Greater Monoamine Oxidase A Binding in Alcohol Dependence

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Abstract

Background—Alcohol dependence (AD) is a multiorgan disease in which excessive oxidative stress and apoptosis are implicated. Monoamine oxidase A (MAO-A) is an important enzyme on the outer mitochondrial membrane that participates in the cellular response to oxidative stress and mitochondrial toxicity. It is unknown whether MAO-A levels are abnormal in AD. We hypothesized that MAO-A V_T , an index of MAO-A level, is elevated in the prefrontal cortex (PFC) during AD, because markers of greater oxidative stress and apoptosis are reported in the brain in AD and a microarray analysis reported greater MAO-A messenger RNA in the PFC of rodents exposed to alcohol vapor.

Methods—Sixteen participants with alcohol dependence and 16 healthy control subjects underwent [^{11}C]-harmine positron emission tomography. All were nonsmoking, medication- and drug-free, and had no other past or present psychiatric or medical illnesses.

Results—MAO-A V_T was significantly greater in the PFC (37%, independent samples t test, $t_{30} = 3.93$, $p < .001$), and all brain regions analyzed (mean 32%, multivariate analysis of variance, $F_{7,24} = 3.67$, $p = .008$). Greater duration of heavy drinking correlated positively with greater MAO-A V_T in the PFC ($r = .67$, $p = .005$) and all brain regions analyzed ($r = .73$ to $.57$, $p = .001$ – $.02$).

Conclusions—This finding represents a new pathological marker present in AD that is therapeutically targetable through direct inhibition or by novel treatments toward oxidative/pro-apoptotic processes implicated by MAO-A overexpression.

Keywords

Addictions; alcohol dependence; monoamine oxidase A; neurotoxicity; oxidative stress; positron emission tomography

Alcohol dependence (AD) is a chronic, relapsing illness with enormous societal impact, accounting for 4% of global death and 5% of the global burden of disease (1). The toxicity

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of chronic, excessive alcohol exposure is associated with diverse organ damage across the liver, heart, pancreas, and brain, implicating several processes including acetaldehyde formation, disturbed calcium and iron regulation, epigenetic modifications, and oxidative stress (2,3). However, despite advances in understanding the consequences of excessive alcohol intake, a paucity of neural targets have been identified with translatable potential for pharmacotherapy.

Monoamine oxidase A (MAO-A) is an important enzyme located on the outer mitochondrial membrane of glia and monoamine releasing neurons, particularly norepinephrine releasing neurons, that participates in the cellular response to mitochondrial toxicity and oxidative stress (4). MAO-A metabolizes monoamines such as serotonin, norepinephrine, and dopamine, and levels of MAO-A in brain tissue show a strong, positive correlation with MAO-A activity (5,6). Previous investigations of MAO-A activity in postmortem brain of AD were primarily negative, with no change reported in prefrontal cortex, and a decrease reported in the hypothalamus and caudate (7,8). Unfortunately, these studies were inconclusive because none of them addressed several recently discovered biases that influence MAO-A levels, such as cigarette smoking (9,10), exposure to current or past major depressive episodes (11–13), and impulsive-aggressive personality traits (14,15). The latter covary with MAO-A levels, likely because of a neurodevelopmental influence of inherited MAO-A levels (16).

Two findings suggest that elevated MAO-A level may occur in AD, particularly in the prefrontal cortex, and hence should be investigated in humans. First, markers of greater oxidative stress (2,17,18) and predisposition to apoptosis (19–21) may be present in brain and other organs in AD, and oxidative stress and mitochondrial toxicity lead to elevated MAO-A levels in neuroblastoma and glioblastoma cell lines (4,22–24). Second, a microarray analysis evaluating the effects of chronic alcohol vapor exposure in rodents reported a 2.5-fold elevation in MAO-A messenger (m)RNA in the prefrontal cortex (25). To investigate MAO-A levels in AD in humans and avoid confounding biases that influence MAO-A levels such as cigarette smoking and major depressive disorder, we chose an in vivo approach by applying [^{11}C]-harmine positron emission tomography (PET). [^{11}C]-harmine PET measures brain MAO-A V_T , an index of MAO-A density, and [^{11}C]-harmine has properties of an excellent PET radiotracer for MAO-A, including high brain uptake, selective and reversible binding, and metabolites that are not brain penetrant (10). On the basis of the association between alcohol dependence and oxidative stress and the involvement of MAO-A in cellular responses to oxidative stress, we hypothesized that MAO-A level would be elevated in the prefrontal cortex during AD. Our second main hypothesis was that greater years of exposure to heavy alcohol use would be associated with the elevation in MAO-A level.

