

Elevated Monoamine Oxidase A Binding During Major Depressive Episodes Is Associated with Greater Severity and Reversed Neurovegetative Symptoms

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Inadequate treatment response occurs in approximately 40% of major depressive episodes (MDEs), and one approach to solve this is careful matching of treatment to the specific pathologies of MDE. One such biological abnormality is elevated monoamine oxidase A (MAO-A) levels, which occurs in the prefrontal and anterior cingulate cortex (PFC and ACC) during MDE; however, the subtypes for which this abnormality is most prominent are unknown. We hypothesized that MAO-A levels in the PFC and ACC are most elevated in MDE with greater severity and reversed neurovegetative symptoms (hypersomnia and either hyperphagia or weight gain). MAO-A V_T (an index of MAO-A density) was measured using [¹¹C]harmine positron emission tomography (PET) in 42 subjects with MDEs secondary to major depressive disorder and 37 healthy controls. The effect of severity and reversed neurovegetative symptoms on MAO-A V_T in the PFC and ACC was analyzed using a multivariate analysis of variance (MANOVA). Greater severity and reversed neurovegetative symptoms were associated with elevated MAO-A V_T in the PFC and ACC (MANOVA, severity: $F_{(2,38)} = 5.44$, $p = 0.008$; reversed neurovegetative symptoms: $F_{(2,38)} = 5.13$, $p = 0.01$). Increased MAO-A level, when greater severity and reversed neurovegetative symptoms are present, may explain the association of these clinical features with a preferential response to MAO inhibitors, which is especially well-evidenced for reversed neurovegetative symptoms in MDE. As MAO-A creates oxidative stress, facilitates apoptosis, and metabolizes monoamines, therapeutics opposing these processes are predicted to best treat MDE with greater severity and reversed neurovegetative symptoms.

Neuropsychopharmacology (2014) **39**, 973–980; doi:10.1038/npp.2013.297; published online 13 November 2013

Keywords: oxidative stress; apoptosis; serotonin; monoamine oxidase; major depressive disorder

INTRODUCTION

Major depressive disorder (MDD) is the leading cause of death and disability in moderate to high income countries (WHO, 2008). A key reason for this is treatment resistance, as fewer than 40% of major depressive episodes (MDEs) have an optimal response to initial treatment (Trivedi *et al*, 2006). One strategy to improve treatment response is to apply clinical measures or biomarkers to identify pathological subtypes so as to guide treatment selection. Although there are no commonly applied clinical subtyping strategies or biomarkers to select first- or second-line treatments for

MDE, this strategy is anticipated to improve MDE response to treatment because many different biological abnormalities have been identified, including reduced glial cell density, deficient signal transduction, deficient neurogenesis, reduced hippocampal volume, elevated cytokine levels, greater glucocorticoid secretion, greater oxidative stress, markers of increased apoptosis, and indices reflecting lowered monoamines (Meyer, 2012; Rajkowska and Stockmeier, 2013; Schmidt *et al*, 2011).

Among markers of MDE pathology, there are several key reasons to investigate indices of central monoamine oxidase A (MAO-A) level as a biomarker for subtyping: MAO-A has diverse functions, including facilitating a pro-oxidant state through the production of hydrogen peroxide, participating in apoptosis, influencing mitochondrial function, and metabolizing monoamines (Youdim *et al*, 2006), each of which have sequelae that may be targeted by a variety of therapeutics. MAO-A level is highly correlated with MAO-A activity in brain (Nelson *et al*, 1979; Saura *et al*, 1992) and indices of MAO-A level in the prefrontal (PFC) and anterior cingulate cortex (ACC) were elevated by 25–40% across the

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Received 30 July 2013; revised 16 October 2013; accepted 17 October 2013; accepted article preview 24 October 2013

three studies that investigated the most common, early onset MDD (Johnson *et al*, 2011; Meyer *et al*, 2006; Meyer *et al*, 2009). Elevated MAO-A level in these regions in MDE is consistent with other specific findings: MDE is a high-stress state, and glucocorticoid administration increases transcription rate and catalytic activity of MAO-A in human neuroblastoma and glioblastoma cell lines (Ou *et al*, 2006a). Furthermore, glucocorticoid administration and/or chronic stress increase MAO-A mRNA and/or MAO-A protein levels in the PFC of rodents (Filipenko *et al*, 2002; Slotkin *et al*, 1998). In humans, during MDE, greater whole-brain serotonin turnover has been reported (Barton *et al*, 2008) and this is consistent with greater MAO-A level and/or activity as MAO-A is the primary route of serotonin metabolism in brain. Also, reduced mRNA and levels of nuclear transcription factor R1, which inhibits MAO-A transcription, and elevated levels of TIEG2, a nuclear transcription factor that promotes transcription of MAO-A, were found in postmortem PFC in MDE (Grunewald *et al*, 2012; Johnson *et al*, 2011; Ou *et al*, 2006a, b).

