depletion of serotonin and alpha-methyl-p-tyrosine depletion of dopamine have been shown to lead to low mood in otherwise healthy individuals and to lead to a re-occurrence of symptoms in individuals with MDD that are in remission (Young, Smith et al. 1985; Leyton, Young et al. 1997; Leyton, Young et al. 1997; Berman, Narasimhan et al. 1999; Neumeister, Nugent et al. 2004; Hasler, Fromm et al. 2008). Neuroimaging data also suggested lowered monoamine availability in the brain (Meyer, Kruger et al. 2001; Ordway, Schenk et al. 2003; Meyer 2008). Furthermore, all currently available antidepressant medications result in an increase in monoamines in the brain. Until recently, the etiology of this loss of monoamines was not understood, but it has been found that a potential underlying mechanism for this decrease in available monoamines may be due to increased monoamine metabolism in the brain (Meyer, Ginovart et al. 2006; Barton, Esler et al. 2008; Johnson, Stockmeier et al. 2011).
Although all currently available antidepressant medications increase monoamines in the brain, it is well known that there is a lag in the relief of depressive symptoms following antidepressant treatment. Since the increase in monoamine availability following treatment is thought to be immediate, much research has focused on the potential neuroadaptations that take place following treatment (Gardier, Malagie et al. 1996; Feighner 1999; Schmidt, Warner-Schmidt et al. 2012; Able, Liu et al. 2014). It is thought that the elevation in monoamines caused by antidepressant medications may lead to secondary changes, such as changes in postsynaptic and presynaptic receptors (Gardier, Malagie et al. 1996; Serres, Millan et al. 2012; Gray, Milak et al. 2013), which are in turn responsible for restoring the monoamine balance long term and improving depressive symptoms. This lag in recovery from MDE after treatment also suggests the importance of a number of potential pathologies leading to the presence of depressive symptoms, where decreased monoamines may only represent a part of the pathology of MDD.

Since MDD is known to be a very heterogeneous disorder, it is logical that there be a number of pathologies contributing to the manifestation of the disease across individuals. Among many other factors, decreases in brain derived neurotrophic factor (BDNF) (mainly in the hippocampus) have been shown (Karege, Perret et al. 2002; Shimizu, Hashimoto et al. 2003; Castren and Rantamaki 2010), as well as decreases in markers of neural plasticity and anti-inflammatory response, such as cyclic AMP response element binding protein (CREB) (Yamada, Yamamoto et al. 2003), elevated cytokines and immune response, such as increases in TNF-alpha, interleukin-6 and C-reactive protein.
hyperactivity of the HPA-axis (Bardeleben and Holsboer 1989; Rush, Giles et al. 1997; Duval, Mokrani et al. 2001), and changes in glial cell density and grey matter (Rajkowska 2000; Stockmeier and Rajkowska 2004; Rajkowska and Stockmeier 2013).

In MDD, one of the most replicated findings, through Magnetic Resonance Imaging (MRI), is decreased hippocampal volume (Bremner, Narayan et al. 2000; MacQueen, Campbell et al. 2003; Sheline, Gado et al. 2003; Campbell, Marriott et al. 2004; Videbech and Ravnkilde 2004). The vast amount of human and animal literature highlighting the importance of decreased hippocampal volume in the pathology of MDD as well as existing literature suggesting increased hippocampal neurogenesis in the effectiveness of the treatment of MDD will be discussed in greater detail below (Malberg, Eisch et al. 2000; Kempermann and Kronenberg 2003; Jayatissa, Bisgaard et al. 2006; Warner-Schmidt and Duman 2006; Sahay and Hen 2007). Many of the above listed potential MDD pathologies can be altered with antidepressant treatment, such as the HPA-axis hyperactivity (Nemeroff and Owens 2004) and some changes in immune response (Hannestad, DellaGioia et al. 2011), pointing to the relationship between these pathologies and dysregulation of monoamines.

1.3.2 Monoamine Oxidase (MAO): A Player in MDD

Monoamine Oxidase (MAO) was first coined in the 1950s and was called tyramine oxidase, based on its ability to metabolize tyramine. It was first recognized for its role in metabolizing monoamines both neuronally and extraneuronally (likely in glial cells) but more recently recognized for its role in facilitating oxidative stress and apoptosis (Ou,
Chen et al. 2006; Fitzgerald, Ufer et al. 2007). There are two subtypes of MAO, MAO-A and MAO-B. These subtypes were identified based on their inhibitor sensitivities and substrate selectivities, where MAO-A primarily metabolizes serotonin and norepinephrine and is inhibited by clorgyline while MAO-B primarily metabolizes dopamine and phenelethylamine and is inhibited by benzylamine (Johnston 1968; O'Carroll, Bardsley et al. 1986; Konradi, Svoma et al. 1988). MAO-A and MAO-B are located on the outer mitochondrial membrane of the cell in both neuronal and non-neuronal cells, such as glia (Schnaitman, Erwin et al. 1967; Westlund, Denney et al. 1988; Saura, Kettler et al. 1992). Studies have shown that MAO-A and MAO-B are important in the pathology of a number of diseases, including not only mood disorders, such as MDD, but neurodegenerative disorders, such as Alzheimer’s disease (Zheng, Youdim et al. 2010; Bolea, Gella et al. 2012; Zheng, Amit et al. 2012).

In the brain, MAO-A is strongly localized in regions rich in noradrenergic neurons, such as the locus coeruleus and MAO-B has the highest density in serotonergic neurons, such as the dorsal raphe (Westlund, Denney et al. 1985). In the periphery, MAO-A is primarily located in the gastrointestinal tract and MAO-B is largely in the liver (Saura, Bleuel et al. 1996; Rodriguez, Saura et al. 2001). Interestingly, MAO-A and MAO-B in the brain are mainly localized to neurons which are not responsible for the metabolism of the monoamines contained within the neuron. Here, they are thought to function to prevent the entrance of false exogenous neurotransmitters (Youdim, Edmondson et al. 2006).
MAO-A and MAO-B share many similarities in structure but differences in their substrate selectivity and regulation highlight their importance in different disease states; whereas MAO-B is thought to be important in neurodegenerative disorder pathology, MAO-A plays a greater role in the pathology of MDD. MAO-A and MAO-B share 70% of their amino acid sequence identity (Cawthon, Pintar et al. 1981; Pintar, Barbosa et al. 1981). Differences in the binding sites of MAO-A and MAO-B allow for their substrate selectivity however, they both contain a flavin adenine dinucleotide (FAD) binding site and small amino acids changes in this binding site can switch the substrate selectivities (Geha, Rebrin et al. 2001; Edmondson, Binda et al. 2004). Furthermore, MAO-A and MAO-B are regulated differently. Whereas MAO-A is thought to be primarily regulated by hormones, such as estrogen, progesterone and glucocorticoids (Youdim, Banerjee et al. 1989; Gundlah, Lu et al. 2002; Ou, Chen et al. 2006), MAO-B is primarily regulated by second messenger pathways through protein kinase C and mitogen kinase pathways (Wong, Ou et al. 2002; Shih and Chen 2004). Importantly, MAO-A has binding sites for glucocorticoids located directly on the promoter region and glucocorticoids are capable of both directly and indirectly altering the transcription of MAO-A by directly acting at the MAO-A core promoter region and by decreasing the R1 repressor of and increasing the activator of MAO-A transcription (TIEG-2) (Ou, Chen et al. 2006; Grunewald, Johnson et al. 2012). The ability of glucocorticoids to regulate the transcription of MAO-A may further point to the greater importance of MAO-A over MAO-B in the pathology of MDD, since dysregulation of the HPA-axis is a known contributor to MDD pathology.
1.3.2.1 Functional Role of MAO-A

MAO-A facilitates oxidative stress and apoptosis and metabolizes serotonin, norepinephrine and dopamine. Through the metabolism of monoamines, MAO-A produces hydrogen peroxide, which when it reacts with iron, produces reactive oxygen species that lead directly to damage of the electron transport chain of mitochondrial DNA (Hauptmann, Grimsby et al. 1996). The resultant sequela of events leads not only to oxidative damage but may also lead to cell death (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007; Fitzgerald, Ugun-Klusek et al. 2014) (Figure 1.1). The role of MAO-A in relation to markers associated with apoptosis has been shown in human neuroblastoma cell lines, where inducing an apoptotic-like state increases MAO-A levels and leads to an increase in apoptotic markers, such as caspase 3 and induces apoptotic morphology of cells, an effect which can be blocked by inhibiting the enzyme (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007). Therefore, the role of MAO-A in contributing to monoamine dysregulation in MDD may be primarily due to its function in metabolizing monoamines involved in regulating mood, however, the role of MAO-A in facilitating oxidative stress and apoptosis may be linked to other underlying pathologies of MDD, such as structural changes (i.e. decreased hippocampal volume and cortical thickness).
Figure 1.1 The mechanism of neurotoxicity induced by iron and hydrogen peroxide, via the Fenton reaction (Adapted from Youdim et al., 2006)*. MAO produces hydrogen peroxide ($H_2O_2$) through the metabolism of monoamines. Normally the $H_2O_2$ is then inactivated by glutathione peroxidase (GPO) but it can be converted, chemically, by $Fe^{2+}$ ions (Fenton reaction) into the highly reactive hydroxyl radical, with consequent increases in oxidative damage to neurons. Inhibition of MAO decreases the formation of $H_2O_2$ and iron chelation removes the $Fe^{2+}$ ions, decreasing the formation of hydroxyl radical and the levels of oxidative stress (Youdim and Bakhle 2006).

1.3.2.2 Genetics of MAO-A

The genetic variations of MAO-A have provided insights into its functional role. The gene for MAO-A is located on the X-chromosome and there are variants of the MAO-A gene associated with decreased or increased expression (Sabol, Hu et al. 1998). The variant associated with lower expression is a two repeat allele and has been repeatedly implicated in aggressive behaviours (Manuck, Flory et al. 2000; Alia-Klein, Goldstein et al. 2008; Buckholtz and Meyer-Lindenberg 2008; McDermott, Tingley et al. 2009). A family study determined that those with this genetic variant show greater impulsive behaviour and more aggressive behaviour when provoked (Brunner, Nelen et al. 1993). This increased aggression associated with the two repeat variant of MAO-A is true across species; e.g. MAO-A knock-out mice also show increased aggressive behaviours as well as increased reactivity to stress and increased monoamines (Cases, Seif et al. 1995).

There is some literature to suggest that the MAO-A variant associated with increased expression may be associated with depressive symptoms (Schulze, Muller et al. 2000; Doornbos, Dijck-Brouwer et al. 2009). It has also been suggested that this variant may be more common in females rather than males in relation to disease state (Schulze, Muller et al. 2000). Specifically, post-partum women with the four repeat variant score greater on MDE rating scales relative to those without this allele (Doornbos, Dijck-Brouwer et al. 2009). Epigenetic variation of MAO-A may also be more prevalent in women compared to men (Pinsonneault, Papp et al. 2006), where methylation of the MAO-A gene may be associated with nicotine and alcohol dependence in women (Philibert, Gunter et al. 2008). Furthermore, the methylation status of the MAO-A promoter, as detected in white blood cells, may predict the brain MAO-A total distribution volume ($V_T$), as measured by
[\textsuperscript{11}C] clorgyline, in males, suggesting an interesting peripheral marker of central MAO-A (Shumay, Logan et al. 2012).

1.3.2.3 The Importance of MAO-A in MDD Pathology

The functional roles of MAO-A, including facilitating oxidative stress, the association to markers of apoptosis and regulation of monoamines are all thought to contribute to the etiology of MDD. This suggests the importance of investigating MAO-A in MDD. Decreased monoamine availability is considered an important mechanism leading to sad mood in MDD. Until recently, an explanation for lowered monoamines in MDD was not clear. Recently, it has been found through multiple techniques that MAO-A levels are elevated during a MDE (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Johnson, Stockmeier et al. 2011). Specifically, a 34% elevation in MAO-A specific distribution volume, a measure of MAO-A density in the brain, was found across the brain using [\textsuperscript{11}C]harmine PET (Meyer, Ginovart et al. 2006). This was further replicated showing an approximate increase of 25% of MAO-A \( V_T \) in individuals experiencing a MDE compared to healthy controls (Meyer, Wilson et al. 2009). This replication data was interesting because it showed that those individuals that went on to have a reoccurrence of depressive symptoms had the highest MAO-A \( V_T \) during the MDE, perhaps suggesting a subtype of MDE with greater elevation in MAO-A \( V_T \) (Meyer, Wilson et al. 2009). An elevation of MAO-A levels in post-mortem orbitofrontal cortex has also been shown in those that suffered from MDD (Johnson, Stockmeier et al. 2011). Interestingly, when comparing those that had previous treatment with a selective serotonin reuptake inhibitor to those without treatment, it was shown that MAO-A levels
did not differ, suggesting that treatment with a SSRI does not normalize elevated MAO-A levels in MDD (Johnson, Stockmeier et al. 2011). Data supporting elevated MAO-A levels suggests a greater serotonin turnover rate during MDE (Barton, Esler et al. 2008). However, whether the greatest elevation in MAO-A levels is related to the severity of the disease state or a particular subgroup of MDD is yet to be determined.

Elevated MAO-A $V_T$ has also been shown in other disease states associated with low mood, specifically, post-partum depression and nicotine and alcohol withdrawal (Sacher, Wilson et al. 2010; Bacher, Houle et al. 2011; Matthews, Kish et al. 2014). Using $^{[11]C}$harmine PET, Sacher and colleagues showed that at 4 days post-partum, following large decreases in estrogen and during a period at which depressive symptoms are likely, there is a spike in MAO-A $V_T$ of approximately 40% across the brain (Sacher, Wilson et al. 2010). Furthermore, using the same technique, MAO-A $V_T$ was shown to be elevated in both nicotine and alcohol withdrawal, two conditions during which depressive symptoms or low mood are often prominent and may be related to relapse to drug use (Bacher, Houle et al. 2011; Matthews, Kish et al. 2014). MAO-A $V_T$ elevation during a MDE and during low mood states represents a robust finding and may provide a potential explanation of the lowered monoamine availability thought to contribute to the pathology of MDD. Elevated MAO-A levels represents a pathology of MDD that can be targeted with existing therapeutic agents and therefore may be an interesting target to study towards a more personalized medicine approach to MDD.
1.3.2.4 Potential Mechanism of Elevated MAO-A Levels

There has been some research investigating potential mechanisms of elevated MAO-A levels. Specifically, it is known that MAO-A is primarily regulated through hormones, and recently it has been shown that glucocorticoids are capable directly regulating MAO-A levels (Ou, Chen et al. 2006; Ou, Chen et al. 2006). Since it is well known that a hyperactive HPA-axis is associated with MDD, increased glucocorticoids in MDD may be a likely mechanism by which MAO-A levels are elevated in MDE. In line with this, it has been shown that a dexamethasone challenge results in an elevation in MAO-A levels in rats and human skeletal muscle cells (Slotkin, Seidler et al. 1998; Manoli, Le et al. 2005). There are glucocorticoid binding sites located on the MAO-A promoter and glucocorticoid administration can lead to increased MAO-A transcription as well as a decrease in R1 (repressor of MAO-A transcription) and an increase in TIEG-2 (activator of MAO-A transcription) (Ou, Chen et al. 2006; Grunewald, Johnson et al. 2012).

Furthermore, inducing apoptosis in human neuroblastoma cell lines through serum starvation results in an increase in MAO-A mRNA and catalytic activity and increasing MAO-A level can in turn increase markers associated with apoptosis, an effect that is blocked by inhibiting the enzyme (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007). The potential role of MAO-A in apoptosis may also suggest a possible relationship to decreased grey matter volume seen in MDD.
1.3.3 Structural Brain Changes in MDD Pathology

1.3.3.1 Decreased Grey Matter and Neurogenesis in MDD Pathology

Decreased grey matter volume, particularly in the hippocampus, is a highly replicated finding in MDD (Vakili, Pillay et al. 2000; Campbell, Marriott et al. 2004; Videbech and Ravnilde 2004; McKinnon, Yucel et al. 2009). Interpretation of the existing animal research suggests that hippocampal volume and neurogenesis is decreased in animal models of MDD (Czeh, Michaelis et al. 2001; Malberg and Duman 2003).

Antidepressant treatment may increase neurogenesis and treatment efficacy may rely on this increase (Magarinos, Declandes et al. 1999; Malberg, Eisch et al. 2000; Santarelli, Saxe et al. 2003; Li, Zhang et al. 2004; Dranovksy and Hen 2006; Song and Wang 2011). In animal models of MDD, animals that were treated with antidepressants had increases in hippocampal volume compared to those that were untreated, which may point to a neuroprotective effect of antidepressants (Malberg and Duman 2003; Santarelli, Saxe et al. 2003). Using a chronic mild stress rat model of MDE, where a number of depressive symptoms are exhibited, including anhedonia (as expressed by a decrease in sucrose consumption), a decrease in hippocampal cytogenesis has been demonstrated (Jayatissa, Bisgaard et al. 2006). Following treatment with escitalopram, this decrease in cytogenesis was reversed, but only in animals that recovered from the depressive symptoms, suggesting that lack of cytogenesis is associated with treatment resistance (Jayatissa, Bisgaard et al. 2006).
Decreased hippocampal volume has been highly replicated in human neuroimaging and post-mortem studies as well (Bremner, Narayan et al. 2000; Sheline, Gado et al. 2003; Campbell and Macqueen 2004; Campbell, Marriott et al. 2004; Stockmeier, Mahajan et al. 2004; Stockmeier and Rajkowska 2004; Videbech and Ravnkilde 2004; Neumeister, Wood et al. 2005; Cobb, Simpson et al. 2013; Rajkowska and Stockmeier 2013). For example, a meta-analysis of 32 MRI studies examining hippocampal volume in MDE patients and healthy controls showed a reduction in volume of approximately 4% of individuals with MDD (McKinnon, Yucel et al. 2009). The decreased hippocampal volume seen in MDD has been shown to be correlated with a number of factors, such as duration of illness (Sheline, Wang et al. 1996; Sheline, Sanghavi et al. 1999), severity of illness (Vakili, Pillay et al. 2000) and number of previous episodes (Videbech and Ravnkilde 2004). Moreover, MDEs left untreated may also be associated with reductions in hippocampal volumes (Sheline, Gado et al. 2003), suggesting that antidepressants may have a neuroprotective effect, congruent with research in animal models of MDD. Post-mortem research has given insights into the exact cellular changes associated with hippocampal atrophy (Stockmeier, Mahajan et al. 2004). Specifically, hippocampal volumes in individuals with MDD were seen to be reduced by up to 18% compared to healthy controls (Stockmeier, Mahajan et al. 2004). Despite the atrophy seen in the hippocampus, the packing density of glia, pyramidal neurons, granules cell neurons and neuron soma size are significantly increased in MDD (Stockmeier, Mahajan et al. 2004). These findings suggest that a possible reduction in the neuropil rather than a decrease in the number of neurons may account for the observed decrease in hippocampal volume (Stockmeier, Mahajan et al. 2004).
The exact mechanism by which hippocampal volume is reduced in MDD is not clear. Much research has implicated the role of BDNF, since it is decreased in the hippocampus in MDD, suggesting a decrease in neurogenesis (Shimizu, Hashimoto et al. 2003). It has also been suggested that lowered hippocampal volume may be due to dysregulation of the HPA-axis and the resultant increases in circulating stress hormones may lead to oxidative damage and cell loss in the brain (Dranovsky and Hen 2006). The suggestion that increased activity of the HPA-axis may be related to decreases in hippocampal volume in MDD may point to a relationship between structural brain changes and increased MAO-A levels. To date, there has been no investigation towards a potential relationship between these two pathologies of MDD.