Methods and Materials

Study Participants

Sixteen individuals with alcohol dependence (mean age, 35; SD, 8) were compared to 16 healthy controls (mean age, 34; SD, 9). Demographics are listed in Table 1. Some of the

healthy participants ($n = 14$) participated in earlier studies (10,12). Participants were within the age range of 18 to 50 years and in good physical health.

The key inclusion criteria for the AD group was an AD diagnosis using the Structured Clinical Interview for DSM-IV (26). Severity of AD was assessed using the Alcohol Dependence Scale (27). Our intent was to recruit subjects with AD who had not acquired additional psychiatric or medical illnesses, and we chose a minimum drinking cutoff commonly associated with AD that required consuming at least 4 (for women) or 5 (for men) drinks per day at least 5 days of the week (28,29). This cutoff is a well-accepted level for hazardous drinking because consumption beyond this level across several years has an extremely high probability of leading to AD (30). In a previous study, moderate to heavy cigarette smoking was associated with greater MAO-A V_T in early withdrawal and reduced MAO-A V_T during active smoking (10); hence, cigarette smoking was an exclusion criterion in the current study. Although 50% of people with AD smoke cigarettes (31), this cutoff of alcohol consumption ensures a representative sampling of alcohol intake behavior in AD subjects, regardless of smoking status (30). Plasma aspartate aminotransferase, alanine aminotransferase, and urine ethyl glucuronide were also measured.

To avoid factors that could bias MAO-A levels in each group, exclusion criteria included any current or past Axis I or Axis II disorder (26) (apart from AD in the AD group), cigarette smoking, herbal, drug or medication use within 8 weeks of scanning, history of psychiatric or medical illness, or any other substance abuse or dependence. Lifetime history of comorbid Axis I disorders, including past major depressive episodes and anxiety disorders, were exclusionary. Screening included exhaled carbon monoxide level (MicroSmokerlyzer; Bedfont Scientific Ltd., Kent, United Kingdom), plasma cotinine levels, and a urine drug test at screening and on the day of PET scan. Those with positive results for other substances were excluded. For women, phase of menstrual cycle was recorded by self-report. In a previously collected sample, there was no relationship between phase of menstrual cycle and MAO-A V_T (32). To avoid confound from variations in plasma estrogen levels, women in early postpartum, perimenopause, or menopause were excluded. Participants were required not to drink tea or coffee on the day of scanning. All subjects reported no previous head injury and had no neurological disorders associated with alcohol dependence (Korsakoff syndrome, Wernicke's encephalopathy). For each participant, written consent was obtained after the procedures were fully explained. The study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto.

Protocol on Day of PET Imaging

All study participants underwent a single [^{11}C]-harmine PET scan. Alcohol metabolism may lead to the formation of a beta-carboline compound called harman (33,34), and alcoholic beverages frequently contain harman (35), which has moderate affinity for MAO-A. To avoid temporary occupancy effects of harman (10), [^{11}C]-harmine PET scanning was timed at a point when harman levels were negligible (36), which was verified by plasma sampling. Subjects were instructed to maintain their usual alcohol intake behavior and to stop drinking at 12 A.M. the evening before the [^{11}C]-harmine PET scan. A breathalyzer screen was taken

and results were consistent with recent cessation of alcohol intake. Also, before scanning, 12-cm visual analog scales for mood (i.e., happy–depressed), energy (i.e., most–least), and anxiety (i.e., relaxed–tense), and the Clinical Institute Withdrawal Assessment for measurement of severity of withdrawal symptoms, were completed. For the visual analog scales, participants were instructed to draw a vertical line crossing the 12-cm linear scale at the point corresponding to the strength of their experience of the given dimension of the mood state.