Additional reasons to pursue clinical subtyping in relation to MAO-A level include the availability of irreversible monoamine oxidase inhibitors (MAOIs) for clinical use and the recent success of new MAOIs that overcome the restriction of dietary tyramine associated with the original MAOIs (Youdim *et al*, 2006). The latter is best exemplified by the design of compounds with greater selectivity for MAO-A or MAO-B and/or greater reversibility (such as moclobemide and EMSAM) of MAOIs in which the parent medication is inactive peripherally but then metabolized to be active in the brain, thereby increasing the ratio of brain to peripheral concentrations of the drug (Youdim *et al*, 2006). This latter set of compounds is being tested in phase 2 trials in humans.

In the present study, we apply [¹¹C]harmine PET to determine which subtypes of MDE have the highest MAO-A V_T , an index of regional brain MAO-A level, with our primary regions of interest being the PFC and ACC (for review of [¹¹C]harmine properties see Meyer *et al* (2006, 2009)). Although all regions sampled to date tend to demonstrate elevated MAO-A V_T during MDE, we prioritized these two regions because the accumulated evidence is greatest for elevated MAO-A level in these two regions (Johnson *et al*, 2011; Meyer *et al*, 2006, 2009) and subregions of these structures are activated during sad mood and negative cognition (Liotti *et al*, 2002; Ressler and Mayberg, 2007).

For the present study, we focus on two subtypes of MDE: severity (ie, moderate to severe versus mild level of symptoms) and reversed neurovegetative symptoms (presence of hypersomnia and either hyperphagia or weight gain). The rationale for investigating severity was based on a data set from an earlier study in which we observed that in MDE subjects, 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 PFC relative to those MDE with a HRSD of less than 20 (specific subanalysis not published). The rationale for choosing MDE with reversed neurovegetative symptoms as the second subtype is that these symptoms are associated with a greater likelihood of response to MAOIs (Lonnqvist *et al*, 1994; Quitkin *et al*, 1993), which could be explained by greater expression of the MAO-A target, ie, treatments

which best match the pathology should have a higher likelihood of response. Moreover, a latent class analysis found that atypical MDE is primarily identified by the symptoms of increased appetite, weight gain, and hypersomnia (Sullivan *et al*, 1998), and among atypical symptoms, this cluster is associated with the greatest inter-rater reliability (kappa values: hyperphagia, 1.0; hypersomnia, 0.90; mood reactivity, 0.83; rejection sensitivity, 0.75; and leaden paralysis, 0.78; Posternak and Zimmerman, 2001). Thus, the hypotheses of the present study are that subtypes of MDE with greater severity of symptoms (as defined by a HRSD ≥ 20) and reversed neurovegetative symptoms will have greater MAO-A V_T in the PFC and ACC.