### 1.3.3.2 Changes in Cortical Thickness in MDD

Measures of cortical thickness obtained from MR images can provide interesting structural information about brain regions often implicated in MDD, such as the PFC and ACC. To date, not much research has investigated changes in cortical thickness in MDD. The most consistent findings show decreased cortical thickness in the prefrontal regions, such as the medial orbital frontal cortex, and the anterior cingulate cortex (table 1.2) (Peterson, Warner et al. 2009; Jarnum, Eskildsen et al. 2011; Tu, Chen et al. 2012; van Eijndhoven, van Wingen et al. 2013; Papmeyer, Giles et al. 2014). Interestingly, the regions that show most consistent decreases in cortical thickness are also the regions that are repeatedly and most consistently shown to have the greatest elevations in MAO-A levels in MDD (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Johnson,
Stockmeier et al. 2011). It is possible that there is a relationship between decreased
cortical thickness in the prefrontal cortex and anterior cingulate cortex and elevated
MAO-A levels, due to the role of MAO-A in oxidative stress.
Table 1.2. Summary of Literature on Cortical Thickness in Major Depressive Disorder

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of Participants</th>
<th>Main Finding</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Peterson, Warner et al. 2009)</td>
<td>131 with (n=66) and without (n=65) family history (FH+ v. FH-) of MDD</td>
<td>-Decreased cortical thickness in right hemisphere by 28% in FH+ v. FH-&lt;br&gt;-Increased in ACC and PCC in left hemisphere&lt;br&gt;-Decreased in left mOFC</td>
<td>N/A</td>
</tr>
<tr>
<td>(Jarnum, Eskildsen et al. 2011)</td>
<td>23 MDE, 26 healthy controls</td>
<td>-Decreased in OFC MDD v. HC&lt;br&gt;-Decreased PCC non-remitters v. remitters</td>
<td>N/A</td>
</tr>
<tr>
<td>(Tu, Chen et al. 2012)</td>
<td>36 MDD, 36 healthy controls</td>
<td>-Largest decrease in PFC</td>
<td>-No. of episodes (&gt;4 v. &lt;4) inversely correlated to cortical thickness in left PFC&lt;br&gt;-No correlation to symptom severity or duration of illness</td>
</tr>
<tr>
<td>(van Eijndhoven, van Wingen et al. 2013)</td>
<td>20 medication naive, 20 medication free, recovered, 31 healthy controls</td>
<td>-Decreased in left mOFC&lt;br&gt;-Increased in ACC and PCC</td>
<td>Symptom severity inversely correlated to left mOFC cortical thickness</td>
</tr>
</tbody>
</table>
1.3.4 Pharmacological Treatments of MDD

A number or treatments for MDD exist, including pharmacological treatments, such as tricyclic antidepressants, SSRIs and MAOIs and non-pharmacological treatments, such as cognitive behavioural therapy and interpersonal therapy. Each type of pharmacological treatment has been proven effective in treating MDD, although studies have shown differences in effectiveness across subtypes of MDE (Thase, Frank et al. 1992; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993). It is known that SSRIs do not normalize MAO-A levels in the brain (Meyer, Wilson et al. 2009; Johnson, Stockmeier et al. 2011). Therefore, MAOIs may represent a viable treatment option to target the functional consequences of elevated MAO-A levels in the brain, including not only the metabolism of monoamines, but increased oxidative stress.

1.3.4.1 Monoamine Oxidase Inhibitors

MAOIs were the first developed antidepressant medications and their discovery was serendipitous. As previously mentioned, the first MAOI was originally a treatment for tuberculosis and its antidepressant effects were recognized when patients being treated with this medication reported euphoric mood (Bloch, Dooneief et al. 1954; Loomer, Saunders et al. 1957). On this basis, MAOIs were thought to be a novel treatment for MDD. However, at the time, it was noted that these medications also caused severe adverse events such as liver toxicity, hypertensive crisis (“cheese reaction”), and hemorrhage and therefore the use of MAOIs was discontinued until such time as MAOIs
without these adverse effects were developed. At the present time, both irreversible, nonselective inhibitors (phenelzine and tranylcypromine) and reversible, selective inhibitors (moclobemide and selegeline) exist.

1.3.4.2 The Tyramine Reaction of MAOIs Leading to the Underutilization

The “cheese reaction” is a result of the inability of MAO to break down tyramine during irreversible, unselective blockade. When both MAO-A and MAO-B are irreversibly blocked there may be a buildup of tyramine from consuming certain foods high in this amine (such as aged cheese). Tyramine is a potent releaser of norepinephrine and can therefore elevate blood pressure when a high amount is ingested (McCabe and Tsuang 1982; Magyar, Szatmary et al. 2007). Therefore, patients taking older generation MAOIs (such as phenelzine or tranylcypromine) to treat their depressive symptoms must be maintained on a special diet in order to avoid these side effects. Some newer MAOIs (i.e. selective and reversible) are not associated with these severe side effects at average therapeutic doses. There is an initiative to develop more well-tolerated MAOIs that avoid the tyramine effect such that they have high selectivity for MAO-A or B or they are pro-drugs that are metabolized in the brain and by-pass the peripheral effects.

Despite the proven efficacy of MAOIs they remain highly under-prescribed (Hemels, Koren et al. 2002). Recently published Canadian data has shown that over a ten year period there were only 348 new users of irreversible MAOIs (Shulman, Fischer et al. 2009). In addition, although the prescription rate of antidepressants increased
tremendously between the years of 1981 to 2000, MAOIs were only prescribed at a rate of 2.1% of all antidepressant medications in Canada (Hemels, Koren et al. 2002). Further, over a 10-year period (from 1997 to 2007) the yearly incidence of MAOI prescriptions remained low and also decreased from 3.1/100,000 to 1.4/100,000, which is contrary to some recommendations (Shulman, Fischer et al. 2009). This may be because many clinicians and patients do not feel that the risk to benefit ratio of an MAOI is advantageous. However, current advances in the development of novel MAOIs point to medications that obviate the necessity for dietary restrictions, thus maximizing the benefits and decreasing the risks associated with these medications.

1.3.4.3 Efficacy of Phenelzine and Moclobemide Compared to Other Antidepressants in Treating MDD

Phenelzine is an irreversible, nonselective, inhibitor of MAO and is therefore associated with dietary restrictions but is a very efficacious antidepressant (McGrath, Stewart et al. 1987; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993; Birkenhager, van den Broek et al. 2004). Specifically, it has been shown that phenelzine has at least equal efficacy when compared to tricyclic antidepressants such as imipramine, a finding which has been reported in several studies (McGrath, Stewart et al. 1987; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993). Unfortunately, since the discovery of SSRIs, phenelzine has been a largely underutilized treatment for MDD. There is evidence to suggest that the efficacy of phenelzine is at least approximately equal to commonly prescribed SSRIs (Pande, Birkett et al. 1996) and a large benefit to treatment of MDD with phenelzine is that it is thought to be quite efficacious at treating more treatment-
resistant MDE cases and MDE with atypical features (i.e. increased appetite or hyperphagia and increased sleeping or hypersomnia) (McGrath, Stewart et al. 1987; Thase, Frank et al. 1992; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993).

Moclobemide is a reversible inhibitor that is selective at MAO-A. Unlike the irreversible MAOIs, reversible inhibitors of MAO-A (RIMAs) do not bind MAO-A covalently, instead, they are displaced from MAO-A when high concentrations of tyramine are present and therefore do not necessitate dietary restrictions at average therapeutic doses (Riederer, Laux et al. 1988; Versiani, Nardi et al. 1990). It has been shown that RIMAs are effective antidepressants with milder side effects than irreversible MAOIs but may be equally as effective at treating MDD as irreversible MAOIs, as well as tricyclics, such as imipramine and clomipramine and SSRIs, such as fluoxetine (Gabelic and Kuhn 1990; Lecrubier and Guelfi 1990; Rossel and Moll 1990; Angst, Amrein et al. 1995; Lapiere, Joffe et al. 1997; Lotufo-Neto, Trivedi et al. 1999).

1.3.4 Efficacy of Phenelzine and Moclobemide in Treating Subtypes of MDD

Phenelzine and moclobemide may be effective in treating more treatment-resistant cases of MDD and MDE with atypical-type symptoms (see Table 1.3 below) (McGrath, Stewart et al. 1987; Thase, Frank et al. 1992; McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993). In a sample of atypical-type MDD, there was a 67% response rate to phenelzine and only a 43% response rate to imipramine (Liebowitz, Quitkin et al. 1984). Furthermore, data has shown that those that have the greatest severity of MDE symptoms
also have the greatest response to treatment with phenelzine (Thase, Frank et al. 1992). Similarly, moclobemide may be more effective in treating MDE with atypical-type symptoms. When comparing moclobemide and fluoxetine, those individuals with atypical-type symptoms had a greater response to moclobemide as measured by the clinical global rating scale (Lonnqvist, Sihvo et al. 1994). Interestingly, the symptoms that most improved with treatment were reversed neurovegetative features (i.e. increased sleeping and increased appetite) (Lonnqvist, Sihvo et al. 1994). The etiology of reversed neurovegetative features of MDE is not well established but it has been suggested that the severity of symptoms is associated with an inverse correlation to plasma cortisol and decreased serum TNF-alpha and interleukin-6, which is opposite to MDE with melancholic features (Karlovic, Serretti et al. 2012).

The explanation as to why these medications may be more efficacious in treating these subgroups of MDE is not known. In line with the existing literature, exploration of an existing data set taken from Meyer and colleagues showed that MDE patients with a score of greater or equal to 20 on the 17-item HRSD had a 15% elevation in the MAO-A V_T in the prefrontal cortex relative to those MDE with a HRSD of less than 20 (Figure 1.2, data taken from Meyer et al., 2006, specific subanalysis not published). These data, in combination with existing literature suggesting that individuals with severe or atypical-type MDE symptoms show greater response to treatment with MAOIs, may suggest that significantly elevated MAO-A levels may be responsible for the increased responsiveness of these individuals to MAOIs. It is possible that MDD with a greater severity or atypical-type symptoms of MDD may represent two subgroups of MDD in which
elevated MAO-A may serve as a central biomarker and therefore by treating these individuals with a MAOI, the treatment is being better matched to the pathology.
<table>
<thead>
<tr>
<th>Author</th>
<th>Number of participants (completers)</th>
<th>Trial Design</th>
<th>Treatment</th>
<th>Significant Predictor</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Asgharnejad 2010) (Abstract)</td>
<td>285(235)</td>
<td>Double blind placebo-controlled trial</td>
<td>CX157 (TriRima) (180mg)</td>
<td>No</td>
<td>Most effective in those with the most severe symptoms</td>
</tr>
<tr>
<td>(Sogaard, Lane et al. 1999)</td>
<td>218 (197)</td>
<td>Double blind trial</td>
<td>Moclobemide (mean: 410.2mg) v. Sertraline (mean: 83.1mg)</td>
<td>Yes</td>
<td>Both improved symptoms of MDE (HRSD) but 77.5% sertraline ~ 67.5% moclobemide on CGI-I</td>
</tr>
<tr>
<td>(Pande, Birkett et al. 1996)</td>
<td>42 (38)</td>
<td>Double blind trial</td>
<td>Phenelzine (45-90mg) v. Fluoxetine (20-60mg)</td>
<td>Yes</td>
<td>Phenelzine = fluoxetine</td>
</tr>
<tr>
<td>(Lonnqvist, Sihvo et al. 1994)</td>
<td>209 (n=53 atypical, n=156 other)</td>
<td>Double blind trial</td>
<td>Moclobemide (300-450mg) vs. Fluoxetine (20-40mg)</td>
<td>Yes</td>
<td>Moclobemide &gt; fluoxetine</td>
</tr>
<tr>
<td>(McGrath, Stewart et al. 1993)</td>
<td>89</td>
<td>Double blind crossover</td>
<td>Phenelzine (75mg) v. Imipramine (274mg)</td>
<td>Yes</td>
<td>Phenelzine &gt; imipramine</td>
</tr>
<tr>
<td>(Thase, Frank et al. 1992)</td>
<td>42 (40)</td>
<td>Open label trial</td>
<td>Phenelzine (60mg) OR Tranylcypromine (20-60mg, mean: 38.5mg)</td>
<td>Yes</td>
<td>Reversed vegetative symptoms, severity and treatment resistance predict response to MAOIs</td>
</tr>
<tr>
<td>(Quitkin, Harrison et al. 1991)</td>
<td>86 (64)</td>
<td>Double blind trial</td>
<td>Phenelzine (45-60mg) v. Imipramine (150-200mg)</td>
<td>Yes</td>
<td>Phenelzine &gt; imipramine</td>
</tr>
<tr>
<td>(Quitkin, McGrath et al. 1990)</td>
<td>115 (90)</td>
<td>Double blind trial</td>
<td>Phenelzine (60-90mg) v. Imipramine (200-300mg)</td>
<td>Yes</td>
<td>83% Phenelzine &gt; 50% imipramine (Replication of n=120)</td>
</tr>
<tr>
<td>Author</td>
<td>Number of participants (completers)</td>
<td>Trial Design</td>
<td>Treatment</td>
<td>Significant Predictor</td>
<td>Finding</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------------------------------</td>
<td>--------------</td>
<td>------------------------------------</td>
<td>-----------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Quitkin, McGrath et al. 1989)</td>
<td>80 (60)</td>
<td>Double blind trial</td>
<td>Phenelzine (60-90mg v. Imipramine 200-300mg)</td>
<td>Mood reactivity v. Atypical</td>
<td>No  Phenelzine &gt; imipramine (atypical MDE) Phenelzine = imipramine (mood reactivity)</td>
</tr>
<tr>
<td>(Quitkin, Stewart et al. 1988)</td>
<td>74 (60)</td>
<td>Double blind trial</td>
<td>Phenelzine (60-90mg mean: 74mg) v. Imipramine (200-300mg mean:277mg)</td>
<td>Reactive mood + one associated symptom</td>
<td>No  Phenelzine &gt; imipramine</td>
</tr>
<tr>
<td>(Liebowitz, Quitkin et al. 1988)</td>
<td>163 (19)</td>
<td>Double blind trial</td>
<td>Phenelzine (60mg) vs. Imipramine (200mg)</td>
<td>Yes</td>
<td>No  71% phenelzine &gt; 50% imipramine</td>
</tr>
<tr>
<td>(Liebowitz, Quitkin et al. 1984)</td>
<td>106 (60) (preliminary report to 1988 publication)</td>
<td>Double blind trial</td>
<td>Phenelzine (60-90mg) v. Imipramine (200-300mg)</td>
<td>Yes + panic attacks or hysteroid dysphoric features</td>
<td>No  Phenelzine &gt; imipramine</td>
</tr>
</tbody>
</table>
Figure 1.2. MAO-A specific distribution volume (DV) s in the prefrontal cortex in individuals with a major depressive episode. Those with a higher severity of MDE symptoms as rated by a score of greater or equal to 20 on the 17-item Hamilton Rating Scale for Depression (HRSD) had significantly high MAO-A DVs compared to those with a lower severity of depressive symptoms (p<0.04).

1.3.4.5 Pharmacokinetics of Phenelzine and Moclobemide

Phenelzine is an irreversible, non-selective inhibitor of MAO. It has approximately equal affinity for MAO-A (IC50 of 0.015uM) and MAO-B (0.033uM) with slightly greater preference for MAO-A (Kettler, Da Prada et al. 1990). Phenelzine undergoes first-pass hepatic metabolism and reaches Cmax in 2-4 hours, with the plasma steady state concentration increasing over chronic dosing (Mallinger and Smith 1991). It has an elimination half-life of approximately 12 hours but its action in the brain lasts much longer
(up to 2 weeks) due to its irreversible mechanism of action (Robinson 1985). Not only is phenelzine an inhibitor or MAO, but it is also a substrate, thereby operating on zero-order kinetics and allowing for bioaccumulation (Robinson, Nies et al. 1978).

Moclobemide is a reversible inhibitor, which is selective at MAO-A (Haefely, Burkard et al. 1992). Moclobemide is thought to be a relatively weak MAO-A inhibitor in vitro, however, it is more potent in vivo than other reversible inhibitors (Kettler, Da Prada et al. 1990). It has low in vitro affinity for MAO-A, with an IC50 of approximately 6μM, however, the effect of moclobemide in the brain persists for up to 16 hours (Kettler, Da Prada et al. 1990; Haefely, Burkard et al. 1992; Haefely, Burkard et al. 1993). It is thought that moclobemide causes a conformational change in MAO-A, thereby allowing for its effect in vivo to be stronger (Haefely, Burkard et al. 1993; Abell and Kwan 2001). The elimination half-life of moclobemide is approximately 2-4 hours and is mainly renal (Gram, Guentert et al. 1995). The bioavailability of moclobemide is 60% after the first dose and increases to over 80% after repeated doses. Moclobemide is primarily metabolized by CYP2C19 and therefore its metabolism may be susceptible to genetic variation (Gram, Guentert et al. 1995).

1.3.4.6 Pharmacodynamics of Phenelzine and Moclobemide

Until recently, it was not possible to study the action of MAOIs in vivo in humans due to the lack of available brain imaging techniques and therefore the brain occupancy of MAO-A at a range of clinical doses of MAOIs was not known. Occupancy data has been demonstrated for SSRIs as well as some antipsychotics (Kapur, Zipursky et al. 2000; Meyer, Wilson et al.
For five commonly used SSRIs, there is an 80% occupancy of the serotonin transporter in the striatum at doses that are clinically significant from placebo. This finding has important implications for treating with an SSRI and the development of novel SSRIs. Since there is robust evidence implicating MAO-A and its functional consequences as a strong pathology in MDD and there is an initiative for the development of novel MAOIs, phenelzine and moclobemide give an opportunity to study the pharmacodynamics of two pharmacologically different but functionally similar medications. This can provide important information necessary for treatment and the development of novel medications of this type. This information can also improve the treatment of MDD with currently available MAOIs by understanding the dose-occupancy relationship. Since it has been shown that certain patients are more responsive to MAOIs, identification of the optimal MAOI occupancy may provide an improved treatment algorithm for these individuals. Identification of the optimal MAO-A occupancy of MAOIs and improving treatment with these medications may allow for better targeting of multiple MDD pathologies since the functional consequences of elevated MAO-A levels in MDD may be related to other MDD pathologies, such as increased oxidative stress.