PET Image Acquisition

The PET images were obtained using a High Resolution Research Tomograph PET camera (in-plane resolution; full width at half maximum, 3.1 mm; 207 axial sections of 1.2 mm; Siemens Molecular Imaging, Knoxville, Tennessee) in a manner described previously (12). A dose of 370 MBq of intravenous [^{11}C]-harmine was administered as a bolus. The [^{11}C]-harmine was of high radiochemical purity ($99.16\% \pm 1.12\%$) and high specific activity ($94.27 \pm 33.71 \text{ GBq}/\mu\text{mol}$) at the time of injection. The emission scan was reconstructed in 15 frames of 1 minute, followed by 15 frames of 5 minutes. An automatic blood sampling system was used to measure arterial blood radioactivity continuously for the first 10 minutes after injection. Manual samples were obtained at 2.5, 7.5, 15.0, 20.0, 30.0, 45.0, 60.0, and 90.0 minutes. The radioactivity in whole blood and plasma were measured as described previously (37).

Image Analysis

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, .78, .78, and 1.5 mm; GE Medical Systems, Milwaukee, Wisconsin). Regions of interest (ROIs) were delineated on these magnetic resonance images using a semiautomated method based on linear and nonlinear transformations of an ROI template in standard space to the individual magnetic resonance image (MRI), followed by a refinement process based upon the gray matter probability (38,39). The MRI was coregistered to the summated [^{11}C]-harmine PET image using a mutual information algorithm (40), and the resulting transformation was applied to sample the ROIs from the PET image. The location of the ROI was verified by visual assessment on the summated [^{11}C]-harmine PET image.

The primary ROI was the whole prefrontal cortex, but additional regions with high MAO-A density were sampled, including the anterior cingulate cortex, dorsal putamen, ventral striatum, thalamus, hippocampus, and midbrain. The definitions of the ROIs were similar to our previous investigations (11,41,42) and are based on a neuroanatomy atlas of structural MRI and postmortem tissue (43), with the exception of the divisions of dorsal putamen and ventral striatum, which are described by Malawi (44). Refer to Supplement 1 for additional details. Subregions of the prefrontal cortex, including the dorsolateral, medial, orbitofrontal, and ventrolateral prefrontal cortices, were sampled to evaluate whether the effects observed in the main analyses of the prefrontal cortex were consistent within these subregions. The subregions were defined based on their cytoarchitectural differentiation from surrounding tissue, which was mapped onto the external morphology of the cortex (45–47).

MAO-A total distribution volume (V_T) was measured via [^{11}C]-harmine PET. MAO-A V_T equals the ratio of tissue to plasma concentration of [^{11}C]-harmine at equilibrium, of which 85% represents radioligand specifically bound to MAO-A (37,48). Hence, changes in MAO-A V_T may be interpreted as representing changes in harmine binding to MAO-A. The V_T can be expressed in terms of kinetic rate parameters as follows: $V_T = (K_1/k_2) \times (k_3/k_4) + (K_1/k_2)$, where K_1 and k_2 are influx and efflux rate parameters, respectively, for radiotracer passage across the blood-brain barrier, and k_3 and k_4 describe the radioligand transfer between the free and nonspecific compartment and the specific binding compartment. Among different groups, K_1/k_2 is similar [for details, see Ginovart *et al.* (37)]. MAO-A V_T can be measured validly and reliably with an unconstrained two-tissue compartment model or the Logan model with arterial sampling; the latter technique was applied in the current study (37).

Statistical Analysis

The primary analysis was an independent samples *t* test to assess the group effect (i.e., AD vs. health) on MAO-A V_T in the prefrontal cortex. In addition, to further characterize the comparison between the AD group and healthy control subjects, a multivariate analysis of variance (MANOVA) was used to assess the group effect upon MAO-A V_T in all brain regions assayed. Independent samples *t* tests were then applied at each ROI to compare groups.

The main secondary analysis was a Pearson correlation coefficient to assess the relationship between years of heavy alcohol use and MAO-A V_T within the prefrontal cortex. In addition, to further characterize the relationship between years of heavy alcohol use and MAO-A V_T , Pearson correlation coefficients were determined for each ROI.