MATERIALS AND METHODS

Participants

Forty-two participants were recruited who met criteria for current MDE and MDD (mean age = 31.6 years, SD = 7.5) as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) and verified by subsequent consultation with a psychiatrist (RGC, RDL, or JHM). The presence of reversed neurovegetative symptoms of depression were verified by the SCID-I. Data from 15 subjects had been previously compared with healthy subjects but had not been examined in relation to subtype (and were not the data reviewed in the introduction to justify the first hypothesis). Inclusion criteria were early onset MDD (onset prior to age 45) and current age from 18 to 50 years as the intent of this study was to subtype early onset MDD. Other inclusion criteria were good physical health and a HRSD of at least 14 on the 17-item HRSD. Exclusion criteria included co-morbid psychiatric or medical illness, current or past substance abuse, report of cigarette smoking within the past year (since recent heavy cigarette smoking may bias MAO-A V_T (Bacher *et al*, 2011; Fowler *et al*, 1996)), positive urine toxicology drug screen, and herbal drug or medication use within the past 8 weeks with the exception of SSRIs for which the exclusion period was 2 weeks (37 participants had no SSRI use in the past month). Although SSRIs, through reducing extracellular clearance of serotonin, likely counter a downstream effect of greater serotonin metabolism from elevated MAO-A level, we previously demonstrated that SSRIs do not affect MAO-A V_T *in vivo* (Meyer *et al*, 2009), a finding consistent with the pharmacological specificity of SSRIs. Specific exclusion criteria for women included current perimenopause, current postmenopause, and a positive urine pregnancy test. Phase of menstrual cycle was recorded by self-report and was consistent with estrogen and progesterone plasma samples obtained. We did not control for phase of menstrual cycle, as in a previously collected sample there was no relationship between phase of menstrual cycle and MAO-A V_T (Meyer *et al*, 2009). In addition, all participants were screened to rule out borderline and antisocial personality disorder using the Structured Clinical Interview for DSM-IV for Axis II disorders.

Participants were also screened to rule out common medical conditions associated with MDD through plasma sampling for calcium and thyroid-stimulating hormone. Also, participants were required not to consume any caffeine on the day of the PET scan and were required not

Table 1 Demographic and Clinical Characteristics of Individuals with Moderate to Severe Depression and Mild to Moderate Depression

	Moderate to severe depression (n = 25)	Mild to moderate depression (n = 17)
Age, mean (SD), years	32.7 (7.8)	30.9 (7.8)
HRSD score, mean (SD)	23.6 (2.7)	16.6 (2.2) ^a
Age at onset, mean (SD), years	19.4 (9.1)	18.6 (10.1)
No. of previous episodes, mean (SD)	2.2 (1.3)	2.7 (1.8)
Female, No. (%)	15 (60)	10 (59)
Past antidepressant treatment, No. (%)	16 (64)	10 (59)

Abbreviation: HRSD, 17-item Hamilton Rating Scale for Depression.

^aModerate-to-severe depressed participants had significantly higher HRSD scores ($p < 0.001$) but there were no significant differences among any other demographic or clinical variables between groups.

to use any over-the-counter medications or consume any alcohol for 48 h before the PET scan. For a secondary aim of comparing MDE subgroups to health, 37 healthy controls (mean age = 31.9 years, SD = 7.6) were recruited. Criteria and screening were identical to those with MDD except all psychiatric illnesses were exclusionary. Demographic characteristics are shown in Tables 1 and 2. After complete description of the study to the subjects, written informed consent was obtained. The 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.

Imaging

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

Each participant also 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, WI, USA) for the region of interest (ROI) delineation. The ROIs were determined using a semi-automated method in which regions of a template MRI are transformed on to 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 gray matter voxels as previously described (Meyer *et al*, 2009; Rusjan *et al*, 2006).

The primary ROIs were the PFC and ACC because these regions (and their subregions) participate in the affective regulation as demonstrated by paradigms of mood induction and affective cognition (Liotti *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 prefrontal cortex 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 PFC were defined based on their cytoarchitectural differences from adjacent cortex (Rajkowska and Goldman-Rakic, 1995a, b; Uylings *et al*, 2010).

MAO-A V_T represents the total distribution volume of [¹¹C]harmine and it is an index of tissue binding at equilibrium, of which 85% is specific binding to MAO-A, for further details see the Supplementary Methods section. Therefore, differences in MAO-A V_T may be interpreted as representing changes in [¹¹C]harmine binding to MAO-A. The V_T can be expressed in terms of kinetic rate parameters of a two-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* (2006)). For [¹¹C]harmine PET, V_T may be validly and reliably measured with either an unconstrained two-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 *et al*, 2006), and the latter was applied in this study. This method has been previously described in detail (Ginovart *et al*, 2006; Meyer *et al*, 2009).

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

Table 2 Demographic and Clinical Characteristics Data of Individuals with and without Reversed Neurovegetative Symptoms of Depression^a

	With reversed neurovegetative symptoms (n = 10)	Without reversed neurovegetative symptoms (n = 32)
Age, mean (SD), years	31.3 (9.0)	32.2 (7.5)
HRSD score, mean (SD)	18.8 (2.9)	21.4 (4.4)
Age at onset, mean (SD), years	16.5 (6.8)	19.9 (10.0)
No. of previous episodes, mean (SD)	2.4 (1.3)	2.4 (1.6)
Female, No. (%)	5 (50)	20 (63)
Past antidepressant treatment, No. (%)	8 (80)	18 (56)

Abbreviation: HRSD, 17-item Hamilton Rating Scale for Depression.