1.3.5 Target Imaging Through Positron Emission Tomography (PET)

PET is a versatile technique that can be used to directly measure indices related to neurochemical, receptor, enzyme and drug actions in the living brain (Fowler, Volkow et al. 1999; Phelps 2000). This technique can provide substantial benefits towards understanding the neurochemistry involved in psychiatric disorders.
PET relies on the radiochemical properties of positron emitting isotopes combined with a drug known to bind at the target of interest, such as $^{11}$C-harmine at MAO-A. These isotopes can be injected into volunteers to allow for the visualization of the target in vivo. The decay of these radiochemicals results in the emission of positrons through gamma decay, which in turn collide with electrons, in time, resulting in the production of two photons that travel in opposite directions (for review see: Phelps 2000; Lindsey, Gatley et al. 2003). These photons are detected by the PET camera and following reconstruction and correction of the resultant image, can allow for the determination of the location and quantitation of the target of interest (Phelps 2000). This is determined using a small amount of the radiolabeled drug (tracer dose) which results in occupancy of a low proportion of the receptor population (Mintun, Raichle et al. 1984; Innis, Cunningham et al. 2007) and does not result in pharmacological effects.

Since PET samples only a portion of the receptor population of interest in vivo, a number of assumptions must be considered when calculating the occupancy of the radiotracer at the target of interest and involves sophisticated kinetic modeling and simulations (Ichise, Meyer et al. 2001; Meyer and Ichise 2001). Kinetic models are developed with the production of each reliable PET tracer. The kinetic model that best suits each tracer often becomes the gold standard of image analysis specific to quantitating the target of interest for that tracer. Many reviews have been published on this topic and cover in-depth the assumptions necessary for the final outcome of which model best suits the radiotracer (Ichise, Meyer et al. 2001; Meyer and Ichise 2001). $^{11}$C-harmine quantitation is best exemplified by an
unconstrained 2-tissue compartment model to which the Logan plot is highly correlated and can be used to quantitate MAO-A binding in the brain (Logan, Fowler et al. 1990; Ginovart, Meyer et al. 2006).

1.3.5.1 Imaging MAO-A using PET

$^{[11}C]$ harmine PET is thought to be the gold standard in quantification of MAO-A in vivo, although other tracers do exist, such as $^{[11}C]$-clorgyline and $^{[11}C]$-befloxatone (see Table 1.4). The ideal PET tracer has high affinity and is selective for the target, is reversible at the target site, and has metabolites that do not cross the blood brain barrier as to not interfere with the measurement (Tweedie and Burke 1987; Bergstrom, Westerberg et al. 1997; Bergstrom, Westerberg et al. 1997). $^{[11}C]$harmine exemplifies these qualities for MAO-A. $^{[11}C]$harmine has high affinity for MAO-A ($K_i=2nM$) and it is selective for MAO-A (Tweedie and Burke 1987; Bergstrom, Westerberg et al. 1997; Bergstrom, Westerberg et al. 1997). No known metabolites of harmine cross the blood brain barrier and it is highly reversible at MAO-A (Bergstrom, Westerberg et al. 1997). The reversibility of a tracer allows for easier, more reliable and more stable quantitation of the target. Irreversibility of a tracer allows the radiochemical to be susceptible to influences of changes in blood flow and therefore can less reliably predict the outcome, especially in situations where blood flow changes are expected (i.e. disease states) (Ichise, Meyer et al. 2001; Meyer and Ichise 2001).
Table 1.4: Comparison of PET Radiotracers for Monoamine Oxidase A*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selectivity</strong></td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
<tr>
<td><strong>Reversibility</strong></td>
<td>not reversible</td>
<td>highly reversible</td>
<td>highly reversible</td>
</tr>
<tr>
<td></td>
<td>(Fowler, MacGregor et al. 1987)</td>
<td>(Ginovart, Meyer et al. 2006)</td>
<td>(Bottlaender, Valette et al. 2010)</td>
</tr>
<tr>
<td><strong>Modeling</strong></td>
<td>2 tissue compartment</td>
<td>2 tissue compartment</td>
<td>2 tissue compartment</td>
</tr>
<tr>
<td></td>
<td>(Fowler, MacGregor et al. 1987)</td>
<td>(Ginovart, Meyer et al. 2006)</td>
<td>(Bottlaender, Valette et al. 2010)</td>
</tr>
<tr>
<td><strong>Reliability</strong></td>
<td>very good</td>
<td>Excellent</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td>(Fowler, Volkow et al. 1996)</td>
<td>(Sacher, Rabiner et al.)</td>
<td></td>
</tr>
<tr>
<td><strong>Metabolites Crossing the Blood Brain Barrier?</strong></td>
<td>unlikely</td>
<td>no brain penetrant metabolites</td>
<td>Unlikely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Wilson, Meyer et al. 2003)</td>
<td></td>
</tr>
</tbody>
</table>

*Reproduced with permission from Springer Science and Business Media and Springer-Verlag Berlin Heidelberg, Copyright (2014), p713, Chapter 25. Monoamine Oxidase A and Serotonin Transporter Imaging, Jeffrey H. Meyer, Figure 25.1.
1.3.5.2 Utilizing Neuroimaging to Identify Central Biomarkers of MDD: Matching Treatment to Pathology

A very important approach toward the treatment of psychiatric disorders in general is to recognize the heterogeneity within each disorder and better match treatment to the pathology. Identifying biomarkers of particular disease subtypes would be ideal for reaching this goal (Holsboer 2008). A potential approach to match treatment to specific pathology is to identify central biomarkers, using neuroimaging, that are associated with easily identifiable clinical measures and have already existing treatments to target the process. For example, it has been shown that individuals who have the melancholic subtype of MDE have increased GABA and decreased glutamate as demonstrated through magnetic resonance spectroscopy (Sanacora, Gueorguieva et al. 2004). However, treatments that directly target this change are not available, and in order to advance the approach to personalized treatment in MDD it may be that focusing on biomarker abnormalities that can be treated with currently available medications may be the best approach.

Fortunately, MAOIs currently exist to target MAO-A in the brain. To date, no research has been done towards optimizing the treatment of MDD with these medications. The best approach to this would be to identify clinical predictors associated with increased MAO-A and define the dose-occupancy relationship of currently available MAOIs in order to optimize treatment and minimize side effect with these medications. The dose-occupancy information would also inform the initiative for the development of novel MAOIs. Furthermore, it is known that individuals with a greater severity of MDE and atypical type
symptoms of MDE show a greater response to treatment with MAOIs. In line with this, as previously mentioned, it was noted in a previously collected data set, that individuals with a higher severity of MDE symptoms had a significant 15% elevation in the MAO-A $V_T$ in the prefrontal cortex (data examined from Meyer et al., 2006, specific subanalysis not published). $[^{11}C]$harmine PET allows for the investigation into a potential relationship between these subgroups of MDD and the well-known pathology of elevated MAO-A levels in the brain. Finally, by combining multiple imaging techniques, it is possible to determine a potential relationship between the replicated findings of elevated MAO-A level in the brain and structural brain changes (i.e. decreased hippocampal volume and cortical thickness) since these biomarkers may share underlying pathology. Determining clinical predictors of elevated MAO-A, determining the optimal MAO-A occupancy of MAOIs and understanding the relationship between increased MAO-A $V_T$ and structural changes in MDD can lead to a more personalized approach to the treatment of MDD with MAOIs.

1.4. Restatement of purpose, objectives and hypotheses

1.4.1 Overall Thesis Purpose, Objective and Hypothesis

*Purpose:* To better understand the role of MAO-A in MDD by determining if MAO-A total distribution volume ($V_T$) is significantly elevated in MDE with specific symptoms, the relationship between MAO-A $V_T$ and structural changes in MDD and assessing the targeting of MAO-A by MAOIs.
Objectives:

1. To identify potential subtypes of MDE with elevated MAO-A $V_T$.

2. To identify a relationship between elevated MAO-A $V_T$ and structural changes in MDD.

3. To assess the targeting of MAO-A by MAOIs.

Overall Hypothesis: MAO-A $V_T$ is significantly elevated in MDE with a high severity and reversed neurovegetative MDE symptoms, elevated MAO-A $V_T$ is correlated to decreases in hippocampal volume and prefrontal and anterior cingulate cortical thickness and currently available MAOIs at therapeutic doses target an occupancy of MAO-A of at least 80%.

1.4.2 Specific Research Objectives and Hypotheses

Study 1. MAO-A Elevation in MDEs with Severe or Reversed Neurovegetative Symptoms

Specific Objective: To determine if those individuals with a high severity or reversed neurovegetative MDE symptoms have significantly greater elevation in MAO-A $V_T$.

Primary hypothesis: MAO-A $V_T$, an index of MAO-A density, will be elevated in the prefrontal and anterior cingulate cortices of individuals with severe and/or reversed neurovegetative MDE symptoms relative to those without these symptoms.
Secondary Hypothesis: MAO-A $V_T$ will be elevated in all regions sampled (including the thalamus, midbrain, hippocampus, putamen and ventral striatum, as well as subregions of the prefrontal cortex) in individuals with severe and/or reversed neurovegetative MDE symptoms compared to those without these symptoms.

**Study 2. The Relationship between MAO-A Total Distribution Volume, Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness**

Specific Objective: To determine the relationship between elevated MAO-A $V_T$ and decreased hippocampal volume and prefrontal and anterior cingulate cortical thickness in those with MDD.

Primary Hypothesis: MAO-A $V_T$ will be inversely correlated to hippocampal volume and prefrontal and anterior cingulate cortical thickness in MDD.

Secondary Hypothesis: Hippocampal volume and prefrontal and anterior cingulate cortical thickness will be decreased in MDD relative to healthy controls.
Study 3. Dose-occupancy Relationship of Currently Available MAOIs

Specific Objective: To determine the dose-occupancy relationship of a selective, reversible inhibitor of MAO-A (moclobemide) and a non-selective, irreversible inhibitor of MAO (phenelzine).

Primary Hypothesis: There will be a dose-dependent decrease in MAO-A $V_T$ after 6 weeks of treatment with moclobemide and phenelzine across all brain regions sampled.

Secondary Hypothesis: Phenelzine and doses of moclobemide that require tyramine restrictions will have a higher MAO-A occupancy relative to average clinical doses of moclobemide.
Section 2.0. MATERIALS AND METHODS

2.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive Episodes is associated with Greater Symptom Severity and Reversed Neurovegetative Symptoms

2.1.1 Study Design

This study aimed to identify if those with a greater severity of depressive symptoms, as defined by a Hamilton Rating Scale for Depression (HRSD) score ≥ 20, had significantly elevated MAO-A $V_T$ relative to those with mild to moderate symptoms of depression (HRSD score ≤ 19). This hypothesis was based on a previous separate data set that suggested that individuals with a HRSD score greater or equal to 20 have significantly elevated MAO-A $V_T$ in the prefrontal cortex relative to those with a score less than or equal to 19 (specific subanalysis not published). The study further aimed to identify if those with reversed neurovegetative symptoms of depression, mainly increased sleeping or hypersomnia and increased appetite or hyperphagia, also had significantly elevated MAO-A $V_T$ relative to those without these symptoms. This was based on historical evidence that these individuals are more responsive to treatment with MAOIs (Thase, Frank et al. 1992; Quitkin, Stewart et al. 1993; Lonnqvist, Sihvo et al. 1994).

Participants attended the Centre for Addiction and Mental Health (CAMH) on three separate occasions. The first visit was for assessment of eligibility criteria and the second to undergo a $[^{11}\text{C}]$harnine PET scan for determination of MAO-A $V_T$ and one visit for an MRI scan at Toronto General Hospital which was used to determine regions of interest.
2.1.2 Subject Selection

Participants were eligible for study participation if they met all of the inclusion and exclusion criteria listed below.

Inclusion Criteria:

1. Current MDE secondary to MDD as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (First 1995), which was verified by subsequent consultation with a treating psychiatrist at CAMH.
2. Early onset MDD (onset prior to age 45).
3. Physically healthy.
5. HRSD score of at least 14 on the 17-item HRSD. The presence of reversed neurovegetative symptoms of depression were verified by the SCID-I (First 1995).

Exclusion Criteria:

1. Co-morbid psychiatric or medical illness.
2. Current or past substance abuse including cigarette smoking within the past year (since recent cigarette smoking may bias MAO-A VT (Fowler, Volkow et al. 1996; Bacher, Houle et al. 2011)).
4. Current perimenopause or postmenopause.
5. Positive urine pregnancy test (women)

6. Herbal drug or medication use within the past eight weeks with the exception of SSRIs for which the exclusion period was 2 weeks (thirty-seven out of forty-two participants had no SSRI use in the past month). It has been previously demonstrated that SSRIs do not affect MAO-A $V_T$ in vivo (Meyer, Wilson et al. 2009).

7. Borderline and antisocial personality disorder assessed by the Structured Clinical Interview for DSM-IV for Axis II disorders (Blais and Norman 1997).

For a secondary aim of comparing MDE subgroups to health, data from a group of previously collected healthy control participants was used. These participants met identical eligibility criteria to those with MDD except all psychiatric illnesses were exclusionary.

2.1.3 Subject Recruitment

Forty-two participants who met criteria for current MDE and MDD were recruited through advertisements in local newspapers, internet advertisement sites and treating psychiatrists at CAMH. Fifteen of these participants had been previously recruited and had been previously compared with healthy subjects but had not been examined in relation to subtype (and were not part of the data used to justify the severity hypothesis).
2.1.4 Sample Size Justification

It was seen in the previously collected sample that there was a difference of 15\% in MAO-A $V_T$ in the prefrontal cortex between those with a greater severity of depression (HRSD $\geq 20$) compared to those with a mild to moderate severity of depression (HRSD $\leq 19$). The mean MAO-A $V_T$ was 21.41 with a standard deviation of 3.15, therefore a sample size of 15 per group would be necessary to detect a 15\% difference with an alpha $= 0.05$ and a power of 80\% (GPower3.1) (Faul, Erdfelder et al. 2007; Faul, Erdfelder et al. 2009).

2.1.5 Assessment Day Procedures

All participants that met eligibility criteria and were deemed a potential suitable study candidate through telephone screening were invited to CAMH for an assessment visit. Upon arrival, participants were given a detailed description of the study and if interested asked to sign the informed consent form. Participants were screened for psychiatric illnesses using the SCID-I. Participants were also asked to fill out a standard battery of questionnaires related to mood and personality used in the laboratory, including HRSD, Beck Depression Inventory, a Visual Analog Scale, Dysfunctional Attitude Scale, the Structured Clinical Interview for DSM-IV for Axis II disorders, and the NEO-PIR. On the day of assessment, participants were also asked to provide a urine sample for urine toxicology purposes. If deemed eligible following this assessment visit, participants were scheduled for the PET scan.
2.1.6 Study Day Procedures

Each participant arrived at the CAMH PET centre approximately 1.5 hours prior to the scheduled PET scan start time. Mood symptoms were assessed using the HRSD, a self-reported visual analog scale and Beck Depression Inventory (BDI), with the primary outcome measure being the HRSD. All participants provided a urine sample for toxicology purposes on the day of the PET scan. For women, this sample was also used to confirm that they were not pregnant using a urine pregnancy test. Following this, an arterial line was inserted by a respiratory therapist into the radial artery, in addition to an intravenous catheter inserted into the antecubital vein of each individual.

Participants were also screened to rule out common medical conditions associated with MDD through plasma sampling, such as tests for thyroid stimulating hormone. Furthermore, they were asked not to consume any caffeine on the day of the PET scan and were asked not to use any over-the-counter medications or consume any alcohol for 48 hours prior to the PET scan.

2.1.7 PET Image Acquisition

Each participant underwent one $[^{11}\text{C}]$harmine PET scan to determine MAO-A $V_T$. For each PET scan, 370MBq of $[^{11}\text{C}]$harmine was administered as a bolus intravenously. An automatic blood sampling system was used to measure arterial blood radioactivity over the first 10 minutes of the scan. Manual samples were obtained at 2.5, 7.5, 15, 20, 30, 45, 60 and 90 minutes post injection. The method of measuring radioactivity in whole blood and parent
compound in plasma has been previously described (Ginovart, Meyer et al. 2006). PET images were acquired using an HRRT PET camera (in-plane resolution; full width half maximum, 3.1mm; 207 axial sections of 1.2mm; Siemens Molecular Imaging, Knoxville, Tennessee, U.S.A.) as previously described (Meyer, Wilson et al. 2009). The frames consisted of 15 frames of 1 minute followed by 15 frames of 5 minutes. $[^{11}\text{C}]$harmine doses were of high specific activity (mean: 1572.60 mCi/μmol, standard deviation 1024.61 mCi/μmol) and high radiochemical purity (mean: 98.7%, standard deviation: 0.95%).

2.1.8 MR Image Acquisition

Each participant underwent magnetic resonance imaging (GE Signa 1.5-T scanner; fast spoiled gradient echo, T$_1$-weighted image; x, y, z voxel dimensions, 0.78, 0.78, and 1.5 mm, GE Medical Systems, Milwaukee, Wis.) for the region of interest (ROI) delineation. The MRI scan usually took place on a separate day, either prior to or following the PET scan day. The ROIs were determined using a semi-automated method in which regions of a template MRI are transformed onto the individual MRI based on a series of transformations and deformations that match the template image to the individual co-registered MRI as well as segmentation of the individual MRI to select the grey matter voxels as previously described (Rusjan, Mamo et al. 2006; Meyer, Wilson et al. 2009),
2.1.9 Primary Behavioural Measure Outcome

**Hamilton Rating Scale for Depression (HRSD):** The HRSD was developed in 1960 (Hamilton 1960). It is a 17-item questionnaire and each item is measured on a 5 point scale (rated from 0-4). The items include; depressed mood, feelings of guilt, suicide, insomnia (early), insomnia (middle), insomnia (delayed), work and activities, motor retardation, agitation, anxiety (psychic), anxiety (somatic), somatic symptoms (gastrointestinal), somatic (general), somatic symptoms (genital), hypochondriasis, insight and loss of weight. The maximum possible total score is 52.