Results

Difference in MAO-A V_T Between the Alcohol Dependent Group and Healthy Control Subjects

The primary finding was a significant elevation in MAO-A V_T , an index of MAO-A density, in the prefrontal cortex of alcohol dependent subjects compared to healthy controls (magnitude 37%, independent samples *t* test, $t_{30} = 3.93$, $p < .001$), with an effect size of 1.4. There was also a significant elevation in all of the brain regions analyzed (MANOVA, $F_{7,24} = 3.67$, $p = .008$). The mean difference across all regions was 32%, range 21% to 40%, see Figure 1. In addition, subregions of the prefrontal cortex (dorsolateral, medial, orbitofrontal, ventrolateral) were assessed and the results were similar (MANOVA, $F_{4,27} = 2.97$, $p = .038$; mean difference 27%, range 21%–31%). Post hoc tests reveal the group difference was significant in each prefrontal region (independent samples *t* tests, $p = .004$ –.02).

Relationship Between MAO-A V_T and Duration of Heavy Alcohol Use

A positive correlation between MAO-A V_T and the duration of heavy alcohol use was found in the prefrontal cortex (Pearson correlation coefficient, $r = .67$, $p = .005$). There was also a significant positive correlation found in all other brain regions analyzed ($r = .73$ –.57, $p = .001$ –.02), see Figure 2.

Relationship Between MAO-A V_T and Other Clinical Features

For this analysis, we focused on depressed mood measured with the visual analog scale on the PET scan day and MAO-A V_T in the prefrontal and anterior cingulate cortices. A stepwise regression was applied that included three predictor variables: the visual analogue score for depressed mood, group (AD vs. healthy, because it is well established that depressed mood is associated with alcohol dependence) (49,50), and angry-hostility, a personality facet score (from the NEO Personality Inventory—Revised) previously associated with MAO-A V_T (14,15). All factors were significant (multivariate analysis of covariance, effect of depressed mood, $F_{2,26} = 4.88$, $p = .02$; effect of group, $F_{2,26} = 5.38$, $p = .01$; effect of angry-hostility, $F_{2,26} = 6.02$, $p = .007$) and were related to the dependent variables in a manner consistent with greater MAO-A V_T when depressed mood is elevated and lower MAO-A V_T when angry-hostility is present, see Figure 3. See Table 2 for an overview of all statistical analyses of MAO-A V_T .

Additional Characteristics of the AD Group

There were no significant differences in radioligand binding to plasma protein between groups ($t_{28} = .92$, $p = .36$). As expected, given the 12-hour period since previous drinking of alcoholic beverages, the plasma level of beta-carboline harman was negligible for all subjects on the day of the scan (<5 pg/mL). There were several post hoc analyses not specifically hypothesized but that were of interest to understand the relationship between indices of alcohol dependence and prefrontal cortex MAO-A V_T . The correlations between prefrontal cortex MAO-A V_T and number of drinks per day in the week before scanning, as well as the Clinical Institute Withdrawal Assessment score before scanning, were not significant (Pearson correlation coefficient, $r = .30$, $p = .20$, $r = .17$, $p = .54$, respectively). Additionally, angry hostility did not correlate with depression ($r = .17$, $p = .53$) or AD duration ($r = .26$, $p = .33$). Depression scores did not correlate with AD duration ($r = .19$, $p = .47$).

Discussion

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

This finding represents an important direction for understanding the underlying pathology of AD because MAO-A levels influence key processes related to many of the brain pathologies typically observed later in AD, such as abnormal mitochondrial function, reduced glial cell density, and thinned cortex (51–53). For example, reduced mitochondrial respiration, an

index of reduced mitochondrial functioning, occurs when MAO-A activity is increased (54), and AD is associated with reduced *N*-acetylaspartate levels, an index of reduced mitochondrial function (55,56). Also, in contrast to many other brain proteins, MAO-A is present homogeneously throughout the cortex, including glial cells (6), and when MAO-A activity is greater, glial cell lines are predisposed toward apoptosis (24). MAO-A activity itself creates oxidative stress (4), and markers of oxidative damage to mitochondrial DNA have been reported in AD (51,57). Additionally, inherited vulnerability to oxidative stress has been associated with reduced cortical thickness in AD (58). Given the diverse pathological implications of chronically elevated MAO-A level, intervening on this target has intriguing implications for treating AD.