^aThere were no significant differences among any of the demographic or clinical variables between groups.

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 moderate-to-high severity, mild-to-moderate severity, reversed neurovegetative symptoms present, and reversed neurovegetative symptoms absent.

RESULTS

Effect of Severity upon MAO-A V_T

There was a main effect of severity on MAO-A V_T in the PFC and ACC (MANOVA, $F_{(2,38)} = 5.44$, $p = 0.008$) and in the subregions of the PFC (OFC, VLPFC, DLPFC, mPFC; MANOVA, $F_{(4,36)} = 4.57$, $p = 0.004$). The whole-brain analysis did not reach significance (MANOVA, $F_{(7,33)} = 1.90$, $p = 0.10$) but there was a tendency toward a similar finding across all regions sampled (individual ANOVA, $F_{(1,39)} = 5.31$ – 11.01 , $p = 0.03$ – 0.002), and a trend in the hippocampus (ANOVA, $F_{(1,39)} = 2.59$, $p = 0.1$; Figure 1).

Effect of Reversed Neurovegetative 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 severity, there was also an effect of reversed neurovegetative symptoms on MAO-A V_T in the PFC and ACC (MANOVA, $F_{(2,38)} = 5.13$, $p = 0.01$), which was also present in subregions of the PFC (OFC, VLPFC, DLPFC, mPFC; MANOVA, $F_{(4,36)} = 3.23$, $p = 0.02$). There was a similar tendency across all regions sampled (MANOVA, $F_{(7,33)} = 2.56$, $p = 0.03$; Figure 2).

Relationship of MAO-A V_T with Severity as a Continuous Variable

Applying a multivariate analysis of co-variance (MANCOVA) with the dependent variables being MAO-A V_T in the

PFC and ACC, and predictor of HDRS as a covariate and reversed neurovegetative features as a factor, we found both to be significant predictors (severity: $F_{2,38} = 5.09$, $p = 0.01$, reversed neurovegetative symptoms $F_{2,38} = 4.71$, $p = 0.02$). ANOVAs within these two regions, with MAO-A V_T as the dependent variable, 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, $F_{1,39} = 9.64$, $p = 0.004$).

Comparison of MAO-A V_T between Subgroups with Healthy Controls

Participants with moderate-to-severe MDE ($n = 25$) had significantly greater MAO-A V_T in the PFC and ACC compared with healthy controls ($n = 37$; MANOVA, $F_{(2,59)} = 9.75$, $p < 0.001$) and this was also found in all brain regions (MANOVA, $F_{(7,54)} = 3.41$, $p = 0.004$). The subgroup with reversed neurovegetative symptoms ($n = 10$) also had significantly greater MAO-A V_T in the PFC and ACC compared with controls (MANOVA, $F_{(2,44)} = 5.710$, $p < 0.01$), which was also found across all brain regions (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, $F_{(2,51)} = 1.94$, $p = 0.16$).

DISCUSSION

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. Although we focused on the PFC and ACC, there was a tendency for a similar association in all the brain regions sampled. The ability to identify the subtype 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