2.1.10 Ethical Considerations

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, in accordance with the Declaration of Helsinki and all participants signed a written informed consent prior to participation in the study. Subjects were identified by code throughout the study. Each participant was compensated $305.00 CAN ($20.00 for questionnaire completion, $50 for MRI completion and $235.00 for PET scan) for study completion. If a participant did not complete the study they were paid for the portion that was completed.
2.1.11 Data Analysis

2.1.11.1 PET Image Analysis

All PET images were analyzed using the automated image analysis software Regions of Mental Interest (ROMI). Exact procedures used in ROMI have been previously described (Rusjan, Mamo et al. 2006). Briefly, a standard brain template with a set of pre-defined ROIs was transformed to match the individual proton density weighted MR images (based on Talairach et al., 1988 and Kabani et al., 1998 atlases). The primary ROIs were the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) because these regions (and their subregions) participate in the affective regulation as demonstrated by paradigms of mood induction and affective cognition (Liotti, Mayberg et al. 2002; Ressler and Mayberg 2007). Secondary ROIs included regions that are implicated in MDE and/or have moderate to high MAO-A density and included hippocampus, ventral striatum, dorsal putamen, thalamus, and midbrain. In addition several subregions of the PFC were sampled, including dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), medial prefrontal cortex (mPFC), and orbitofrontal cortex (OFC). The borders of the subregions of the prefrontal cortex were defined based upon landmarks on the cortex that were derived based on cytoarchitectural differences from adjacent cortex (Rajkowska and Goldman-Rakic 1995; Rajkowska and Goldman-Rakic 1995; Uylings, Sanz-Arigita et al. 2010). The ROIs that were individually transformed were refined based on the gray matter probability of voxels in the MR images (SPM2 segmentation, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). The individual MR images were then co-registered to the PET images so that the refined ROIs were transformed to the PET image
space. The transformation of the template to match individual MR images and the co-
registration to PET space was done using non-linear iterative algorithms implemented in
SPM2. Time activity curves (TACs) were then extracted into a file compatible with PMOD
software (version 2.6.1; PMOD technologies Ltd., Zurich, Switzerland) in order to derive
MAO-A total distribution volume ($V_T$) values. The Logan method was applied to derive
MAO-A $V_T$ for each ROI using PMOD software (Logan, Fowler et al. 1990).

MAO-A $V_T$ represents the total distribution volume of $[^{11}\text{C}]$harmine and it is an index of
tissue binding at equilibrium, of which 85% is specific binding to MAO-A. Therefore,
changes in MAO-A $V_T$ may be interpreted as representing changes in $[^{11}\text{C}]$harmine binding
to MAO-A. The $V_T$ can be expressed in terms of kinetic rate parameters of a 2-tissue
compartment model: $V_T = (K_1/k_2) \times (k_3/k_4) + (K_1/k_2)$, where $K_1$ and $k_2$ are the influx and
efflux rate constants 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. The ratio of $K_1$ to $K_2$ is similar among different individuals
(for further detail, see Ginovart et al., (Ginovart, Meyer et al. 2006)). For $[^{11}\text{C}]$harmine PET,$
V_T$ may be validly and reliably measured with either an unconstrained 2-tissue compartment
model or with the Logan model with arterial sampling (for which the underestimate of $V_T$ is
negligible at the noise level of time activity curves from the regions of interest) (Ginovart,
Meyer et al. 2006), and the latter was applied in this study. This method has been previously
described in detail (Ginovart, Meyer et al. 2006; Meyer, Wilson et al. 2009).
2.1.11.2 Statistical Analyses

The primary analysis was a multivariate analysis of variance (MANOVA) with predictor variables of severity (greater or equal to 20 on the 17-item HRSD) and reversed neurovegetative symptoms (both hypersomnia and either hyperphagia or weight gain) and the dependent variables were MAO-A $V_T$ in the PFC and ACC. A secondary analysis used a MANOVA applying the same predictor variables however, the dependent variables were MAO-A $V_T$ in all brain regions (to assess whether the predictor variables related to MAO-A $V_T$ globally in the brain). Other secondary analyses applied MANOVA to compare MAO-A $V_T$ in the PFC and ACC between MDE subgroups and the healthy group. The four subgroupings assessed were 1. moderate to high severity, 2. mild to moderate severity, 3. reversed neurovegetative symptoms present, and 4. reversed neurovegetative symptoms absent.

2.2 Study 2. The Relationship between MAO-A $V_T$, Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness

2.2.1 Study Design

The purpose of this study was to utilize an existing database to determine if there was a relationship between elevated MAO-A $V_T$ and reduced hippocampal volume and prefrontal and anterior cingulate cortical thickness in MDD.
2.2.2 Subject Selection

This was a secondary analysis of data using participants with MDD and healthy controls that were previously recruited and had at least one $[^{11}C]harmine$ PET scan as mentioned in study 1. An additional 6 participants with MDD were included in the analysis. A total of 48 participants with MDE secondary to MDD and 37 healthy controls were included in the study. Inclusion and exclusion criteria were identical to study 1 and are restated below.

Inclusion Criteria:

1. Current MDE secondary to MDD as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (First 1995), which was verified by subsequent consultation with a treating psychiatrist at CAMH
2. Early onset MDD (onset prior to age 45)
3. Physically healthy
4. Aged 18-50
5. HRSD score of at least 14 on the 17-item HRSD. The presence of reversed neurovegetative symptoms of depression were verified by the SCID-I (First 1995).

Exclusion Criteria:

1. Co-morbid psychiatric or medical illness
2. Current or past substance abuse including cigarette smoking within the past year (since recent cigarette smoking may bias MAO-A \( \text{V}_T \) (Fowler, Volkow et al. 1996; Bacher, Houle et al. 2011)); 3. Positive urine toxicology drug screen
4. Current perimenopause or postmenopause
5. Positive urine pregnancy test (women)
6. Herbal drug or medication use within the past eight weeks with the exception of SSRIs for which the exclusion period was 2 weeks (thirty-seven participants had no SSRI use in the past month). It has been previously demonstrated that SSRIs do not affect MAO-A \( \text{V}_T \) \textit{in vivo} (Meyer, Wilson et al. 2009), a finding consistent with the pharmacological specificity of SSRIs.
7. Borderline and antisocial personality disorder assessed by the Structured Clinical Interview for DSM-IV for Axis II disorders (Blais and Norman 1997).

\textbf{2.2.3 Sample Size Justification}

In order to determine a correlation between hippocampal MAO-A \( \text{V}_T \) and hippocampal volume or cortical thickness a sample size of 28 is necessary to detect a correlation coefficient of 0.3, with an alpha of 0.05 and 80% power (GPower 3.1) (Faul, Erdfelder et al. 2007; Faul, Erdfelder et al. 2009).

Differences in hippocampal volume in MDE and healthy controls range on average from 4-9\% (Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009). In order to detect a difference of 6\% with an alpha of 0.05 and 80\% power with a known mean hippocampal volume in healthy controls of 2301 \text{mm}^3 and a standard deviation of 264 \text{mm}^3, a sample size
of 44 per group is necessary (GPower 3.1) (Faul, Erdfelder et al. 2007; Faul, Erdfelder et al. 2009).

2.2.3 MR Image Acquisition

Each participant underwent magnetic one resonance imaging scan (GE Signa 1.5-T scanner; fast spoiled gradient echo, T\textsubscript{1}-weighted image; x, y, z voxel dimensions, 0.78, 0.78, and 1.5 mm, GE Medical Systems, Milwaukee, Wis.).

2.2.4 Image Analysis

2.2.4.1 Total Hippocampus and Subfield Segmentation

Total hippocampal volume and subfield segmentation of the T1-weighted MRI images were performed using the Multiple Automatically Generated Templates for different Brains (MAGeT Brain). MAGeT Brain is a novel multi-atlas approach and has been described elsewhere (Chakravarty, Steadman et al. 2013). Briefly, MAGeT Brain is a three-step process which first generates a template library based on the manual segmentation of any given number of subjects in a dataset. Following this, pair-wise registration of each subject’s brain in the dataset to each template generated is automatically conducted to generate multiple segmentations for each participant, which yields a number of segmentations for each participant. Finally, a voxel voting procedure is conducted. The most frequently occurring label at each voxel remains for the final segmentation of each individual, thereby generating the most accurate label at each voxel for each participant. For the purposes of the current work, 11 patients with MDD and 10 healthy control participants were used to generate the template library. Each of the manually labeled atlases was non-linearly warped
to each subject in the template library. All participants in the dataset were then non-linearly warped to each of the subjects in the template library. Following this, each of the generated possible labels was warped to fit each subject in the dataset, yielding 105 candidate labels for each subject that were then fused by the automated voxel voting process. All segmentations were visually inspected.

2.2.4.2 Derivation of Cortical Thickness

The methods used to derive cortical thickness has been previously described (Lerch and Evans 2005). Each participant’s T1-weighted image was segmented into white matter, gray matter and CSF based on a probability map. The inner and outer cortical surfaces were extracted using the automated surface extraction algorithm. This generates a triangular mesh that defines the cortical surface. The mesh was deformed to give the optimal location of the grey-white matter border and the grey matter-CSF border. This allows for measuring of cortical thickness using a distance metric called t-link and has been previously described (Lerch and Evans 2005). Briefly, t-link measures the shortest distance between linked nodes on the inner and outer cortical surfaces at each vertex. The link between the two nodes is created by the deformation of the outer triangular mesh from the inner surface. This method has been shown to be very robust, with low variability (Lerch and Evans 2005). This was an automated procedure that was visually inspected, where any defects were manually corrected. The cortical thickness maps were also smoothed with a 15-mm-full-width at half maximum Gaussian kernel to account for variability and improve the distribution of errors.
From the output of this measure, regions of interest were identified to define subregions of the PFC (including VLPFC, DLPFC and OFC) and cingulate cortex.

### 2.2.5 Statistical Analyses

#### 2.2.5.1 Relationship between Hippocampal MAO-A $V_T$, Total Hippocampal Volume and Hippocampal Subfield Volume in MDD and Healthy Controls

Primary Analysis: A stepwise linear regression was applied to determine significant predictors of total right and left hippocampal volume (and subfields of the hippocampus, including CA1, CA2/3 and CA4DG) in MDD using predictors that have been shown to be related to either hippocampal volume or changes in MAO-A $V_T$ including: age, sex, number of years of education, hippocampus MAO-A $V_T$, age at onset, number of episodes of depression, current medication status, and family history of depression.

Secondary Analysis: A step-wise linear regression was applied to compare right and left total hippocampal volume (and subfields of the hippocampus) in MDE secondary to MDD and healthy controls. The predictors included in the model were group (MDE v. healthy controls), age, gender and number of years of education.

#### 2.2.5.2 Relationship between MAO-A $V_T$ and Prefrontal and Anterior Cingulate Cortical Thickness in MDD and Healthy Controls

Primary Analysis: A stepwise linear regression was applied to determine significant predictors of right and left cortical thickness in subregions of the PFC (VLPFC, DLPFC and OFC) and the cingulate cortex in MDD using predictors that have been shown to be related to either cortical thickness or changes in MAO-A $V_T$, including age, sex, number of years of
Secondary Analysis: A step-wise linear regression was applied to compare right and left total cortical thickness in subregions of the PFC (VLPFC, DLPFC and OFC) and the cingulate cortex in MDE secondary to MDD and healthy controls. The predictors included in the model were group (MDE v. healthy controls), age, gender and number of years of education.

2.3. Study 3. Dose-occupancy Relationship of Phenelzine and Moclobemide: Potential Implications for Novel Antidepressant Development and Optimal Dosing of Existing Monoamine Oxidase Inhibitors

2.3.1 Study Design

The purpose of this study was to identify the dose-occupancy relationship of moclobemide and the occupancy of phenelzine at average therapeutic doses. Participants attended the Centre for Addiction and Mental Health (CAMH) on at least 6 separate occasions. The study design can be seen in Figure 2.1. Participants had follow-up visits approximately every two weeks with the treating psychiatrist while taking medication to ensure tolerance and compliance (the latter verified by metabolites in urine drug screen and plasma sampling). Compliance was additionally verified through a plasma sample, which was taken on the second scan day (phenelzine: $1.15 \pm 1.19$ ng/ml, moclobemide: $3795.4 \pm 2336.5$ ng/ml).
Participants attended the Centre for Addiction and Mental Health (CAMH) for an assessment and psychiatric consultation. After determining eligibility, participants were scheduled for the first Positron Emission Tomography (PET) scan while medication free. Following this, participants were placed on treatment with either moclobemide or phenelzine for 4-6 weeks. During this time, individuals attended CAMH on at least 3 separate occasions for follow up visits and to ensure compliance with medication taking. Following the treatment course, individuals were scheduled for the second PET scan. Magnetic Resonance Imaging (MRI) scans were done at Toronto General Hospital and usually done between the two PET scans.

### 2.3.2 Subject Selection

Inclusion criteria were identical to study 1 and are restated below. Additional to the inclusion and exclusion criteria stated, participants that received a dose of 900-1200mg of moclobemide, or any dose of phenelzine, must have failed at least one previous antidepressant treatment. Target dose was generally chosen by the criteria seen in table 2.1.
Inclusion Criteria:

1. Current MDE secondary to MDD as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (First 1995), which was verified by subsequent consultation with a treating psychiatrist at CAMH
2. Early onset MDD (onset prior to age 45)
3. Physically healthy
4. Aged 18-50
5. HRSD score of at least 14 on the 17-item HRSD. The presence of reversed neurovegetative symptoms of depression were verified by the SCID-I (First 1995).

Exclusion Criteria:

1. Co-morbid psychiatric or medical illness
2. Current or past substance abuse including cigarette smoking within the past year (since recent cigarette smoking may bias MAO-A VT (Fowler, Volkow et al. 1996; Bacher, Houle et al. 2011)); 3. Positive urine toxicology drug screen
4. Current perimenopause or postmenopause
5. Positive urine pregnancy test (women)
6. Herbal drug or medication use within the past eight weeks with the exception of SSRIs for which the exclusion period was 2 weeks (thirty-seven participants had no SSRI use in the past month). It has been previously demonstrated that SSRIs do not affect MAO-A VT in
*vivo* (Meyer, Wilson et al. 2009), a finding consistent with the pharmacological specificity of SSRIs.

7. Borderline and antisocial personality disorder assessed by the Structured Clinical Interview for DSM-IV for Axis II disorders (Blais and Norman 1997).

**Table 2.1. Moclobemide and Phenelzine Target Dose Selection**

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Medication</th>
<th>Clinical Diagnosis/History</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>Moclobemide</td>
<td>MDE and history of side effects to medication</td>
</tr>
<tr>
<td>450</td>
<td>Moclobemide</td>
<td>MDE</td>
</tr>
<tr>
<td>600</td>
<td>Moclobemide</td>
<td>MDE</td>
</tr>
<tr>
<td>900</td>
<td>Moclobemide</td>
<td>MDE and history of non-response or co-morbid axis I disorder</td>
</tr>
<tr>
<td>1200</td>
<td>Moclobemide</td>
<td>MDE and history of non-response or co-morbid axis I disorder</td>
</tr>
<tr>
<td>45</td>
<td>Phenelzine</td>
<td>MDE and history of non-response or co-morbid axis I disorder</td>
</tr>
<tr>
<td>60</td>
<td>Phenelzine</td>
<td>MDE and history of non-response or co-morbid axis I disorder</td>
</tr>
</tbody>
</table>

**2.3.3 Subject Recruitment**

Participants were again recruited through advertisements in local newspapers, internet advertisement sites and treating psychiatrists at CAMH. Twenty-five participants with MDE secondary to MDD were recruited and underwent approximately 6 weeks of treatment with either phenelzine or moclobemide. Five participants were treated with 45-60mg phenelzine
and a total of 20 participants were treated with moclobemide at 300mg (n=4), 450mg (n=1), 600mg (n=6), 900mg (n=4), and 1200mg (n=5).

2.3.4 Sample Size Justification

Previous data suggests an occupancy (or percent difference between occupied and unoccupied scans) of approximately 75% for moclobemide at 600mg (Sacher, Houle et al. 2011). Assuming a difference between the occupied and unoccupied scan of approximately 60% at the lowest dose (at which we would assume the smallest difference between scans), with a mean MAO-A V_7 of approximately 21 and a standard deviation of 3, a sample size of 3 per group would be necessary to detect a difference with an alpha = 0.05 and power of 95% (GPower 3.1) (Faul, Erdfelder et al. 2007; Faul, Erdfelder et al. 2009).

2.3.5 Assessment Day Procedures

All participants were screened for study eligibility on a separate day prior to the first PET scan study day. Furthermore, all participants had a consultation with a psychiatrist (RGC, RL or JHM) prior to the first PET scan. All other assessment day procedures were identical to study 1.

2.3.6 Study Day Procedures

For this study, each participant had two PET scan study days that were identical to study 1 study day procedures.
2.3.7 PET Image Acquisition

Each participant underwent two [\(^{11}\)C]harmine PET scans to determine MAO-A V\(_T\), one at baseline (medication free) and one after 6 weeks of treatment. The image acquisition was identical to study 1. [\(^{11}\)C]harmine doses were again of high specific activity (scan 1 mean: 3156.59 mCi/μmol, standard deviation: 1983.87 mCi/μmol; scan 2 mean: 2720.34 mCi/μmol, standard deviation: 1251.44 mCi/μmol) and high radiochemical purity (scan 1 mean: 98.8%, standard deviation: 0.98%; scan 2 mean: 99.4%, standard deviation: 0.66%).

2.3.8 MR Image acquisition

MRI acquisition was identical to study 1.

2.3.9 Primary Behavioural Measure Outcome

The primary behavioural outcome was the HRSD, identical to study 1.

2.3.10 Ethical Considerations

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, in accordance with the Declaration of Helsinki and all participants signed a written informed consent prior to participation in the study. Subjects were identified by initials and date of birth throughout the study. Each participant was compensated $540.00 CAN ($20.00 for questionnaire completion, $50 for MRI completion and $235.00 for each PET scan) for study completion. If a participant did not complete the study they were paid for the portion that was completed.
2.3.11 Data Analysis

2.3.11.1 PET Image Analysis

The primary ROIs were the prefrontal cortex (PFC), anterior cingulate cortex (ACC), hippocampus, ventral striatum, dorsal putamen, thalamus, and midbrain. All other components of image analysis were identical to study 1.