New therapeutic targets for AD are necessary because cessation rates after common clinical treatments such as naltrexone and acamprosate are 20% to 50% (59). Preclinical models demonstrate promise for MAO-A inhibitors to reduce ethanol consumption (60,61), and MAO-A inhibitors also have the potential to protect mitochondria against ethanol-induced toxicity in multiple organs (4) and to treat the mood symptoms that are often observed in AD (49,50). However, there have been no investigations of MAO-A inhibitors as a pharmacotherapy for AD in humans. Historically, MAO-A inhibitors were not used to treat AD because the original nonselective, irreversible MAO-A/B inhibitor compounds were associated with greater risk for hypertensive crisis from inadequate peripheral metabolism of tyramine, which is ingested through many alcoholic beverages. This issue has been overcome with modern therapeutic development of selective reversible MAO-A inhibitors, as well as MAO inhibitors with high brain to periphery concentration ratios, which do not require dietary restriction of tyramine (4). Thus, MAO-A inhibitors are available and have the potential to resolve both toxicity and mood-related disturbances that result from excessive alcohol use.

The correlation between years of heavy alcohol intake and greater MAO-A V_T suggests that elevated MAO-A V_T was acquired through chronic alcohol exposure, rather than other mechanisms. This perspective is consistent with the effects of chronic alcohol exposure in rodent models, which includes greater MAO-A mRNA in the prefrontal cortex and greater MAO-A activity in the dorsal raphe nucleus (25,62). The specific mechanism through which alcohol exposure might lead to increased MAO-A level is unknown, but it is possible that pro-apoptotic pathways contribute because TIEG2, a transcription factor that initiates a cell-death cascade and promotes expression of MAO-A, is elevated in prefrontal cortex of rodents after chronic ethanol exposure (19). Future investigations in this direction have important implications for developing novel treatment strategies that could reverse elevated MAO-A level through mechanisms other than direct MAO-A inhibition. Another mechanism to consider is whether elevated MAO-A V_T is a preexisting trait. AD is associated with greater levels of aggression and impulsivity (63,64), and such traits are associated with measures of low MAO-A binding in health (14,15), suggesting that a preexisting elevation in MAO-A level is unlikely. Future investigations might also consider gene-by-environment interactions in regard to the effect of AD on MAO-A levels.

The biological mechanism of dysphoria in AD has traditionally been unclear, but the present finding of greater MAO-A V_T in prefrontal and anterior cingulate cortices provides new

insight into the etiology of dysphoric mood and high risk for major depressive episodes in AD (65–67) because subregions of these structures are activated in mood induction studies and implicated in generation of depressed mood (68). As previously mentioned, MAO-A has a role in apoptosis, and abnormal expression of genes related to apoptosis in the prefrontal cortex have been reported in depressed mood (69). Also, when MAO-A density is elevated, monoamine metabolism is increased (4), and monoamine loss via acute monoamine depletions or chronic removal with reserpine is associated with depressed mood (70–75). The association of depressed mood with MAO-A V_T in the present study is supportive of a relationship between MAO-A level and depressed mood in AD, possibly through the aforementioned mechanisms, particularly in AD with long periods of heavy alcohol intake. The question could be raised as to whether dysphoria predisposes to greater MAO-A V_T . Available evidence does not support this direction as a recent study by Soliman *et al.* found that induction of acute stress, which also created dysphoria, was not associated with elevated MAO-A V_T , thereby demonstrating that sad mood need not induce a rise in MAO-A level (76).

Excessive monoamine metabolism may also influence other circuits implicated in AD. For example, decreased ventral striatal dopamine release and insensitivity to this release has been implicated in AD as a mechanism that impairs the ability to develop an adaptive behavior to abstain from drinking (77–79). Dopamine is a substrate of MAO-A, and in the present study elevated MAO-A V_T was found in the ventral striatum. Interestingly, greater 5-HT_{1B} binding was also found in this region in AD using [¹¹C]-P943 PET (80), and depletion of 5-HT through p-chlorophenylalanine has been associated with elevated expression of 5-HT_{1B} receptors (81). 5-HT_{1A} receptor binding was reported as normal in AD in humans (82), whereas studies of 5-HTT are mixed, with some reports of decreased 5-HTT level and/or binding in anterior cingulate cortex, dorsal striatum, and midbrain (83–86) and other reports of no change (82,87). Future work should consider how a monoamine lowering process, such as elevated MAO-A level, might interact with other pathologies observed in AD.