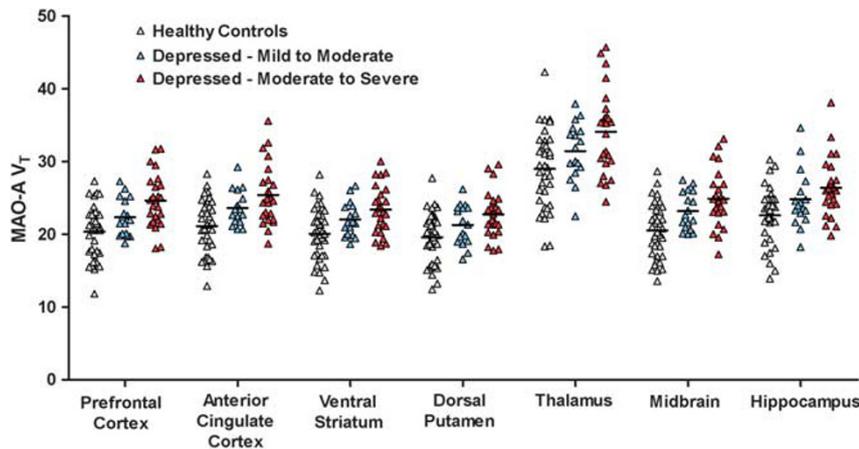


Figure 1 The effect of moderate to severe symptoms of depression ($\text{HRSD} \geq 20$) compared with mild to moderate symptoms ($\text{HRSD} \leq 19$) of depression on MAO-A V_T in regions of interest. Participants with severe depression had significantly higher MAO-A V_T in the prefrontal and anterior 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 toward a similar finding in the other regions sampled (individual ANOVA, $F_{(1,39)} = 5.31$ to 11.01 , $p = 0.002$ – 0.03), and a trend in the hippocampus (ANOVA, $F_{(1,39)} = 2.59$, $p = 0.1$).

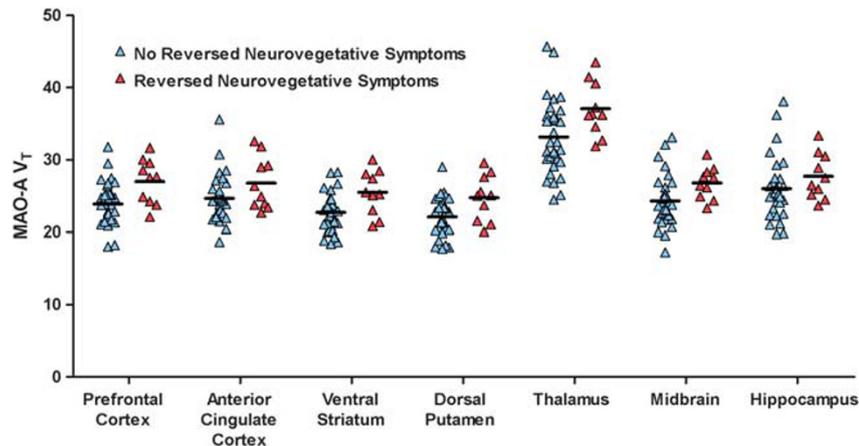


Figure 2 The effect of reversed neurovegetative symptoms of depression compared with no reversed neurovegetative symptoms of depression on MAO-A V_T in regions of interest. Participants with reversed neurovegetative symptoms of depression had significantly higher MAO-A V_T in the prefrontal and anterior cingulate cortices (multivariate analysis of variance (MANOVA), $F_{(2,38)} = 5.13$, $p = 0.01$) and this effect was also present across all the brain regions sampled (MANOVA, $F_{(7,33)} = 2.56$, $p = 0.03$). To visually demonstrate the effect of reversed neurovegetative symptoms of depression 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.

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.

As 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 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 (Lonnqvist *et al*, 1994; McGrath *et al*, 1993; Quitkin *et al*, 1993). However, greater global severity of MDE symptoms has also been associated with greater likelihood of MAOI response (Thase *et al*, 1992). Although 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 and placebo, hence, it would be reasonable to ensure 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 symptoms and/or reversed neurovegetative symptoms. It is therefore possible that a clinical trial with a sample poor in these subtypes

could, on average, have pathology that may be a poorer match to the therapeutic, and therefore be less likely to achieve a superior response to placebo.

We suggest that 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 *et al*, 1992). Elevated MAO-A activity is related to several important downstream effects, as MAO-A participates in the metabolism of serotonin, dopamine, and norepinephrine, produces the pro-oxidant hydrogen peroxide and influences the predisposition toward apoptosis (Fitzgerald *et al*, 2007; Ou *et al*, 2006b; Youdim *et al*, 2006). The specific regions assayed in this study, which 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 production of sad mood induction and the anticipation of negative events (Liotti *et al*, 2002; Ressler and Mayberg, 2007); dorsal striatum influences executive function and psychomotor speed; ventral striatum influences the anticipation of reward (O'Doherty *et al*, 2004) and the midbrain contains monoaminergic nuclei that control the onset and maintenance of sleep (Kajimura *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 α -methylparatyrosine administration is associated with sad mood in humans (Freis, 1954; Verhoeff *et al*, 2003; Young *et al*, 1985). Oxidative stress elicited through overexpression 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 *et al*, 2005; Salim *et al*, 2010). Genetic vulnerability toward apoptosis is associated with greater risk of MDD in some human subpopulations (Harlan *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 (Johnson *et al*, 2011; Thalmeier *et al*, 2008).