2.3.11.2 Occupancy Calculation and Relationship to Dose

Occupancy was calculated using the Lassen Plot as has been previously described elsewhere (Lassen, Bartenstein et al. 1995; Cunningham, Rabiner et al. 2010). Briefly, \( V_T \) at baseline (BL) was plotted as the x-axis and \( V_T BL - V_T \) drug was plotted as the y-axis. The slope of the line gives an overall brain occupancy value and the x-intercept is equal to occupancy*Vns (non-specific distribution volume). With this information, Vns can be calculated. Using Vns, Vs (specific distribution volume) can be calculated using the equation \( V_s = V_T - Vns \). This makes the assumption that Vns is the same for each brain region and both scans. Occupancy was then calculated for each brain region using the equation \( \text{Occupancy} = (V_s BL - V_s drug)/V_s BL \).
2.3.11.3 Moclobemide and Phenelzine Plasma Assays

**Moclobemide:** Buproprion was added as an internal standard to all tubes. Standards, blanks and samples were basified to pH 11.00 with 25% K$_2$CO$_3$ and extracted with ethyl acetate. The organic layer was removed and taken to dryness under vacuum. The residue was reconstituted in toluene and injected on to an Agilent 7890B GC system coupled to a 7000C GC/MS triple quad in the electron impact (EI) mode. An HP5-MS UI (Agilent) column was employed in the assay. The oven temperature was held at 100 degrees C for 1 min and then ramped at 15 degrees C/min for a final temperature of 295 degrees C. Moclobemide and bupropion eluted at 15.15 and 8.6 minutes respectively. The transitions m/z 140>112 and 139>111, for moclobemide and bupropion respectively, were recorded in multiple reaction monitoring mode for identification and quantification. Agilent Mass Hunter software was used for all instrument control and data manipulation.

**Phenelzine:** Phenelzine was assayed by a modification of the method of Rao et al., (Rao, Baker et al. 1987). Briefly, samples, standards and blanks were basified, extracted and derivatized with pentafluorobenzoyl chloride. The organic layer was saved and taken to dryness. The residue was reconstituted in toluene and a portion injected on to a HP-5MS column (Agilent) installed in an Agilent 6890 series GC system coupled to 5973 N mass spectrometer in the negative chemical ionization (NCI) mode. Oven conditions were as follows: initial temperature 100°C, oven ramp 15°C/min, final temperature 295°C (held for 10 min). The retention times of derivatized benzylamine (internal standard) and derivatized phenelzine were 9.1 and 12.48 minutes respectively. Single ion monitoring (benzylamine m/z 281; phenelzine m/z 504) was used for identification and quantification. MSD Chemstation was used for instrument control and data analysis.
2.3.11.4 Statistical Analyses

To determine the dose-occupancy relationship of moclobemide, occupancy data was fit using the hyperbolic equation $F(x) = a(x/(b+x))$ (with a (0,0) point included) for each individual brain region (Sigmaplot v. 11, Systat, USA). Multivariate analysis of co-variance (MANCOVA) was applied with total daily dose as the covariate and the brain regions of interest (including prefrontal cortex, anterior cingulate cortex, ventral striatum, dorsal putamen, thalamus, midbrain and hippocampus) as the dependent variables (IBM SPSS Statistics v. 20).

A multivariate analysis of variance (MANOVA) was used to compare the regional occupancy across three groups: low dose moclobemide (300 to 600mg total daily dose), higher dose moclobemide (900 to 1200mg total daily dose) and phenelzine (45 to 60mg total daily dose). Mean occupancy for higher doses of moclobemide (900-1200mg total daily dose), average clinical doses of phenelzine (45-60mg total daily dose), and average doses of moclobemide (300-600mg total daily dose) were calculated and reported with a 95% confidence interval (CI) of the difference (IBM SPSS Statistics v. 20).
Section 3.0. RESULTS

3.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive Episodes is associated with Greater Severity and Reversed Neurovegetative Symptoms


3.1.1 Demographic and Clinical Characteristics in Subgroups of MDE

There were no significant differences in age, age at onset of MDD, number of previous MDEs, number of males versus females or number of individuals that had received past antidepressant treatment between those with moderate to severe MDE and those with mild to moderate MDE (Table 3.1). There was a significant difference in HRSD score between those with severe MDE and those with mild to moderate MDE (p < 0.001) (Table 3.1). There were no significant differences in any of the above listed variables between those with or without reversed neurovegetative MDE symptoms (Table 3.2).
Table 3.1. Demographic and Clinical Characteristics of Individuals with Moderate to Severe and Mild to Moderate MDE*

<table>
<thead>
<tr>
<th></th>
<th>Moderate to Severe Depression (n=25)</th>
<th>Mild to Moderate Depression (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>32.7 (7.8)</td>
<td>30.9 (7.8)</td>
</tr>
<tr>
<td>HRSD score, mean (SD)</td>
<td>23.6 (2.7)</td>
<td>16.6 (2.2)*</td>
</tr>
<tr>
<td>Age at onset, mean (SD)</td>
<td>19.4 (9.1)</td>
<td>18.6 (10.1)</td>
</tr>
<tr>
<td>No. of previous episodes, mean (SD)</td>
<td>2.2 (1.3)</td>
<td>2.7 (1.8)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>15 (60)</td>
<td>10 (59)</td>
</tr>
<tr>
<td>Past antidepressant treatment, No. (%)</td>
<td>16 (64)</td>
<td>10 (59)</td>
</tr>
</tbody>
</table>

Abbreviations: HRSD, 17-item Hamilton Rating Scale for Depression.
*pModerate to severe MDE participants had significantly higher HRSD scores compared to those with mild to moderate MDE (p < 0.001) but there were no significant differences among any other demographic or clinical variables between groups.

Table 3.2. Demographic and Clinical Characteristics of Individuals with and without Reversed Neurovegetative MDE Symptoms *

<table>
<thead>
<tr>
<th></th>
<th>With Reversed Neurovegetative Symptoms (n=10)</th>
<th>Without Reversed Neurovegetative Symptoms (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>31.3 (9.0)</td>
<td>32.2 (7.5)</td>
</tr>
<tr>
<td>HRSD score, mean (SD)</td>
<td>18.8 (2.9)</td>
<td>21.4 (4.4)</td>
</tr>
<tr>
<td>Age at onset, mean (SD)</td>
<td>16.5 (6.8)</td>
<td>19.9 (10.0)</td>
</tr>
<tr>
<td>No. of previous episodes, mean (SD)</td>
<td>2.4 (1.3)</td>
<td>2.4 (1.6)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>5 (50)</td>
<td>20 (63)</td>
</tr>
<tr>
<td>Past antidepressant treatment, No. (%)</td>
<td>8 (80)</td>
<td>18 (56)</td>
</tr>
</tbody>
</table>

Abbreviations: HRSD, 17-item Hamilton Rating Scale for Depression. There were no significant differences among any of the demographic or clinical variables between groups.

3.1.2 Effect of MDE Severity upon MAO-A V_T

There was a main effect of symptom severity on MAO-A V_T in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) (MANOVA, F(2,38) = 5.44, p=0.008) and in the subregions of the PFC (orbitofrontal cortex (OFC), ventral lateral prefrontal cortex (VLPFC), dorsal lateral prefrontal cortex (DLPFC), medial prefrontal cortex (mPFC)) (MANOVA, F(4,36) =4.57, p=0.004, Figure 3.2). The whole brain analysis, including all brain regions of interest, did not reach significance (MANOVA, F(7,33) = 1.90, p=0.10) but there was a tendency towards a similar finding across most regions sampled (individual ANOVA, F(1,39) =5.31 to 11.01, p=0.03 to 0.002), and a trend in the hippocampus (ANOVA, F(1,39) = 2.59, p=0.1) (Figure 3.1).
Figure 3.1. The effect of severe MDE symptoms (HRSD ≥ 20) compared to mild to moderate MDE symptoms (HRSD ≤ 19) on MAO-A VT in regions of interest. Participants with severe MDE symptoms had significantly higher MAO-A VT in the prefrontal and anterior cingulate cortices (Multivariate Analysis of Variance (MANOVA), $F_{(2,38)} = 5.44$, $p=0.008$). The whole brain analysis did not reach significance (MANOVA, $F_{(7,33)} = 1.90$, $p=0.10$) but there was a tendency towards a similar finding in the other regions sampled (individual ANOVA, $F_{(1,39)} = 5.31$ to 11.01, $p=0.002$ to 0.03), and a trend in the hippocampus (ANOVA, $F_{(1,39)} = 2.59$, $p=0.1$).

Figure 3.2. The effect of severe MDE symptoms (HRSD ≥ 20) compared to mild to moderate MDE symptoms (HRSD ≤ 19) on MAO-A $V_T$ in subregions of the prefrontal cortex. Participants with severe MDE symptoms had significantly higher MAO-A $V_T$ in all subregions of the prefrontal cortex compared to those with mild to moderate MDE symptoms (MANOVA, $F_{(4,36)} = 4.57$, $p=0.004$).
3.1.3 Effect of Reversed Neurovegetative MDE Symptoms upon MAO-A $V_T$

All of the subjects enrolled who had hypersomnia also had either hyperphagia or weight gain (and vice versa). After including the effect of symptom severity, there was an effect of reversed neurovegetative symptoms on MAO-A $V_T$ in the PFC and ACC (MANOVA, $F_{(2,38)} = 5.13$, $p=0.01$, Figure 3.3), which was also present in subregions of the PFC (OFC, VLPFC, DLPFC, mPFC, Figure 3.4) (MANOVA, $F_{(4,36)} = 3.23$, $p=0.02$). This was significant across all regions sampled (MANOVA, $F_{(7,33)} = 2.56$, $p=0.03$) (Figure 3.3).
Figure 3.3. The effect of reversed neurovegetative MDE symptoms compared to no reversed neurovegetative MDE symptoms on **MAO-A V\textsubscript{T} in regions of interest.** Participants with reversed neurovegetative MDE symptoms had significantly higher MAO-A V\textsubscript{T} in the prefrontal and anterior cingulate cortices compared to those without these MDE symptoms (Multivariate Analysis of Variance (MANOVA), F\textsubscript{(2,38)} = 5.13, p=0.01) and this effect was also present across all the brain regions sampled (MANOVA, F\textsubscript{(7,33)} = 2.56, p=0.03). To visually demonstrate the effect of reversed neurovegetative MDE symptoms on MAO-A V\textsubscript{T}, the variance due to symptom severity was removed from the MAO-A V\textsubscript{T} values in this plot. The difference in mean MAO-A V\textsubscript{T} value between low and high severity subtypes was added to the low severity subtypes so severity no longer contributed to the variance. *Reprinted from Neuropsychopharmacology, 39 (4). Chiuccariello et al., “Elevated monoamine oxidase A binding during major depressive episodes is associated with greater severity and reversed neurovegetative symptoms”, pp. 973-980, Copyright (2014), with permission from Nature Publishing Group.*
Figure 3.4. The effect of reversed neurovegetative MDE symptoms compared to no reversed neurovegetative MDE symptoms on MAO-A $V_T$ in subregions of the prefrontal cortex. Participants with reversed neurovegetative MDE symptoms had significantly higher MAO-A $V_T$ compared to those without reversed neurovegetative MDE symptoms in subregions of the prefrontal cortex (Multivariate Analysis of Variance (MANOVA), $(F_{(4,36)} = 3.23, p=0.02)$. To visually demonstrate the effect of reversed neurovegetative MDE symptoms on MAO-A $V_T$, the variance due to severity was removed from the MAO-A $V_T$ values in this plot. The difference in mean MAO-A $V_T$ value between low and high severity subtypes was added to the low severity subtypes so severity no longer contributed to the variance.
3.1.4 Relationship of MAO-A $V_T$ with Severity as a Continuous Variable

Applying a multivariate analysis of co-variance (MANCOVA) with MAO-A $V_T$ in the PFC and ACC as the dependent variables, the predictor of HDRS as a covariate and reversed neurovegetative features as a factor, both predictors were found to be significant (symptom severity: $F_{2,38}=5.09$, $p=0.01$, reversed neurovegetative symptoms: $F_{2,38}=4.71$, $p=0.02$, Figure 3.5). Additional individual ANOVAs with PFC and ACC MAO-A $V_T$ as the dependent variables were also significant (PFC: severity, $F_{1,39}=10.44$, $p=0.003$, reversed neurovegetative symptoms, $F_{1,39}=8.04$, $p=0.007$; ACC: severity, $F_{1,39}=8.26$, $p=0.007$, reversed neurovegetative symptoms, $F_{1,39}=9.64$, $p=0.004$).
Figure 3.5. The relationship of MAO-A VT with symptom severity as a continuous variable in the prefrontal and anterior cingulate cortices. A multivariate analysis of co-variance (MANCOVA) with the dependent variables being MAO-A VT in the (a) PFC and (b) ACC, and HRSD as a covariate and reversed neurovegetative features as a factor showed both variables were significant predictors (symptom severity: $F_{2,38}=5.09$, $p=0.01$, reversed neurovegetative symptoms: $F_{2,38}=4.71$, $p=0.02$).
3.1.5 Comparison of MAO-A $V_T$ between Subgroups with Healthy Controls

Participants with moderate to severe MDE symptoms ($n=25$) had significantly greater MAO-A $V_T$ in the PFC and ACC compared to healthy controls ($n=37$) (MANOVA, $F_{(2,59)}=9.75$, $p<0.001$) and this was also found in all brain regions of interest (MANOVA, $F_{(7,54)}=3.41$, $p=0.004$). Those with reversed neurovegetative MDE symptoms ($n=10$) also had significantly greater MAO-A $V_T$ in the PFC and ACC compared to healthy controls (MANOVA, $F_{(2,44)}=5.71$, $p<0.01$), which was also found across all brain regions of interest (MANOVA, $F_{(7,39)}=3.32$, $p=0.007$). Participants without reversed neurovegetative symptoms ($n=32$) of depression also had significantly greater MAO-A $V_T$ in the PFC and ACC relative to controls (MANOVA $F_{(2,66)}=5.71$, $p<0.01$), but subjects with mild to moderate MDE ($n=17$) had no significant difference in MAO-A $V_T$ in the PFC and ACC relative to controls (MANOVA, $F_{(2,51)}=1.94$, $p=0.16$).

3.1.6 Plasma Free Fraction

The plasma free-fraction of the parent metabolite was collected in 73 of 79 participants. There were no significant differences between those with moderate to severe MDE symptoms and those mild to moderate MDE symptoms ($t=0.184$, $p=0.855$) or those with and without reversed neurovegetative MDE symptoms ($t=0.967$, $p=0.340$).
3.2. Study 2. The Relationship between MAO-A \( V_T \), Hippocampal Volume and Prefrontal and Anterior Cingulate Cortical Thickness

3.2.1 Comparison of Demographic Variables between MDD and Healthy Controls

There were no significant differences in any of the demographic or clinical variables when comparing the MDD and healthy control groups (Table 3.3). All individuals experiencing a MDE had early onset MDD.

Table 3.3. Demographic and Clinical Characteristics of Individuals with MDD and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>MDD (n=48)</th>
<th>Controls (n=37)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>32.77 (8.28)</td>
<td>31.70 (7.62)</td>
<td>p=0.543</td>
</tr>
<tr>
<td>Ratio Male:Female</td>
<td>19:29</td>
<td>15:22</td>
<td></td>
</tr>
<tr>
<td>Years of Education, mean (SD)</td>
<td>3.01 (1.71)</td>
<td>3.12 (1.66)</td>
<td>( X_{(1)}^2 = 0.008, p=0.929 )</td>
</tr>
<tr>
<td>Age of Onset MDD, mean (SD)</td>
<td>19.29 (9.32)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Family History (%)</td>
<td>56.3%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of Episodes, mean (SD)</td>
<td>2.62 (1.74)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Current Antidepressant Treatment, (% with)</td>
<td>64.6%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hamilton Rating Scale for Depression Score, mean (SD)</td>
<td>20.63 (4.30)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
3.2.2 The Relationship of Hippocampal MAO-A $V_T$ to Hippocampal Volume

3.2.2.1 Hippocampal MAO-A $V_T$ and Total Hippocampal Volume in the Combined Sample of MDD and Healthy Controls

A stepwise linear regression was applied. Inclusion of hippocampal MAO-A $V_T$ alone as a predictor variable found no significant relationship between hippocampal MAO-A $V_T$ and total right ($F_{(1,82)} = 0.041, p=0.841$) or left ($F_{(1,82)} = 0.694, p=0.407$) hippocampal volume (Table 3.4). When additional variables including age, sex, group (Figure 3.6 and table 3.5) and number of years of education were added into the model, there was a relationship between sex and total hippocampal volume (right: $F_{(1,68)} = 16.44, p<0.001$, left: $F_{(1,68)} = 16.00, p<0.001$).
Figure 3.6. Hippocampal volume in those with a major depressive episode and healthy controls. There was no significant difference in left or right (right: $F_{(1,82)}=0.042$, $p=0.838$, left: $F_{(1,82)}=0.172$, $p=0.680$) total hippocampal volume between those experiencing a major depressive episode and healthy controls.
3.2.2.2 Hippocampal MAO-A VT and Total Hippocampal Volume in Healthy Controls Alone

There was no significant relationship between hippocampal MAO-A VT and total hippocampal volume in the healthy control group alone (right: F(1,36) = 0.011, p=0.917, left: F(1, 36) = 0.753, p=0.392, Table 3.4). When age, sex and number of years of education were included into the model, only sex predicted total hippocampal volume (right: F(1,25) =5.434, p=0.028, left: F(1,25) =10.493, p=0.003).

3.2.2.3 Hippocampal MAO-A VT and Total Hippocampal Volume in MDD Alone

There was no significant relationship between hippocampal MAO-A VT and total hippocampal volume in the MDD group alone (right: F(1,45) = 0.080, p=0.779, left: F(1,45) = 0.061, p=0.805, Table 3.4). When additional variables (age, age of onset, sex, HRSD score, family history of MDD, number of episodes, current treatment (none, SSRI or MAOI) and years of education) that may relate to MAO-A VT or hippocampal volume in MDD were included in the model, sex, family history of MDD and age of onset predicted total right hippocampal volume (F(4,41) =9.095, p<0.001) and only sex predicted total left hippocampal volume (F(1,41) =7.198, p=0.011).

3.2.3 Hippocampal MAO-A VT and Hippocampal Subfield Volume

3.2.3.1 Hippocampal MAO-A VT and Hippocampal Subfield Volume in the Combined Sample of MDD and Healthy Controls

There was no significant relationship between hippocampal MAO-A VT and CA1, CA23 or CA4DG volume (CA1: right: F(1,82) =0.524, p=0.471, left: F(1,82) =0.003, p=0.955, CA23: right
\( F_{(1,82)} = 0.882, p=0.350, \) left: \( F_{(1,82)} = 0.121, p=0.728, \) CA4DG: right: \( F_{(1,82)} = 0.17, p=0.897, \) left: \( F_{(1,82)} = 0.003, p=0.960, \) Table 3.4). When additional variables including age, sex, group (Table 3.5) and number of years of education were included into the model, only sex predicted CA1, CA23 and CA4DG volume (CA1: right: \( F_{(1,68)} = 16.654, p<0.001, \) left: \( F_{(1,68)} = 19.295, p<0.001, \) CA23: right: \( F_{(1,68)} = 4.519, p=0.037, \) left: \( F_{(1,68)} = 10.815, p=0.002, \) CA4DG: right: \( F_{(1,68)} = 13.425, p<0.001, \) left: \( F_{(1,68)} = 8.074, p=0.006). 

