ORIGINAL ARTICLE

Monoamine Oxidase A Binding in the Prefrontal and Anterior Cingulate Cortices During Acute Withdrawal From Heavy Cigarette Smoking

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Context: Greater prefrontal cortex and anterior cingulate cortex monoamine oxidase A (MAO-A) binding is associated with depressed mood. Substances in cigarette smoke, such as harman, inhibit MAO-A, and cigarette withdrawal is associated with depressed mood. Dysphoria during cigarette withdrawal predicts relapse. It is unknown whether MAO-A binding increases during early cigarette withdrawal.

Objectives: To measure prefrontal and anterior cingulate cortex MAO-A binding during acute cigarette withdrawal and to assess the relationship with smoking severity, plasma levels of harman, and severity of depression.

Design: Study via positron emission tomography of healthy control and cigarette-smoking individuals.

Patients: Twenty-four healthy nonsmoking and 24 otherwise healthy cigarette-smoking individuals underwent positron emission tomography with harmine labeled with carbon 11. Healthy nonsmoking individuals underwent scanning once. Cigarette-smoking individuals underwent scanning after acute withdrawal and after active cigarette smoking. Cigarette smoking was heavy (≥25 cigarettes per day) or moderate (15-24 cigarettes per day).

Setting: Tertiary care psychiatric hospital.

Main Outcome Measure: An index of MAO-A density, MAO-A V_T, was measured in the prefrontal and anterior cingulate cortices.

Results: In heavy-smoking individuals, prefrontal and anterior cingulate cortex MAO-A V_T was greater during withdrawal (23.7% and 33.3%, respectively; repeated-measures multivariate analysis of variance, $F_{1,22}=25.58$, $P<.001$). During withdrawal from heavy smoking, prefrontal and anterior cingulate cortex MAO-A V_T was greater than in healthy controls (25.0% and 25.6%, respectively; multivariate analysis of variance, $F_{2,33}=6.72$, $P=.004$). The difference in MAO-A V_T in the prefrontal cortex and anterior cingulate cortex between withdrawal and active, heavy smoking covaried with change in plasma harman levels in the prefrontal cortex and anterior cingulate cortex (multivariate analysis of covariance, $F_{1,10}=9.97$, $P=.01$). The change in MAO-A V_T between withdrawal and active, heavy smoking also covaried with severity of depression (multivariate analysis of covariance, $F_{1,10}=11.91$, $P=.006$).

Conclusions: The increase in prefrontal and anterior cingulate cortex MAO-A binding and associated reduction in plasma harman level represent a novel, additional explanation for depressed mood during withdrawal from heavy cigarette smoking. This finding resolves a longstanding paradox regarding the association of cigarette smoking with depression and suicide and argues for additional clinical trials on the effects of MAO-A inhibitors on quitting heavy cigarette smoking.

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CIGARETTE SMOKING IS A MAjor public health problem: it is the second leading cause of preventable death and an important risk factor for coronary artery disease, lung disease, suicide, and cancer.[1-3] Prevalence rates are high, typically ranging from 10% to 50%, depending on the country.[2] Although many people who smoke cigarettes would like to quit, the effects of withdrawal frequently lead to relapse. Relapse is particularly problematic in early withdrawal because 50% of people relapse within the first 3 days of quitting.[4,5]

Most biological conceptualizations of early cigarette withdrawal focus on abnormal modulation by nicotine on dopamine-releasing neurons projecting from the ventral tegmental area to the ventral striatum.[6-8] This model is important because it has been applied in therapeutic interventions, which, when combined with therapy, achieve 6-month abstinence rates of as high as 40%.[5,9] However, other neural targets that may be important in cigarette withdrawal are affected by cigarette smoke. For example, several substances in cigarette smoke bind to monoamine oxidase A (MAO-A).[10,11] Moreover, a study

Table 1. Demographic and Clinical Characteristics of the Study Participants[a]