To the best of our knowledge, 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 TNF α levels, and hypothalamic-pituitary-axis-cortisol activity, are suppressed when reversed neurovegetative symptoms are present (Dunjic-Kostic *et al*, 2012; Gold and Chrousos, 2002; Karlovic *et al*, 2012). Thus, the association between elevated MAO-A V_T 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 (Fitzgerald *et al*, 2007; Ou *et al*, 2006a; Sacher *et al*, 2010). We 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).

The current 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 V_T 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 Sp1 (a MAO-A activator), are similarly modulated by glucocorticoids in both glia and neurons (Ou *et al*, 2006a). A second limitation is that we selected MAO-A V_T as a marker. The advantage of this marker is that it is robustly measured with [^{11}C]harmine PET, but the disadvantage is that it represents both specific and free and nonspecific binding. Nevertheless, the free and nonspecific binding for [^{11}C]harmine represents only 15% of MAO-A V_T so the difference in this measure primarily reflects a change in specific MAO-A binding.

In conclusion, greater MAO-A V_T in the PFC and ACC is associated with greater global severity of symptoms and reversed neurovegetative symptoms. A great asset of this work 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 on 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. In addition, the findings unveil new directions toward 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 symptoms). Finally, these results are important as it has been over 25 years since it was known that reversed neurovegetative symptoms are more likely to respond to MAOI (Lonnqvist *et al*, 1994; Quitkin *et al*, 1993), and greater MAO-A expression represents the first explanation that involves identification of a matching biological abnormality in MDE, which is exacerbated when reversed neurovegetative symptoms are present.

FUNDING AND DISCLOSURE

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and salary support from the Ontario Mental Health Foundation and CIHR.

Drs. Meyer, Wilson, and Houle have received operating grant funding for other studies from Eli Lilly, GlaxoSmithKline, Bristol-Myers Squibb, Lundbeck, and SK Life Science in the past 5 years. Dr Meyer has consulted to several of these companies as well as Sepracor, Trius Therapeutics and Mylan Inc. None of these companies participated in the funding, design or execution of this study or writing the manuscript. Dr Meyer is developing natural health products to treat high MAO-A states. Dr Meyer is also applying for patents to implement measures of utilizing MAO to diagnose or treat mood disorders. It is likely that companies which make medications affecting monoamine receptor or monoamine oxidase binding will seek collaborations with these investigators in the future. Dr Kish receives research funding from US NIH NIDA DA25096 and a salary from the Centre for Addiction and Mental Health. The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the Campbell Family Mental Health Research Institute; administrative assistant Natasha Bennett; technicians Alvina Ng and Laura Nguyen; chemistry staff Jun Parkes, Armando Garcia, Winston Stableford, and Min Wong; engineers Terry Bell and Ted Harris-Brandts; and students Shakira Mohammed and Kimberly De Sousa for their assistance with this project. Dr Meyer takes responsibility for the integrity of the data and the accuracy of the data analysis, and that all authors had full access to all the data in the study.