### 3.2.3.2 Hippocampal MAO-VT and Hippocampal Subfield Volume in Healthy Controls Alone

There was no relationship between hippocampal MAO-A VT and CA1, CA23 and CA4DG volume in the healthy control group alone (CA1: right: \( F_{(1,36)} = 0.829, p=0.369, \) left: \( F_{(1,36)} = 0.047, p=0.830, \) CA23: right: \( F_{(1,36)} = 0.524, p=0.474, \) left: \( F_{(1,36)} = 1.487, p=0.231, \) CA4DG: right: \( F_{(1,36)} = 0.378, p=0.543, \) left: \( F_{(1,36)} = 0.203, p=0.655, \) Table 3.4). When age, sex and number of years of education were included into the model, sex was the only predictor of CA1 and CA23 volume (CA1: right: \( F_{(1,25)} = 9.102, p=0.006, \) left: \( F_{(1,25)} = 4.755, p=0.039, \) CA23: right: \( F_{(1,25)} = 7.087, p=0.014, \) left: \( F_{(1,25)} = 6.551, p=0.017). The model that best predicted right CA4DG volume included age and sex \( F_{(2,25)} = 7.821, p=0.003 \) and there were no predictors of left CA4DG volume \( F_{(9,25)} = 1.679, p=0.192). 

### 3.2.3.3 Hippocampal MAO-A VT and Hippocampal Subfield Volume in MDD Alone

There was no relationship between hippocampal MAO-A VT and CA1, CA23 and CA4DG volume in the MDD group alone (CA1: right: \( F_{(1,45)} = 0.001, p=0.072, \) left: \( F_{(1,45)} = 0.075, p=0.786, \) CA23: right: \( F_{(1,45)} = 0.119, p=0.734, \) left: \( F_{(1,45)} = 0.009, p=0.924, \) CA4DG: right: \( F_{(1,45)} = 0.092, p=0.763, \) left: \( F_{(1,45)} = 0.117, p=0.734, \) Table 3.4). When additional variables that may
relate to MAO-A $V_T$ or hippocampal volume in MDD (age, age of onset, sex, HRSD score, family history of MDD, number of episodes, current treatment (none, SSRI or MAOI) and years of education) were included into the model, the model that best predicted left CA1 volume included sex, HRSD score and family history of MDD ($F_{(3,41)} = 9.633$, $p<0.001$) and sex was the only predictor of right CA1 volume ($F_{(1,41)} = 8.431$, $p=0.006$). There were no significant predictors of right CA23 volume ($F_{(9,41)} = 0.917$, $p=0.523$) and sex, HRSD score and family history of MDD predicted left CA23 volume ($F_{(3,41)} = 6.800$, $p=0.001$). Sex was the only significant predictor of right CA4DG volume ($F_{(1,41)} = 5.814$, $p=0.021$) and sex, HRSD score and family history of MDD predicted left CA4DG volume ($F_{(3,41)} = 5.396$, $p=0.003$).
Table 3.4. Relationship of Hippocampal MAO-A \( V_T \) and Hippocampal Volume in MDD and Healthy Controls

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Relationship to MAO-A ( V_T ) in Combined Sample</th>
<th>Relationship to MAO-A ( V_T ) in Healthy Control Group</th>
<th>Relationship to MAO-A ( V_T ) in MDD Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R: ( F_{(1,82)} = 0.041, p = 0.841 )</td>
<td>R: ( F_{(1,36)} = 0.011, p = 0.917 )</td>
<td>R: ( F_{(1,45)} = 0.080, p = 0.779 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.694, p = 0.407 )</td>
<td>L: ( F_{(1,36)} = 0.753, p = 0.392 )</td>
<td>L: ( F_{(1,45)} = 0.061, p = 0.805 )</td>
</tr>
<tr>
<td>Total Hippocampus</td>
<td></td>
<td>R: 0.011, p = 0.917</td>
<td>R: 0.080, p = 0.779</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L: 0.753, p = 0.392</td>
<td>L: 0.061, p = 0.805</td>
</tr>
<tr>
<td>CA1</td>
<td>R: ( F_{(1,82)} = 0.524, p = 0.471 )</td>
<td>R: ( F_{(1,36)} = 0.829, p = 0.369 )</td>
<td>R: ( F_{(1,45)} = 0.001, p = 0.972 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.003, p = 0.955 )</td>
<td>L: ( F_{(1,36)} = 0.047, p = 0.830 )</td>
<td>L: ( F_{(1,45)} = 0.075, p = 0.786 )</td>
</tr>
<tr>
<td>CA23</td>
<td>R: ( F_{(1,82)} = 0.882, p = 0.350 )</td>
<td>R: ( F_{(1,36)} = 0.524, p = 0.474 )</td>
<td>R: ( F_{(1,45)} = 0.119, p = 0.732 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.121, p = 0.728 )</td>
<td>L: ( F_{(1,36)} = 1.487, p = 0.231 )</td>
<td>L: ( F_{(1,45)} = 0.009, p = 0.924 )</td>
</tr>
<tr>
<td>CA4DG</td>
<td>R: ( F_{(1,82)} = 0.170, p = 0.897 )</td>
<td>R: ( F_{(1,36)} = 0.378, p = 0.543 )</td>
<td>R: ( F_{(1,45)} = 0.092, p = 0.763 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.003, p = 0.960 )</td>
<td>L: ( F_{(1,36)} = 0.203, p = 0.655 )</td>
<td>L: ( F_{(1,45)} = 0.117, p = 0.734 )</td>
</tr>
</tbody>
</table>

Table 3.5. Effect of Group on Hippocampal Volume

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Relationship to Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R: ( F_{(1,82)} = 0.042, p = 0.838 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.172, p = 0.680 )</td>
</tr>
<tr>
<td>Total Hippocampus</td>
<td>R: ( F_{(1,82)} = 0.129, p = 0.720 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.090, p = 0.764 )</td>
</tr>
<tr>
<td>CA1</td>
<td>R: ( F_{(1,82)} = 0.572, p = 0.452 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 2.173, p = 0.144 )</td>
</tr>
<tr>
<td>CA23</td>
<td>R: ( F_{(1,82)} = 0.008, p = 0.928 )</td>
</tr>
<tr>
<td></td>
<td>L: ( F_{(1,82)} = 0.040, p = 0.835 )</td>
</tr>
</tbody>
</table>
3.2.3 The Relationship of MAO-A VT to Cortical Thickness in the PFC and ACC

3.2.3.1 MAO-A VT and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in the Combined Sample of MDD and Healthy Controls

Subregions of the Prefrontal Cortex

There was no relationship between MAO-A VT and cortical thickness in the VLPFC (F(4,80) =0.653, p=0.626) DLPFC (F(6,78) =1.166, p=0.33) or OFC (F(4,80) =1.466, p=0.220) in the combined sample (Table 3.6). There was a main effect of group in the VLPFC (F(4,80) =2.441, p=0.05) and OFC (F(4,80) =2.781, p=0.032) but not in the DLPFC (F(6,78) =1.407, p=0.222). When additional variables including age, sex, number of years of education were included in the model, the main effect of group remained in the OFC (F(4,60) =3.454, p=0.013) (Table 3.7) and MAO-A VT also predicted OFC cortical thickness in the overall model (F(4,60) =2.848, p=0.031). When only group and MAO-A VT were included into the model, only the effect of group remained (F(4,79) =2.915, p=0.026). However, this finding was not supported by the voxel-wise analysis and there were no significant difference in cortical thickness across any of the subregions of the PFC through this approach. In the VLPFC, there was a trend to an effect of sex VLPFC (F(4,60) =2.286, p=0.070) when all additional variables were included in the model. When only gender and group were included into the model, there was an effect of both gender (F(4,79) =4.715, p=0.002) and group (F(4,79) =2.527, p=0.047). There were no predictors of DLPFC cortical thickness when age, sex and number of years of education were included in the model.
Cingulate Gyrus

There was no significant relationship between anterior cingulate cortex MAO-A V₆ and cingulate gyrus cortex cortical thickness (F(2,82) =2.450, p=0.09) in the combined sample (Table 3.6). There was no effect of group (F(2,82) =0.215, p=1.564). When age, sex and number of years of education were included in the model, there were no significant predictors of cingulate gyrus cortical thickness.

3.2.3.2 MAO-A V₆ and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in Healthy Controls Alone

Subregions of the Prefrontal Cortex

There was no relationship between MAO-A V₆ and cortical thickness in the VLPFC (F(4,32) =2.349, p=0.08), DLPFC (F(6,30) =0.890, p=0.515) or OFC (F(4,32) =0.891, p=0.481) in the healthy control group alone (Table 3.6). When age, sex and years of education were included in the model there were no significant predictors of VLPFC cortical thickness. In the DLPFC, both age (F(6,16) =3.522, p=0.02) and sex (F(6,16) =3.242, p=0.028) were predictors of cortical thickness when all variables were included in the model. In the OFC, age predicted cortical thickness (F(4,16) =2.914, p=0.05).
**Cingulate Gyrus**

There was a significant relationship between anterior cingulate cortex MAO-A $V_T$ and cingulate gyrus cortical thickness in the healthy control group alone ($F_{(2,34)} = 5.361$, $p=0.009$) (Table 3.6). When age, sex and number of years of education were included in the model, the relationship between MAO-A $V_T$ and cingulate gyrus cortical thickness did not remain ($F_{(2,20)} = 2.854$, $p=0.081$).

### 3.2.3.3 MAO-A $V_T$ and Cortical Thickness in the Prefrontal Cortex and Anterior Cingulate Cortex in MDD Alone

**Subregions of the Prefrontal Cortex**

There was no relationship between MAO-A $V_T$ and VLPFC ($F_{(4,43)} = 0.870$, $p=0.490$), DLPFC ($F_{(6,41)} = 0.867$, $p=0.527$) or OFC ($F_{(4,43)} = 1.443$, $p=0.236$) cortical thickness in the MDD group alone (Table 3.6). When additional variables that may be related to MAO-A $V_T$ or cortical thickness in MDD (age, age of onset, sex, HRSD score, family history of MDD, number of episodes, current treatment (none, SSRI or MAOI) and years of education) were included into the model, no factors predicted VLPFC, DLPFC or OFC cortical thickness in the MDD group alone.

**Cingulate Gyrus**

There was no relationship between anterior cingulate cortex MAO-A $V_T$ and cingulate gyrus cortical thickness in the MDD group alone ($F_{(2,45)} = 0.011$, $p=0.989$) (Table 3.6). When additional
variables that may be related to MAO-A $V_T$ or cortical thickness in MDD (age, age of onset, sex, HRSD score, family history of MDD, number of episodes, current treatment (none, SSRI or MAOI) and years of education) were included into the model, there were no predictors of cortical thickness in the cingulate gyrus.
Table 3.6. The Relationship between MAO-A VT and Cortical Thickness in Prefrontal Cortex Subregions and Cingulate Gyrus

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Cortical Outputs Included</th>
<th>Relationship to MAO-A VT in Combined Sample</th>
<th>Relationship to MAO-A VT in Healthy Control Group</th>
<th>Relationship to MAO-A VT in MDD Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>Inferior Frontal Gyrus</td>
<td>( F_{(6,78)} = 1.166, ) ( p=0.33 )</td>
<td>( F_{(6,30)} = 0.890, ) ( p=0.512 )</td>
<td>( F_{(6,41)} = 0.827, ) ( p=0.53 )</td>
</tr>
<tr>
<td></td>
<td>Rostral Middle Inferior Frontal Gyrus</td>
<td>( F_{(6,78)} = 1.166, ) ( p=0.33 )</td>
<td>( F_{(6,30)} = 0.890, ) ( p=0.512 )</td>
<td>( F_{(6,41)} = 0.827, ) ( p=0.53 )</td>
</tr>
<tr>
<td>VLPFC</td>
<td>Superior Frontal Gyrus</td>
<td>( F_{(4,80)} = 0.653, ) ( p=0.626 )</td>
<td>( F_{(4,32)} = 2.349, ) ( p=0.08^{**} )</td>
<td>( F_{(4,43)} = 0.870, ) ( p=0.49 )</td>
</tr>
<tr>
<td></td>
<td>Rostral Middle Superior Frontal Gyrus</td>
<td>( F_{(4,80)} = 0.653, ) ( p=0.626 )</td>
<td>( F_{(4,32)} = 2.349, ) ( p=0.08^{**} )</td>
<td>( F_{(4,43)} = 0.870, ) ( p=0.49 )</td>
</tr>
<tr>
<td></td>
<td>Caudal Middle Frontal Gyrus</td>
<td>( F_{(4,80)} = 0.653, ) ( p=0.626 )</td>
<td>( F_{(4,32)} = 2.349, ) ( p=0.08^{**} )</td>
<td>( F_{(4,43)} = 0.870, ) ( p=0.49 )</td>
</tr>
<tr>
<td>OFC</td>
<td>Middle Orbitofrontal Gyrus</td>
<td>( F_{(4,80)} = 1.466, ) ( p=0.220 )</td>
<td>( F_{(4,32)} = 0.891, ) ( p=0.481 )</td>
<td>( F_{(4,43)} = 1.433, ) ( p=0.24 )</td>
</tr>
<tr>
<td></td>
<td>Lateral Orbitofrontal Gyrus</td>
<td>( F_{(4,80)} = 1.466, ) ( p=0.220 )</td>
<td>( F_{(4,32)} = 0.891, ) ( p=0.481 )</td>
<td>( F_{(4,43)} = 1.433, ) ( p=0.24 )</td>
</tr>
<tr>
<td>Cingulate Gyrus</td>
<td>Cingulate Gyrus</td>
<td>( F_{(2,82)} = 2.450, ) ( p=0.09 )</td>
<td>( F_{(2,34)} = 5.361, ) ( p=0.009^{**} )</td>
<td>( F_{(2,45)} = 0.011, ) ( p=0.99 )</td>
</tr>
</tbody>
</table>

**The relationship between MAO-A VT and VLPFC cortical thickness did not remain when including age, sex and number of years of education into the model (VLPFC: \( F_{(4,18)} = 1.356, p=0.288 \), cingulate gyrus: \( F_{(2,20)} = 2.845, p=0.08 \)).

Table 3.7. The Effect of Group on Cortical Thickness in the Prefrontal Cortex and Cingulate Gyrus

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Relationship to Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>( F_{(6,78)} = 1.407, p=0.22 )</td>
</tr>
<tr>
<td>VLPFC</td>
<td>( F_{(4,80)} = 2.441, p=0.05 )</td>
</tr>
<tr>
<td>OFC</td>
<td>( F_{(4,80)} = 2.781, p=0.03^{*} )</td>
</tr>
<tr>
<td>Cingulate Gyrus</td>
<td>( F_{(2,82)} = 1.564, p=0.22 )</td>
</tr>
</tbody>
</table>

*The effect of group remained when including age, sex and number of years of education into the model (\( F_{(4,60)} = 3.454, p=0.013 \)) but was not supported by the vertex-wise analysis.
3.3. Study 3. Dose-occupancy Relationship of Phenelzine and Moclobemide: Potential Implications for Novel Antidepressant Development and Optimal Dosing of Existing Monoamine Oxidase Inhibitors

These results are modified from a manuscript that has been accepted by the International Journal of Neuropsychopharmacology (pending minor revisions).

3.3.1 Demographics and Clinical Characteristics

The three groups (Total Daily Moclobemide Dose of 300mg to 600mg, Total Daily Moclobemide Dose of 900mg to 1200mg, Total Daily Phenelzine Dose of 45 to 60mg) had similar scores on the 17-item HDRS prior to treatment ($F_{(2,24)} = 0.04, p=1.0$), similar rates of reversed neurovegetative MDE symptoms ($X^2_{(2)} = 0.05, p=1.0$) and were of similar age ($F_{(2,24)} = 0.9, p=0.4$). They also had similar regional baseline MAO-A $V_T$ values across regions of interest including the PFC, ACC, ventral striatum, dorsal putamen, thalamus, midbrain and hippocampus ($F_{(14,32)} = 1.079, p=0.41$). However, the number of previous treatments ($F_{(2,24)} = 3.1, p=0.06$) and number of classes of previous antidepressant trials differed ($F_{(2,24)} = 3.6, p=0.04$), as would be expected based on the assignment criteria (table 3.8).
Table 3.8. Demographics, Clinical History and Treatment Response for Treatment Groups

<table>
<thead>
<tr>
<th></th>
<th>Moclobemide Group</th>
<th>Phenelzine Group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moclobemide Dose</td>
<td>Phenelzine Dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(300-600mg, n=11)</td>
<td>(45-60mg, n=5)</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>32.6 (8.3)</td>
<td>42.8 (6.2)</td>
<td>F (2, 24) = 2.5, p=0.1</td>
</tr>
<tr>
<td>No. Male, Female</td>
<td>2,9</td>
<td>3,2</td>
<td>X^2 (2) = 3.9, p=0.1</td>
</tr>
<tr>
<td><strong>Clinical History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset, mean (SD)</td>
<td>20.4 (9.8)</td>
<td>27.8 (10.8)</td>
<td>F (2, 24) = 0.9, p=0.4</td>
</tr>
<tr>
<td>No. of previous episodes, mean (SD)</td>
<td>3.6 (4.9)</td>
<td>1.2 (0.5)</td>
<td>F (2, 24) = 1.0, p=0.4</td>
</tr>
<tr>
<td>No. with reversed neurovegetative symptoms</td>
<td>2</td>
<td>1</td>
<td>X^2 (2) = 0.05, p=1.0</td>
</tr>
<tr>
<td>Number of previous antidepressant trials</td>
<td>1.7 (1.9)</td>
<td>5 (4.3)</td>
<td>F (2, 24) = 3.1, p=0.06</td>
</tr>
<tr>
<td>Number of antidepressant classes in previous trials</td>
<td>1.2 (1.3)</td>
<td>3.0 (2.1)</td>
<td>F (2, 24) = 3.6, p=0.04</td>
</tr>
<tr>
<td><strong>Treatment Response</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRSD score pre-treatment, mean (SD)</td>
<td>20.7 (5.3)</td>
<td>21.2 (3.9)</td>
<td>F (2, 24) = 0.04, p=1.0</td>
</tr>
<tr>
<td>HRSD score post-treatment, mean (SD)</td>
<td>12.0 (4.0)</td>
<td>9.0 (6.9)</td>
<td>F (2, 24) = 1.1, p=0.4</td>
</tr>
</tbody>
</table>
### 3.3.2 Dose-Occupancy Relationship of Moclobemide

The dose-occupancy relationship of moclobemide significantly fit the hyperbolic function $F(x) = \frac{a(x)}{b+x}$, where ‘a’ signifies the maximal occupancy and ‘b’ signifies the dose at which half the maximal occupancy is reached ($F_{(1,18)} = 5.57$ to $13.32$, $p=0.002$ to $0.03$). Values for ‘a’ ranged from 85-92% and ‘b’ ranged from 64-79mg (Fig 3.9 and Fig 3.10). The mean occupancy ranged from $71.48\pm 9.12\%$ (total daily dose of 300mg) to $85.40\pm 1.91\%$ (total daily dose of 1200mg). Consistent with this, applying dose as the covariate and the PFC, ACC, ventral striatum, dorsal putamen, thalamus, midbrain and hippocampus occupancy as the dependent variables, a MANCOVA revealed a significant main effect of moclobemide dose on MAO-A occupancy ($F_{(7,12)} = 5.58$, $p=0.006$).
Figure 3.7. The relationship between monoamine oxidase-A occupancy and dose of moclobemide.