One of the limitations of the present study is that there was no PET scan during the intoxication state. This is because alcohol intake has two mechanisms that lead to elevated plasma levels of harman, a beta-carboline compound, which has moderate affinity for MAO-A and may influence available MAO-A binding in humans (9,10). To avoid temporary occupancy effects of harman, [¹¹C]-harmane PET scanning was timed when plasma harman levels were negligible. Also, arterial sampling of the radiotracer necessitates a radial arterial line, and participants could not be safely scanned while intoxicated. Another limitation is that the main outcome measure of [¹¹C]-harmane PET, MAO-A V_T , is an index of MAO-A density and represents total [¹¹C]-harmane in tissue relative to arterial plasma at equilibrium. It is computationally efficient, highly stable, and the least variable measure of [¹¹C]-harmane binding; however, approximately 15% of this measure reflects free and nonspecific binding (37,48). This free and nonspecific component is consistent across individuals, and although tremendous changes in free and nonspecific binding (i.e., 250%) could lead to the changes in MAO-A V_T observed, this is unlikely (37). The elevation in MAO-A V_T may also reflect greater affinity of MAO-A, yet our overall interpretation of the results would not change

because greater affinity of MAO-A would be expected to have similar functional effects as compared with a rise in MAO-A density.

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

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Figure 1.

Greater monoamine oxidase A (MAO-A) V_T in alcohol-dependent subjects compared with healthy control subjects. MAO-A V_T , an index of MAO-A level, was significantly greater in the prefrontal cortex (independent samples t test, $t_{30} = 3.93$, $p < .001$). There was also a significant elevation in all of the brain regions analyzed (multivariate analysis of variance $F_{7,24} = 3.67$, $p = .008$). Independent samples t tests were also applied at each region of interest to compare groups. ^a $p < .001$; ^b $p < .005$; ^c $p < .05$.

Figure 2.

Greater monoamine oxidase A (MAO-A) V_T level in alcohol-dependent subjects correlates with years of heavy alcohol use. MAO-A V_T , an index of MAO-A level, was significantly correlated with years of heavy alcohol use in the prefrontal cortex (Pearson correlation coefficient, $r = .67$, $p = .005$). There was also a significant positive correlation in all other brain regions analyzed ($r = .73-.57$, $p = .001-.02$). Years of heavy alcohol use was defined as the number of years in which participants drank more than five drinks per day, at least 5 days per week, and for which symptoms of alcohol dependence were continuously present.

Figure 3.

Greater monoamine oxidase A (MAO-A) V_T is associated with increased depressed mood and angry-hostility. A multivariate analysis of covariance with factors of depressed mood from the visual analogue scale (VAS), angry-hostility, and group were predictive of MAO-A V_T, an index of MAO-A level, in the prefrontal and anterior cingulate cortex (effect of depressed mood, $F_{2,26} = 4.88$, $p = .02$; group, $F_{2,26} = 5.38$, $p = .01$; angry-hostility, $F_{2,26} = 6.02$, $p = .007$). The residual MAO-A V_T values are shown after regressing for angry-hostility to demonstrate the relationship between depressed mood and MAO-A V_T for control (●) and alcohol-dependent (▲) subjects. When the highest residual value is removed, Pearson correlation coefficients remain significant (pre-frontal cortex: $r = .5$, $p = .007$, anterior cingulate cortex: $r = .5$, $p = .003$).

Table 1Demographic and Clinical Characteristics of Study Participants^a

	Healthy Subjects (<i>n</i> = 16)	Alcohol Dependent Subjects (<i>n</i> = 16)
Age, years	34 (8.8)	35 (7.6)
Sex, Male/Female	14/2	14/2
SCID Diagnosis of Major Depressive Disorder	0	0
HAM-D	.8 (1.2)	3.7 (2.0)
VAS Depressed Mood ^b	2.5 (1.5)	4.2 (2.0)
Angry-Hostility ^c	12.1 (5.1)	12.1 (5.4)
Deliberation ^c	16.4 (3.7)	16.3 (6.0)
Alcohol Intake Behaviors		
No. drinks/day	.14 (.25)	8.0 (3.5)
No. drinking days/week	0 (0)	6.3 (.9)
Duration of alcohol dependence, years	N/A	6.3 (4.1)
Alcohol Dependence Scale	N/A	12.7 (6.9)
CIWA-Ar at scan	N/A	9.8 (1.4)

None of the healthy control subjects drank alcohol on a regular basis or had a diagnosis of alcohol dependence or withdrawal. Independent samples *t* tests showed no significant difference for age ($t_{30} = .56, p = .58$), angry-hostility ($t_{30} = .002, p = .998$), deliberation ($t_{30} = .049, p = .962$). There was a significant difference for HAM-D and VAS scores ($t_{30} = 4.95, p < .001, t_{30} = 2.79, p = .009$, respectively), yet all participants were below the HAM-D threshold for a major depressive episode.