REFERENCES

- Bacher I, Houle S, Xu X, Zawertailo L, Soliman A, Wilson AA *et al* (2011). Monoamine oxidase A binding in the prefrontal and anterior cingulate cortices during acute withdrawal from heavy cigarette smoking. *Arch General Psychiatry* **68**: 817–826.
- Barton DA, Esler MD, Dawood T, Lambert EA, Haikerwal D, Brenchley C *et al* (2008). Elevated brain serotonin turnover in patients with depression: effect of genotype and therapy. *Arch General Psychiatry* **65**: 38–46.
- Dunjic-Kostic B, Ivkovic M, Radonjic NV, Petronijevic ND, Pantovic M, Damjanovic A *et al* (2012). Melancholic and atypical major depression—Connection between cytokines, psychopathology and treatment. *Prog Neuropsychopharmacol Biol Psychiatry* **43C**: 1–6.
- Filipenko ML, Beilina AG, Alekseyenko OV, Dolgov VV, Kudryavtseva NN (2002). Repeated experience of social defeats increases serotonin transporter and monoamine oxidase A mRNA levels in raphe nuclei of male mice. *Neurosci Lett* **321**: 25–28.
- Fitzgerald JC, Ufer C, De Girolamo LA, Kuhn H, Billett EE (2007). Monoamine oxidase-A modulates apoptotic cell death induced by staurosporine in human neuroblastoma cells. *J Neurochem* **103**: 2189–2199.
- Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, Shea C *et al* (1996). Brain monoamine oxidase A inhibition in cigarette smokers. *Proc Natl Acad Sci USA* **93**: 14065–14069.
- Freis ED (1954). Mental depression in hypertensive patients treated for long periods with large doses of reserpine. *N Engl J Med* **251**: 1006–1008.
- Ginovart N, Meyer JH, Boovariwala A, Hussey D, Rabiner EA, Houle S *et al* (2006). Positron emission tomography quantification of [¹¹C]-harmine binding to monoamine oxidase-A in the human brain. *J Cereb Blood Flow Metab* **26**: 330–344.
- Gold PW, Chrousos GP (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry* **7**: 254–275.
- Grunewald M, Johnson S, Lu D, Wang Z, Lomberg G, Albert PR *et al* (2012). Mechanistic role for a novel glucocorticoid-KLF11 (TIEG2) protein pathway in stress-induced monoamine oxidase A expression. *J Biol Chem* **287**: 24195–24206.
- Harlan J, Chen Y, Gubbins E, Mueller R, Roch JM, Walter K *et al* (2006). Variants in Apaf-1 segregating with major depression promote apoptosome function. *Mol Psychiatry* **11**: 76–85.
- Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM *et al* (2005). Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature* **438**: 662–666.
- Johnson S, Stockmeier CA, Meyer JH, Austin MC, Albert PR, Wang J *et al* (2011). The reduction of R1, a novel repressor protein for monoamine oxidase a, in major depressive disorder. *Neuropsychopharmacology* **36**: 2139–2148.
- Kajimura N, Uchiyama M, Takayama Y, Uchida S, Uema T, Kato M *et al* (1999). Activity of midbrain reticular formation and neocortex during the progression of human non-rapid eye movement sleep. *J Neurosci* **19**: 10065–10073.
- Karlovic D, Serretti A, Vrkic N, Martinac M, Marcinko D (2012). Serum concentrations of CRP, IL-6, TNF-alpha and cortisol in major depressive disorder with melancholic or atypical features. *Psychiatry research* **198**: 74–80.
- Liotti M, Mayberg HS, McGinnis S, Brannan SL, Jerabek P (2002). Unmasking disease-specific cerebral blood flow abnormalities: mood challenge in patients with remitted unipolar depression. *Am J Psychiatry* **159**: 1830–1840.
- Lonnqvist J, Sihvo S, Syvalahti E, Kiviruusu O (1994). Moclobemide and fluoxetine in atypical depression: a double-blind trial. *J Affect Disord* **32**: 169–177.
- Lowell BB, Shulman GI (2005). Mitochondrial dysfunction and type 2 diabetes. *Science* **307**: 384–387.
- McGrath PJ, Stewart JW, Nunes EV, Ocepek-Welikson K, Rabkin JG, Quitkin FM *et al* (1993). A double-blind crossover trial of imipramine and phenelzine for outpatients with treatment-refractory depression. *Am J Psychiatry* **150**: 118–123.
- Meyer JH (2012). Neuroimaging markers of cellular function in major depressive disorder: implications for therapeutics, personalized medicine, and prevention. *Clin Pharmacol Ther* **91**: 201–214.
- Meyer JH, Ginovart N, Boovariwala A, Segrati S, Hussey D, Garcia A *et al* (2006). Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. *Arch General Psychiatry* **63**: 1209–1216.
- Meyer JH, Wilson AA, Segrati S, Miler L, Rusjan P, Bloomfield PM *et al* (2009). Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence. *Arch General Psychiatry* **66**: 1304–1312.
- Nelson DL, Herbet A, Glowinski J, Hamon M (1979). [³H]Harmaline as a specific ligand of MAO A-II. Measurement of the turnover rates of MAO A during ontogenesis in the rat brain. *J Neurochem* **32**: 1829–1836.
- O'Doherty J, Dayan P, Schultz J, Deichmann R, Friston K, Dolan RJ (2004). Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science* **304**: 452–454.
- Ou XM, Chen K, Shih JC (2006a). Glucocorticoid and androgen activation of monoamine oxidase A is regulated differently by R1 and Sp1. *J Biol Chem* **281**: 21512–21525.
- Ou XM, Chen K, Shih JC (2006b). Monoamine oxidase A and repressor R1 are involved in apoptotic signaling pathway. *Proc Natl Acad Sci USA* **103**: 10923–10928.
- Patti ME, Corvera S (2010). The role of mitochondria in the pathogenesis of type 2 diabetes. *Endocr Rev* **31**: 364–395.
- Posternak MA, Zimmerman M (2001). Symptoms of atypical depression. *Psychiatry Res* **104**: 175–181.