The data were fit using the hyperbolic equation $F(x) = a(x / b + x)$, where ‘a’ signifies the maximal occupancy and ‘b’ signifies the dose at which half the maximal occupancy is reached. (a) Depicted here are a selected number of brain regions however, the model significantly fit the data in each brain region tested. (b) The hyperbolic equation for each brain regions of interest.
3.3.3 MAO-A Occupancy of Three Groupings (Total Daily Dose of 300mg to 600mg Moclobemide, Total Daily Dose of 900mg to 1200mg Moclobemide, Total Daily Phenelzine Dose of 45 to 60mg)

A MANOVA revealed a significant main effect of higher doses of moclobemide (1200mg and 900mg) and phenelzine (45 and 60mg) on MAO-A occupancy across brain regions sampled when compared to the average clinical doses of moclobemide (300 - 600mg) (MANOVA main effect: $F_{(7,16)}=3.94$, $p=0.01$, regional comparisons: $t= 2.27-4.28$, $p = <0.001-0.03$). The mean brain MAO-A occupancy by moclobemide at average daily dose (300-600mg total daily dose, $n = 11$) was 74.23±8.32% (CI: 68.64-79.82%). The mean MAO-A occupancy by moclobemide at higher doses (900-1200mg total daily dose, $n=9$) was 83.75±5.52% (CI: 79.50 – 88.0). The mean MAO-A occupancy by phenelzine (45-60mg total daily dose, $n=4$) was 86.82 ± 6.89% (CI: 75.86-97.78) (Fig 3.10). The participant taking a total daily dose of 22.5 mg of phenelzine had a MAO-A occupancy of 35.26%, and was excluded from analyses as this dose is below the minimum therapeutic dose supported by clinical trials (McGrath, Stewart et al. 1987; Birkenhager, van den Broek et al. 2004). There was a trend to a relationship between plasma level of moclobemide and overall brain MAO-A occupancy across the entire range of doses ($F_{(1,19)}=4.117$, $p=0.058$). In the 300-900mg groups, numerical dose ($F_{(1,15)}=9.050$, $p=0.011$) and time since last dose ($F_{(1,15)}=8.808$, $p=0.012$) significantly predicted plasma concentration of moclobemide. Plasma level did not predict overall brain occupancy across the phenelzine doses (45-60mg, $F_{(1,2)} = 4.081$, $p=0.181$).
Figure 3.8. Monoamine oxidase-A occupancy is higher with high doses of moclobemide (900-1200mg) and phenelzine (45-60mg) compared to low doses of moclobemide (300-600mg). There was a significant main effect of dose of moclobemide (1200mg and 900mg) and phenelzine (45 and 60mg) on MAO-A occupancy across brain regions sampled when compared to the average clinical doses of moclobemide (300, 450 and 600mg) (MANOVA, $F_{(7,16)}=3.94$, $p=0.01$).
3.3.4 Post-hoc Analysis of the Relationship between MAO-A Occupancy in the Prefrontal and Anterior Cingulate Cortex and Remission

Post-hoc univariate analysis with MAO-A occupancy in the PFC and ACC as the independent variables was predictive of post-treatment remission (less than 7 on the 17-item HRSD) (PFC: $F_{(1,24)}=6.21$, $p=0.02$) ACC: $F_{(1,24)}=7.08$, $p=0.01$). As MAO-A occupancy in ROIs are highly correlated, there was a similar relationship between MAO-A occupancy and post treatment remission for all other regions tested ($F_{(1,24)}=6.69-7.30$, $p=0.01-0.02$).

3.3.5 Post-hoc Analysis Comparison of MAO-A $V_{NS}$ across Treatment Groups as Determined from the Lassen Plot

The Lassen plots were of high quality (range $R^2 = 0.8 – 0.99$). There were no significant differences in MAO-A $V_{NS}$ across the treatment groups (moclobemide range: 300-1200mg and average dose phenelzine range: 45-60mg, $F_{(2,19)}=2.325$, $p = 0.08$). There was no significant difference in MAO-A $V_{NS}$ in those with a moderate to severe MDE versus those with a mild to moderate MDE ($F_{(1,23)}=0.071$, $p=0.792$) or in those with reversed neurovegetative features versus those without these features of MDE ($F_{(1,23)}=1.616$, $p=0.216$).
4.0. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1. Study 1. Elevated Monoamine Oxidase A Binding during Major Depressive Episodes is associated with Greater Severity and Reversed Neurovegetative Symptoms

This discussion has been modified from Chiuccariello et al., 2014 Neuropsychopharmacology (Chiuccariello, Houle et al. 2014) *Reprinted from Neuropsychopharmacology, 39 (4).

Chiuccariello et al., “Elevated monoamine oxidase A binding during major depressive episodes is associated with greater severity and reversed neurovegetative symptoms”, pp. 973-980, Copyright (2014), with permission from Nature Publishing Group.

This is the first study to investigate the relationship between symptoms of MDE and MAO-A $V_T$. The main findings are that MAO-A $V_T$ is significantly greater in the PFC and ACC in moderate to severe MDE and in MDE with reversed neurovegetative symptoms. While the primary regions of interest were the PFC and ACC, there was a tendency for a similar association in all the brain regions sampled. The ability to identify the type of MDE with the highest level of MAO-A $V_T$ offers an important opportunity to improve the development and application of therapeutics that target MAO-A or the sequelae of elevated MAO-A level. In addition, these findings suggest new mechanistic relationships between MAO-A level and the symptom clusters of severity and reversed neurovegetative symptoms.

Since optimal matching of therapeutic to illness pathology should increase the likelihood of response, the association between greater severity and reversed neurovegetative symptoms with higher MAO-A $V_T$ in the PFC and ACC has the potential to be applied to improve treatments that target functions of MAO-A, or the sequelae of elevated MAO-A level. Treatments that
counter functions of MAO-A include not only MAO inhibitors, but also several other medication classes in development such as multiple monoamine reuptake inhibitors, and neuroprotective medications with antioxidant, anti-apoptotic or mitochondrial protecting properties. To date, among the symptoms correlated with elevated MAO-A $V_T$, the best evidence for a relationship to clinical response is the association between reversed neurovegetative symptoms with response to MAOIs (McGrath, Stewart et al. 1993; Quitkin, Stewart et al. 1993; Lonnqvist, Sihvo et al. 1994). However, greater global severity of MDE symptoms has also been associated with greater likelihood of MAOI response (Thase, Frank et al. 1992). While the current findings argue in support of clinical trials investigating the relationship between these symptom clusters and response to specific types of antidepressants, they also have implications for the general development of these classes of medications for phase 2 and 3 trials. Approximately 50% of clinical trials with marketed antidepressants fail to differentiate between the active therapeutic drug and placebo, hence it would be reasonable to recommend that future trials of medications targeting elevated MAO-A level or the sequelae of elevated MAO-A level sample a reasonable proportion of participants with a high severity of MDE symptoms and/or reversed neurovegetative MDE symptoms. A clinical trial in which these subtypes are under-represented could, on average, result in a poorer match of the pathology to the therapeutic drug and therefore, be less likely to achieve a superior response to placebo.

The most likely explanation for the association between greater MAO-A $V_T$ in the PFC and ACC and greater severity is that elevated MAO-A level in these regions leads to a greater severity of symptoms. In brain tissue, MAO-A level is highly correlated with MAO-A activity and MAO-A $V_T$ is primarily an index of MAO-A level (Saura, Kettler et al. 1992). Enhancement of MAO-
A activity is related to several important downstream effects since MAO-A participates in the metabolism of serotonin, dopamine and norepinephrine, produces the pro-oxidant hydrogen peroxide (Youdim, Edmondson et al. 2006) and influences the predisposition towards apoptosis (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007). The specific regions assayed in this study, which also have a reasonably high MAO-A density, also participate in neural systems dysregulated during MDE and elevations of MAO-A $V_T$ in these regions could potentially account for the individual symptoms of MDE. Specifically, subregions of the PFC and ACC are active during the induction of sad mood and during the anticipation of negative events (Liotti, Mayberg et al. 2002; Ressler and Mayberg 2007); Dorsal striatum influences executive function and psychomotor speed; Ventral striatum influences the anticipation of reward (O'Doherty, Dayan et al. 2004) and the midbrain contains monoaminergic nuclei that control the onset and maintenance of sleep (Kajimura, Uchiyama et al. 1999). Hence the locations of excessively elevated MAO-A $V_T$ have the potential to influence many of the neural systems that function abnormally during MDE, and thereby influence illness severity. Empirically, chronic depletion of multiple monoamines through reserpine administration, or acute depletion of subsets of monoamines like serotonin through tryptophan depletion, or norepinephrine and dopamine through alphamethylparatyrosine administration is associated with sad mood in humans (Freis 1954; Young, Smith et al. 1985; Verhoeff, Christensen et al. 2003). Oxidative stress elicited through over expression of glyoxalase 1 and glutathione reductase 1 genes or through other methods such as xanthine and xanthine oxidase treatment is associated with anxiety behavior in preclinical models (Hovatta, Tennant et al. 2005; Salim, Asghar et al. 2010). Genetic vulnerability towards apoptosis is associated with greater risk of MDD in some human subpopulations (Harlan, Chen et al. 2006) and both mRNA and protein levels of R1 (a nuclear
transcription factor which facilitates resistance against apoptosis) are reduced in the PFC in MDD (Thalmeier, Dickmann et al. 2008; Johnson, Stockmeier et al. 2011).

The present study represents the first example of an abnormality in MDE that is increased in the presence of reversed neurovegetative symptoms. Most biological markers of MDE, such as increased plasma TNFα levels, and hypothalamic-pituitary-axis cortisol activity, are suppressed when reversed neurovegetative symptoms are present (Gold and Chrousos 2002; Dunjic-Kostic, Ivkovic et al. 2012; Karlovic, Serretti et al. 2012). Thus, the association between elevated MAO-A VT in the PFC and ACC and reversed neurovegetative symptoms provides new directions to investigate their etiology, particularly for weight gain. A number of factors may lead to elevated MAO-A level or activity, including glucocorticoid administration, estrogen depletion and mitochondrial toxicity/dysfunction (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007; Sacher, Wilson et al. 2010). This may suggest that the latter marker, if present throughout the body and brain, may be the best theoretical direction to investigate for weight gain because mitochondrial dysfunction in muscle has been identified in obesity and impaired mitochondrial function reduces the ability of muscle to be active and expend energy (Lowell and Shulman 2005; Patti and Corvera 2010).

This study has the advantage of measuring an index of MAO-A density in vivo but has disadvantages inherent to PET imaging. The resolution of PET does not allow investigation of the cellular specificity of the changes in MAO-A VT as MAO-A can be present in both glia and neurons. Even so, given that the most likely mechanism for elevated MAO-A in MDD is excessive glucocorticoid secretion, and glucocorticoids promote transcription of MAO-A in both
of these general cell types, it is likely that severity is related to MAO-A level in both cell types. In addition, levels of the transcription factors implicated in greater MAO-A expression in MDD, such as reduced expression of R1 (a MAO-A repressor) and greater expression of TIEG-2 (a MAO-A activator), are similarly modulated by glucocorticoids in both glia and neurons (Ou, Chen et al. 2006; Grunewald, Johnson et al. 2012). A second limitation is that we selected MAO-A Vₜ as a marker. The advantage of this marker is that it is robustly measured with [¹¹C]harmine PET, but the disadvantage is that the measure represents both specific as well as free and non-specific binding. Nevertheless, the free and non-specific binding for [¹¹C]harmine represents only 15% of MAO-A Vₜ so the differences in this measure primarily reflects a change in specific MAO-A binding.

In conclusion, greater MAO-A Vₜ in the PFC and ACC is associated with greater global severity of MDE symptoms and reversed neurovegetative MDE symptoms. An advantage of these findings is that these symptoms are straightforward to measure clinically and it is rare for clinical markers of MDE to be strongly associated with biological markers of MDE. Based upon the principle that optimal matching of treatment to pathology should result in a greater therapeutic response, these findings can be applied to improve sampling of patients for phase 2 and phase 3 trials for treatments that specifically target MAO-A or the sequelae of elevated MAO-A level. In addition, these findings unveil new directions towards understanding the symptoms of MDE suggesting either a causal link (such as the association of greater MAO-A level in the PFC and ACC with greater severity of symptoms) or a correlative link (such as a marker of associated pathology in the case of the reversed neurovegetative MDE symptoms). Finally, these results are important as it has been over 25 years since it was known that reversed neurovegetative MDE
symptoms are more likely to respond to MAOI (Quitkin, Stewart et al. 1993; Lonnqvist, Sihvo et al. 1994). Greater MAO-A expression represents the first explanation that involves identification of a matching biological abnormality in MDE that is exacerbated when reversed neurovegetative MDE symptoms are present.

4.2 Study 2. Monoamine Oxidase A Total Distribution Volume (\(V_T\)) in Relation to Hippocampal Volume and Cortical Thickness in Major Depressive Disorder

This is the first study to investigate a potential relationship between elevated MAO-A \(V_T\), which is a biomarker in MDE with the largest magnitude of change, decreased hippocampal volume, which is the most replicated biomarker in MDE, and cortical thickness in MDD. No relationship was found between hippocampal MAO-A \(V_T\) and hippocampal volume in MDD and healthy controls. Additionally, there was no significant difference in hippocampal volume between those experiencing a MDE and healthy controls. MAO-A \(V_T\) was not associated with cortical thickness in the PFC or ACC in MDD. The findings of this study may provide evidence toward a multiple phenotype model of MDD, suggesting that different individuals with MDE may be better identified by different biomarkers. The lack of relationship between MAO-A \(V_T\) and structural changes in MDD may suggest that these represent two distinct biological markers or markers that are present at different stages of disease progression. These findings may provide a better understanding of MDD and more clearly identify elevated MAO-A \(V_T\) as an early stage biomarker of MDE.
Contrary to the hypothesis, there was no significant relationship between hippocampal MAO-A \( V_T \) and total hippocampal volume or hippocampal subfield volume (including CA1, CA23 and CA4DG) in MDD and healthy controls, the MDD group alone, or the healthy control group alone. Elevated MAO-A \( V_T \) and levels in MDD, as determined through PET and postmortem data, is a highly replicated finding (Meyer, Ginovart et al. 2006; Meyer, Wilson et al. 2009; Johnson, Stockmeier et al. 2011), as is decreased hippocampal volume (Stockmeier, Mahajan et al. 2004; Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009), as seen through both MRI and postmortem data. It was hypothesized that if these two pathologies share underlying mechanisms (Malberg and Duman 2003; Ou, Chen et al. 2006; Espinosa-Oliva, de Pablos et al. 2011) they may represent related central biomarkers of MDD. Since an overactive HPA-axis is a common feature of MDD (Raadsheer, Hoogendijk et al. 1994; Pariante and Lightman 2008; Zhu, Liu et al. 2014), increased action of glucocorticoids is the postulated mechanism by which MAO-A levels are likely elevated in MDE (Ou, Chen et al. 2006; Grunewald, Johnson et al. 2012) and by which volume is decreased. However, the lack of a relationship between elevated MAO-A \( V_T \) and decreased hippocampal volume in this study may suggest that these represent two mutually exclusive central biomarkers of MDD or represent different stages of disease progression.

Given the shared postulated mechanism for elevated MAO-A \( V_T \) and decreased hippocampal volume in MDD, it may not be certain that these two central biomarkers are completely mutually exclusive. It may be that elevated MAO-A \( V_T \) in MDE represents a biomarker of earlier stages of MDD, whereas decreased hippocampal volume may represent a later stage biomarker. This fits well with some, but not all, literature, suggesting that greater hippocampal volume decreases
are seen with increasing number of MDEs and only in older age groups (MacQueen, Campbell et al. 2003; Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009). Furthermore, some literature suggests no difference in hippocampal volume in those with a first episode of depression versus healthy controls (MacQueen, Campbell et al. 2003), suggesting that hippocampal volume decreases with increasing illness duration (Sheline, Sanghavi et al. 1999; MacQueen, Campbell et al. 2003). A number of potential mechanisms have been proposed for the decreases in hippocampal volume seen in MDD, such as decreased neurogenesis (Czeh, Michaelis et al. 2001; Pham, Nacher et al. 2003; Dranovsky and Hen 2006; Jayatissa, Bisgaard et al. 2006), decreased BDNF (Erickson, Prakash et al. 2010; Hajek, Kopecek et al. 2011), and changes in glia cell density (Stockmeier, Mahajan et al. 2004; Stockmeier and Rajkowska 2004). Therefore, given the role MAO-A in apoptosis (Ou, Chen et al. 2006; Fitzgerald, Ufer et al. 2007), it may be that elevated MAO-A V_T in early stages of MDD may contribute to the decreases in hippocampal volume seen in later stages of disease progression.

No difference in hippocampal volume between MDD and healthy controls was found in this study. Decreased hippocampal volume in MDD is a highly replicated finding, but the lack of difference in hippocampal volume between MDE and healthy controls seen in this study is supported by some research (McKinnon, Yucel et al. 2009). Although the existing literature assessing the difference in hippocampal volume between MDD and healthy controls is quite comprehensive it is often associated with large variations in the magnitude of effect (Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009) and the relationship to clinical variables, such as duration of illness (Sheline, Wang et al. 1996; Sheline, Sanghavi et al. 1999), number of episodes (Videbech and Ravnkilde 2004) or treatment (Sheline, Gado et al. 2003; Malykhin,
A recent meta-analysis of 32 MRI studies assessing hippocampal volume in MDD versus healthy controls found that in MDD, the hippocampal volume was approximately 4% lower (McKinnon, Yucel et al. 2009). Interestingly, this decrease in hippocampal volume was only found in those that had a duration of episode longer than 2 years and had more than one MDE (McKinnon, Yucel et al. 2009). Another interesting finding from the study by McKinnon and colleagues was that this significant decrease in hippocampal volume in MDD was only seen in older adults, with only a 1.5% decrease in hippocampal volume in young adults with MDD (McKinnon, Yucel et al. 2009). The lack of difference in hippocampal volume in MDE and healthy controls may be accounted for by the clinical characteristics in the sample included in this research, for which less evidence exists to suggest hippocampal volume changes.