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

^aValues are expressed as mean (SD).

^bEndorsement of depressed mood on the VAS at the time of the scan.

^cPersonality facet within the Neuroticism Extroversion Openness Personality Inventory—Revised questionnaire.

Overview of Analyses of MAO-A V_T

Table 2

Analysis Ranking	Comparison of MAO-A V _T Values	Region(s)	Statistic	p Value ^a	Mean % Change	Direction of Change
Primary	Difference in PFC between AD and health	PFC	Independent samples <i>t</i> test, $\xi_0 = 3.93$	.000	37%	Increase
Subanalyses Related to Primary Analysis	Difference in all regions between AD and health	PFC, ACC, dorsal putamen, ventral striatum, thalamus, hippocampus, midbrain	MANOVA, $F_{7,24} = 3.67$	.008	32%	Increase
	Individual regional comparisons	PFC, ACC, dorsal putamen, ventral striatum, thalamus, hippocampus, midbrain	$\xi_0 = 3.93$	.000	37%	Increase
			$\xi_0 = 4.02$	.000	40%	Increase
			$\xi_0 = 3.20$	.003	33%	Increase
			$\xi_0 = 2.32$	.027	24%	Increase
			$\xi_0 = 3.15$	.004	34%	Increase
			$\xi_0 = 2.53$	.017	21%	Increase
			$\xi_0 = 3.51$	.001	33%	Increase
	Difference in subregions of the PFC between AD and health	dIPFC, OFC, mPFC, vIPFC	MANOVA, $F_{4,27} = 2.97$	.038	27%	Increase
	Individual PFC subregion comparisons	dIPFC, OFC, mPFC, vIPFC	$\xi_0 = 2.80$	.009	27%	Increase
			$\xi_0 = 2.61$	.014	26%	Increase
			$\xi_0 = 2.47$	.020	22%	Increase
			$\xi_0 = 3.15$	.004	31%	Increase
Secondary	Correlation with years of heavy drinking in PFC	PFC	Pearson correlation, $R = .67$	.005	N/A	Positive correlation
Subanalyses Related to Secondary Analysis	Correlation with years of heavy drinking in all other regions	ACC, dorsal putamen, ventral striatum, thalamus, hippocampus, midbrain	$R = .67$	.005	N/A	Positive correlations
			$R = .71$	.002		
			$R = .73$	.001		
			$R = .70$	.002		
			$R = .69$	.003		
			$R = .57$	.020		
Additional	Relationship to depressed mood in PFC and ACC	PFC, ACC	MANCOVA, $F_{2,26} = 4.88$ (effect of depressed mood) $F_{2,26} = 5.38$, (effect of group) $F_{2,26} =$	.02	N/A	Increased with greater depressed mood, AD group
				.01		Decreased with greater angry-hostility

Analysis Ranking	Comparison of MAO-A V _T Values	Region(s)	Statistic	p Value ^a	Mean % Change	Direction of Change
			6.02 (effect of angry-hostility)	.007		

Values across the regions analyzed were highly correlated in the alcohol-dependent and healthy groups ($r = .99-.70$). For correction of multiple comparisons, there were three main independent sets of analyses of MAO-A V_T: AD versus health, relationship of MAO-A V_T with years of heavy alcohol use, and relationship between MAO-A V_T in the PFC and ACC with depressed mood.

ACC, anterior cingulate cortex; AD, alcohol dependence; dlPFC, dorsolateral prefrontal cortex; MANCOVA, multivariate analysis of covariance; MAO-A, monoamine oxidase A; mPFC, medial prefrontal cortex; N/A, not applicable; OFC, orbitofrontal cortex; PFC, prefrontal cortex; vIPFC, ventrolateral prefrontal cortex; VT, an index of MAO-A level.

^aUncorrected *p* values shown.