- Quitkin FM, Stewart JW, McGrath PJ, Tricamo E, Rabkin JG, Ocepek-Welickson K *et al* (1993). Columbia atypical depression. A subgroup of depressives with better response to MAOI than to tricyclic antidepressants or placebo. *Br J Psychiatry Suppl* 21: 30–34.
- Rajkowska G, Goldman-Rakic PS (1995a). Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Remapping of areas 9 and 46 using quantitative criteria. *Cereb Cortex* 5: 307–322.
- Rajkowska G, Goldman-Rakic PS (1995b). Cytoarchitectonic definition of prefrontal areas in the normal human cortex: II. Variability in locations of areas 9 and 46 and relationship to the Talairach Coordinate System. *Cereb Cortex* 5: 323–337.
- Rajkowska G, Stockmeier CA (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr Drug Targets* 14: 1225–1236.
- Ressler KJ, Mayberg HS (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci* 10: 1116–1124.
- Rusjan P, Mamo D, Ginovart N, Hussey D, Vitcu I, Yasuno F *et al* (2006). An automated method for the extraction of regional data from PET images. *Psychiatry Res* 147: 79–89.
- Sacher J, Wilson AA, Houle S, Rusjan P, Hassan S, Bloomfield PM *et al* (2010). Elevated brain monoamine oxidase A binding in the early postpartum period. *Arch General Psychiatry* 67: 468–474.
- Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K (2010). Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. *Brain Res* 1359: 178–185.
- Saura J, Kettler R, Da Prada M, Richards JG (1992). Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 *in vitro*: localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. *J Neurosci* 12: 1977–1999.
- Schmidt HD, Shelton RC, Duman RS (2011). Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology* 36: 2375–2394.
- Slotkin TA, Seidler FJ, Ritchie JC (1998). Effects of aging and glucocorticoid treatment on monoamine oxidase subtypes in rat cerebral cortex: therapeutic implications. *Brain Res Bull* 47: 345–348.
- Sullivan PF, Kessler RC, Kendler KS (1998). Latent class analysis of lifetime depressive symptoms in the national comorbidity survey. *Am J Psychiatry* 155: 1398–1406.
- Thalmeier A, Dickmann M, Giegling I, Schneider B, M Hartmann A, Maurer K *et al* (2008). Gene expression profiling of post-mortem orbitofrontal cortex in violent suicide victims. *Int J Neuropsychopharmacol* 11: 217–228.
- Thase ME, Frank E, Mallinger AG, Hamer T, Kupfer DJ (1992). Treatment of imipramine-resistant recurrent depression, III: Efficacy of monoamine oxidase inhibitors. *J Clin Psychiatry* 53: 5–11.
- Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L *et al* (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* 163: 28–40.
- Uylings HB, Sanz-Arigita EJ, de Vos K, Pool CW, Evers P, Rajkowska G (2010). 3-D cytoarchitectonic parcellation of human orbitofrontal cortex correlation with postmortem MRI. *Psychiatry Res* 183: 1–20.
- Verhoeff NP, Christensen BK, Hussey D, Lee M, Papatheodorou G, Kopala L *et al* (2003). Effects of catecholamine depletion on D2 receptor binding, mood, and attentiveness in humans: a replication study. *Pharmacol Biochem Behav* 74: 425–432.
- WHO (2008). The Global Burden of Disease: 2004 Update World Health Organization.
- Youdim MB, Edmondson D, Tipton KF (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci* 7: 295–309.
- Young SN, Smith SE, Pihl RO, Ervin FR (1985). Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)* 87: 173–177.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)