There was no relationship between MAO-A V_T and cortical thickness in the PFC or ACC in MDD and healthy controls, the MDD group alone, or the healthy control group alone when controlling for age, sex and number of years of education. Furthermore, cortical thickness was not decreased in the PFC or ACC in MDD relative to healthy controls. To date, there has not been much research to determine a relationship between cortical thickness and MDD. However, recent literature suggests the most replicated differences in cortical thickness in MDD versus healthy controls are in PFC and ACC regions (Jarnum, Eskildsen et al. 2011; Tu, Chen et al. 2012). Often, these decreases in cortical thickness are related to a family history of MDD (Peterson, Warner et al. 2009), number of MDEs (Tu, Chen et al. 2012), or symptom severity (van Eijndhoven, van Wingen et al. 2013). The literature also suggests that there are areas of cortical thickening in the PFC in MDD compared to healthy controls (Peterson, Warner et al. 2009; Papmeyer, Giles et al. 2014; Qiu, Lui et al. 2014). It may be that a pattern of cortical
thinning and thickening in MDD is emerging and it may relate to differences in synaptic plasticity or a lack thereof in MDD (Peterson, Warner et al. 2009; Pampmeyer, Giles et al. 2014; Qiu, Lui et al. 2014). The lack of relationship between MAO-A Vₜ and cortical thickness may again represent MAO-A as an earlier stage biomarker of MDD that may be related to changes in cortical thickness at a later stage of disease progression.

Although the findings of the current study are in line with some existing literature, there are potential limitations that may have restricted our ability to detect a relationship between elevated MAO-A Vₜ and structural changes in MDD or a difference in structural changes between MDD and healthy controls. First, as previously mentioned, PET does not allow investigation of the cellular specificity of the changes in MAO-A Vₜ as MAO-A can be present in both glia and neurons. Glucocorticoid secretion can promote transcription of MAO-A in both of these cell types, however, it may be that changes in one cell type versus another may be more related to structural changes in MDD. Secondly, since this was a cross-sectional study design, it was not possible to assess whether long durations of elevated MAO-A level could eventually contribute structural differences (i.e. decreased hippocampal volume and cortical thickness). Furthermore, since this was a secondary analysis of data, the restricted age range of the current study could be considered a potential limitation. It was not possible to investigate a relationship between MAO-A Vₜ and structural changes at a later stage of disease progression and this may have limited the ability to detect a difference in structural changes between MDD and healthy controls. However, overall this study was able to provide increased evidence of elevated MAO-A Vₜ as an early stage central biomarker on MDD.
In conclusion, this research found no direct relationship between MAO-A $V_T$ and decreases in hippocampal volume or cortical thickness in the PFC or ACC in MDD and healthy controls. There were no significant differences in hippocampal volume, subfields of the hippocampus or PFC or ACC cortical thickness between those with MDD compared to healthy controls. The lack of relationship between MAO-A $V_T$ and decreased hippocampal volume and decreased PFC and ACC cortical thickness may suggest that elevated MAO-A $V_T$ represents an early stage central biomarker of MDD, whereas structural changes may represent a later stage marker of disease progression. The finding that there were no significant differences in hippocampal volume or cortical thickness in the PFC and ACC between MDD and healthy controls may suggest that structural changes seen in MDD are more common in an older age range, relative to young adults, similar to the findings of a recent meta-analysis (McKinnon, Yucel et al. 2009). Taken together, the findings of this study contribute to the understanding of MAO-A as a central biomarker of early stage MDD and support the possibility that structural changes are more pronounced during later stages of MDD.

4.3. Study 3. Monoamine Oxidase-A Occupancy by Moclobemide and Phenelzine: Implications for the Development of Monoamine Oxidase Inhibitors

This discussion is modified from a manuscript that has been accepted by the International Journal of Neuropsychopharmacology (pending minor revisions).

This is the first study to determine the MAO-A occupancy of multiple MAOIs at a range of therapeutic doses. A main finding of this study is that MAO-A occupancy is significantly increased with increasing doses of moclobemide. Furthermore, MAO-A occupancy of two
different antidepressant regimens applied in treatment-resistant MDE (higher doses of moclobemide (900-1200mg total daily dose) or phenelzine (45-60mg total daily dose)) were significantly greater than MAO-A occupancy of a regimen applied in MDE with minimal histories of treatment-resistance (lower dose moclobemide (300-600mg total daily dose)). These results have important implications for choosing optimal MAO-A occupancy for novel antidepressants, the use of moclobemide treatment and the treatment algorithm of MAOIs in MDD.

The dosing of moclobemide typically applied in MDE with minimal histories of treatment-resistance was associated with a MAO-A occupancy of 74% and the dose of moclobemide or phenelzine applied in MDE with histories of treatment-resistance was associated with a MAO-A occupancy of 84%. These finding suggest that when designing new MAO-A inhibitors, reaching a 74% occupancy is a suitable target for a novel first-line MDE antidepressant but reaching a 84% occupancy is a suitable target for MDE with histories of treatment-resistance. Given that the doses of moclobemide at 900mg and 1200mg are associated with a requirement of mild dietary tyramine restriction and the other clinically available MAOIs phenelzine and tranylcypromine, require stringent dietary tyramine restrictions at all doses, there is presently a therapeutic gap such that there is no MAOI that achieves an 85% occupancy without requiring some level of tyramine restriction (Simpson and Gratz 1992; Dingemanse, Wood et al. 1998; Magder, Aleksic et al. 2000; Marcason 2005). While it might seem contradictory to recommend a higher MAO-A occupancy for treatment of MDD since MAO-A density in MDE is elevated 35 to 40%, these numbers need not match for at least a couple of reasons: First, the elevation in MAO-A level may have been present for months to years with a number of downstream effects
and an antidepressant clinical trial is only six weeks. Therefore, it may be necessary to target an occupancy higher than the increase in MAO-A $V_T$ seen in MDE to improve the effectiveness of the treatment over the short duration of treatment course. Second, there are a number of therapeutic targets in MDE, some of which may not be influenced solely by lowering available MAO-A, therefore raising monoamines excessively to reach other targets such as key signal transduction molecules may be important (Dwivedi 2009).

The current findings also have important implications for treatment algorithm design with MAOIs. With regards to prescribing moclobemide, the data demonstrates that for moclobemide, the typical dose occupancy curve has not reached a plateau across the doses tested so after non-response at a lower dose, it is logical to expect greater target engagement at a higher dose. With respect to switching from a higher dose of moclobemide to phenelzine (since MAO-A occupancies are reasonably similar) the best rationale would be to obtain additional targets with phenelzine, such as MAO-B or semicarbazide-sensitive amine oxidase inhibition or elevation of GABA (Baker, Wong et al. 1991; Baker, Coutts et al. 1992; Holt, Berry et al. 2004). Another clinically relevant point is that low doses of irreversible MAOIs are unlikely to achieve 100% occupancy, or even a substantial occupancy, since the phenelzine occupancy for doses between 22.5mg and 60mg daily ranged from 35% to 88%. This is an important issue since there is a widespread assumption that irreversible MAOI treatments obtain high occupancy at low dose, an assumption inherent in the selection of low dose tranylcypromine for the STAR*D trial (McGrath, Stewart et al. 2006; Nolen, van den Broek et al. 2007).
It is interesting that none of the dosing regimens reached an occupancy of 100% and the occupancy hyperbolic curve fits suggest that 100% occupancy would not be achievable at even very high doses of moclobemide. This is consistent with in vivo occupancies for some medications for other targets: For example maximal D₂ occupancies have been observed for clozapine at 60% (Nordstrom, Farde et al. 1995) and 90% at the serotonin transporter for most SSRIs (Meyer, Wilson et al. 2004; Voineskos, Wilson et al. 2007). There are a number of reasons as to why these medications may not reach 100% occupancy in the brain which may include presence of a subpopulation of MAO-A proteins that are accessible to [¹¹C]harmine but not moclobemide or phenelzine or a dramatically increased synthesis of MAO-A under high occupancy states.

A potential limitation is that this study was not designed to examine the relationship between occupancy and therapeutic response. While this is theoretically possible, most clinical trials differentiating antidepressants from placebo investigation require 100 or more subjects in each treatment group (Thase 1999; Gibertini, Nations et al. 2012), which is not generally feasible for PET imaging studies due to the cost. However, it was found in a post-hoc analysis that occupancy was predictive of remission as measured by the HRSD and it is notable that one out of five subjects that remitted post-treatment had an occupancy below 82%, whereas four out of 5 subjects the remitted post-treatment had an occupancy greater than 82%.

In conclusion, this is the first study to evaluate the relationship between dose and MAO-A occupancy for moclobemide and to investigate the occupancy of typical clinical doses of phenelzine. These findings have direct implications for MAO-A inhibitor development. To
design a MAO-A inhibitor as first line for MDE, an occupancy of at least 74% is recommended since this corresponds to the occupancy of low doses of moclobemide, whereas to design a MAO-A inhibitor as second line treatment of MDE or for more treatment resistant MDE, an occupancy of at least 84% is desirable because this corresponds to the occupancy of moclobemide and phenelzine dosing used in these clinical situations. The current data also demonstrates that a plateau in the dose occupancy relationship for moclobemide has not yet been reached across typical therapeutic doses. Therefore, in order to attempt to engage more target with treatment, there is a rationale for raising the dose of moclobemide from a 300 to 600mg daily dose to a 900 to 1200mg total daily dose. Also, since MAO-A occupancy with phenelzine treatment of 45 to 60mg daily is comparable to moclobemide at 900 to 1200mg daily, the main rationale for switching across these treatments is to engage additional targets with phenelzine rather than greater occupancy of MAO-A. Future studies of new MAO-A inhibitors should incorporate MAO-A occupancy measurement to assess whether they offer distinct advantages of target engagement relative to their side effects compared to presently available treatments.

4.4 **Overall Conclusions**

Overall, the current body of research has identified clinical predictors of elevated MAO-A, the optimal MAO-A occupancy by MAOIs and has suggested MAO-A as a distinct biomarker in comparison to structural changes seen in MDE. Specifically, those with reversed neurovegetative features and a high severity of MDE symptoms have the greatest elevation in MAO-A $V_T$, the optimal target MAO-A occupancy of MAOIs ranges from 74-84% based on the target population and MAO-A represents a distinct target from structural changes in MDE. Taken together, this
research suggests a model of identifying clinical predictors that are associated with distinct central biomarker abnormalities and can be targeted by existing therapeutics to improve treatment outcomes for MDD.

A high severity or reversed neurovegetative symptoms of MDE are easily identifiable clinical predictors. The advantage to the use of clinical biomarkers is that they are inexpensive and readily clinically applicable. A high severity and reversed neurovegetative MDE symptoms have individual sensitivities of 64% and 80% respectively to predict high MAO-A $V_T$ (greater or equal to 25) in the PFC or ACC. This suggests the potential for identification of complementary markers, such as genetic factors, to increase the sensitivity to predict high MAO-A $V_T$ in the brain. Together, the sensitivity of these symptoms to predict high MAO-A $V_T$ is 100%, suggesting that the utilization of these clinical predictors in the clinic may improve treatment outcomes. These findings, taken with historical evidence suggesting individuals with these symptoms of MDE respond better to treatment with MAOIs, provides compelling evidence to increase the use of MAOIs in the clinic for a better match of treatment to pathology for these individuals. This research also highlights the importance of identifying clinical predictors of biomarker abnormalities as they have the potential to improve treatment outcomes in an easily applicable, cost-effective manner.

The lack of relationship between elevated MAO-A $V_T$ and hippocampal volume or changes in cortical thickness may provide some evidence toward a multiple phenotype model of MDD, suggesting that the presentation of MDD symptoms may be related to different underlying central biomarkers of disease for different individuals or at different stages of the disease. The
lack of relationship between MAO-A V$_T$ and structural changes in MDE does not necessarily rule out some relationship between these two replicated central biomarkers of MDD, but may suggest that elevated MAO-A V$_T$ may be an early stage marker of MDD. It may be that elevated MAO-A V$_T$ during this stage of disease may relate to structural changes at later stages of disease progression. Overall, this research would suggest the importance of identifying the distinctiveness or relatedness of different biomarkers of MDD to better understand the disease pathology.

The findings of the MAOI occupancy study provide important implications for the treatment algorithm with MAOIs, suggesting that for a first-line approach, a lower dose of moclobemide (300-600mg) may be used, however, for more treatment resistant cases of MDE, high doses of moclobemide (900-1200mg) or an average dose of phenelzine (45-60mg) may be best. It is important to establish these occupancy thresholds because empirically these are chosen more arbitrarily and there is an initiative for the development of novel, well-tolerated MAOIs. Recently, CX-157, which is a novel selective MAO-A inhibitor, has been shown to have a MAO-A occupancy of 47-72%, however, to date there has been no publication of a positive clinical trial (Fowler, Logan et al. 2010). The occupancies determined in the trial of CX-157 might be considered suboptimal based on the findings of the current research, which would suggest that an occupancy of approximately 84% may be the best target for the development of novel therapeutics to ensure a positive clinical trial since most individuals with MDE require second-line antidepressant treatment.
MDD is a very heterogeneous disorder and a number of pathologies may lead to the presentation of depressive symptoms. One method to improve the treatment efficacy of MDD is to identify clinical predictors of MDE based on known central or peripheral biomarkers that could be targeted with existing therapeutics. A number of advantages to clinical practice emerge from this body of research. Firstly, this research has provided an approach to optimize treatment targeting MAO-A in MDD. Specifically, if a high severity or reversed neurovegetative MDE symptoms are present, it might suggest optimal targeting of MAO-A using a MAOI. Secondly, the research suggests that an occupancy of 74% (or doses of moclobemide ranging from 300-600mg) may be the best target for individuals with little or no exposure to antidepressant medication and an occupancy of 84% (or high dose moclobemide (900-1200mg) or average dose phenelzine) for more treatment resistant cases of MDE. This may increase the chances of a beneficial outcome for the patient and limit the number of different medication trials necessary as it has been shown that non-response to multiple medications may decrease the chances of a favourable outcome.

4.5 Future Directions

A number of potential future directions emerge from the body of research described. The overall model suggests the importance of identifying particular clinical predictors that relate to central or peripheral biomarkers of MDE and can be targeted by existing therapeutics. In addition to improving the clinical predictors associated with elevated MAO-A $V_T$, it could be possible to identify predictors associated with other biomarkers of MDE. Since MAO-A $V_T$ is a replicated central biomarker of MDE, and this research has shown it to be distinct from structural changes
in MDE during early stage disease progression, it may be interesting to see the relationship of MAO-A $V_T$ to structural changes at later stages of disease progression. The identification of the optimal target occupancy of MAOIs provides important implications in the development process of novel MAOIs.

Clinical predictors of MDE subtypes may result from a number of disease pathologies. Although the current research has identified the subtypes with the greatest elevation in MAO-A $V_T$, identification of multiple clinical predictors associated with changes in biomarkers of MDE can enhance the applicability of this research in the treatment of more mild cases of MDE. Moreover, identification of clinical predictors related to multiple disease pathologies might increase the understanding of the heterogeneity and improve the treatment approach to MDE. For example, it has been shown that those with the greatest severity of MDE symptoms also have the greatest elevation in TSPO $V_T$ in the ACC, a marker of the density of the translocator protein associated with activation of microglia in the brain (Setiawan, Wilson et al. 2015). This might suggest that treatments that can also decrease inflammation may be beneficial in the treatment of MDE with a high severity of symptoms as well. This could also suggest the use of clinical predictors (i.e. a high severity of MDE symptoms) to determine a collection of different phenotypes of MDE. Overall, this line of work could contribute to better identifying particular clinical predictors that are associated with the greatest changes in specific central biomarkers of MDE and lead to a better match of patient and treatment, thereby in part, addressing the heterogeneity of MDD.
Although the current research did not find a relationship between MAO-A $V_T$ and structural changes commonly seen in MDD, it does not confirm that these central biomarkers are completely mutually exclusive. As previously stated, it could be that elevated MAO-A levels represent an early stage biomarker of MDD that may be more directly related to structural changes in MDD at a later stage of disease progression. To date, no research has investigated changes in MAO-A $V_T$ in early onset MDD at ages above 50 years old. Perhaps in this older age range, there is a relationship between MAO-A $V_T$ and structural changes given the potentially shared common underlying mechanism of an overactive HPA-axis. Furthermore, perhaps an ideal way to understand MDD pathology is to work toward identification of the relatedness or distinctiveness of a peripheral and central biomarker panel. Since it is known that a number of factors are implicated in the pathology of MDD, and MAO-A is a marker associated with a large magnitude of change, it may be of interest to determine the relationship of this biomarker to other peripheral and central markers in MDD, such as genetic factors or markers associated with oxidative stress.

Identification of the optimal MAO-A occupancy for MAOIs through this research provides important information for better utilizing these medications in the clinic. The findings suggest that dose is the best predictor of occupancy for available MAOIs. The findings have important implications for the treatment algorithm approach in attempting to minimize unnecessary side effects and maximizing the benefit of these medications. This research would suggest that in the initiative for the development of novel MAOIs, future drug development should aim for the targets proposed here to maximize the potential for the medication to be effective and result in a positive clinical trial. Development of novel medications is often not successful for a number of
reasons, one being a lack of power to determine a significant difference between active drug and placebo in clinical trials. Incorporation of a population with reversed neurovegetative features of MDE and a high severity of MDE symptoms could thereby improve the efficacy of clinical trials of novel MAOIs. Furthermore, these trials might identify those that respond best to treatment with the novel MAOI, thereby potentially replicating the findings presented here: i.e. those with the highest elevation in MAO-A $V_T$ are the individuals that respond best to treatment with MAOIs.

### 4.6 Final Comment

The current body of research is the first to identify clinical predictors associated with increased MAO-A $V_T$, the optimal MAO-A occupancy of MAOIs and MAO-A as a distinct biomarker in relation to structural changes associated with MDE. MDD, like many psychiatric illnesses, is a very heterogeneous disorder and it is suggested that by the year 2030 it will be the leading cause of death and disability worldwide. It is associated with a high rate of treatment resistance and up to 60% of individuals will have a recurrence of symptoms within 2 years of illness onset. There is a need to improve the understanding of MDD as a heterogeneous disorder and to tailor treatments to better match the treatment to the patient in order to attempt to overcome treatment resistance. The model presented in this body of research suggests an approach to optimize the treatment of MDD, by identification of clinical predictors associated with distinct biomarkers of disease which are targetable by existing therapeutics.
5.0 REFERENCES


Liu, Y., R. C. Ho, et al. (2012). "Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression." J Affect Disord 139(3): 230-9.


Papmeyer, M., S. Giles, et al. (2014). "Cortical Thickness in Individuals at High Familial Risk of Mood Disorders as They Develop Major Depressive Disorder." Biol Psychiatry.