Characteristic	Healthy Nonsmoking Individuals (n=24)	Cigarette-Smoking Individuals (n=24)
Age, y	33.23 (8.32)	36.00 (6.69)
Age of onset of smoking, y	NA	18.08 (6.12)
Duration of smoking, y	NA	16.78 (6.70)
No. of cigarettes per day	NA	31.27 (11.36)
Fagerström Test for Nicotine Dependence score	NA	6.67 (1.63)
Nicotine Dependence Syndrome Scale, overall score	NA	0.39 (0.97)
Tobacco dependence score	NA	7.29 (1.74)
Male sex, No. (%)	20 (83)	21 (88)
Female sex, No. (%)	4 (17)	3 (13)
HAM-D score	0.78 (1.31)	1.25 (1.65)
Neuroticism[b]	68.52 (18.87)	71.83 (23.20)
Angry-hostility[b]	10.52 (3.53)	10.54 (5.08)
Conscientiousness[b]	119.04 (15.43)	115.46 (23.01)
Deliberation[b]	17.13 (4.00)	18.42 (4.72)

Abbreviations: HAM-D, 17-Item Hamilton Rating Scale for Depression; NA, not applicable.
[a] Percentages may not total 100 because of rounding. Values are expressed as mean (SD) except where indicated.
[b] Personality dimension and/or facet within the NEO Personality Inventory–Revised questionnaire.

using carbon 11–labeled clorgyline positron emission tomography (PET)[12] found that cigarette smoking was associated with globally reduced MAO-A binding in active-smoking participants compared with nonsmoking control individuals in a between-group design.

Monoamine oxidase A binding, particularly in the prefrontal and anterior cingulate cortices, is strongly implicated in affect regulation.[13-19] Negative affect is an important component of cigarette withdrawal because its severity is strongly predictive of relapse.[20-22] Monoamine oxidase A has a functional role tightly related to mood because it is an enzyme that metabolizes serotonin, norepinephrine, and dopamine.[23,24] Depletion of these monoamines is associated with depressed mood.[25-29] In addition, elevations in MAO-A binding in affect modulating regions, particularly the prefrontal cortex and anterior cingulate cortex, occur during major depressive episodes, before recurrence of major depressive episodes, and shortly after pregnancy during postpartum blues (ie, when symptoms are within healthy range).[13-15] Conversely, MAO-A inhibition is a well-established property for a longstanding class of antidepressants.

Although it is often described that MAO-A binding is reduced in those who smoke cigarettes irrespective of their active smoking or withdrawal state, there is reason to suspect that regional MAO-A binding may change between active smoking and withdrawal because the plasma half-life of the MAO-A–binding substances found in cigarette smoke is short. For example, harman and norharman are 2 substances present in cigarette smoke, and their plasma half-lives are 70 and 50 minutes, respectively, in humans.[30] Therefore, during acute cigarette withdrawal, it is possible that these substances quickly leave the plasma and then the brain, resulting in a rapid elevation in MAO-A binding. Whether this sequence of events occurs is uncertain because some brain-penetrant substances clear from plasma much faster than from the brain.[31] Currently, it is unknown whether MAO-A binding increases during acute cigarette withdrawal because MAO-A binding has not been investigated during withdrawal, to our knowledge.

Positron emission tomography with carbon 11–labeled harmine ([^{11}C]harmine) is an excellent method to measure changes in brain MAO-A binding. This radiotracer has high brain uptake, selective binding, reversible binding, and metabolites that are not brain penetrant.[32-34] The main hypothesis of this study is that MAO-A binding increases during acute cigarette withdrawal in regions implicated in affect regulation, such as the prefrontal cortex and the anterior cingulate cortex. The second hypothesis is that the anticipated increase in MAO-A binding during acute withdrawal will be greater in those who smoke heavily compared with those who smoke moderately. The third hypothesis is that the anticipated increase in prefrontal and anterior cingulate cortex MAO-A binding during acute withdrawal will be greater in those who have greater reductions in plasma levels of harman and norharman, 2 MAO-A brain-penetrant substances present in cigarette smoke with reasonably high affinity for MAO-A.[10,35] The fourth hypothesis is that given the inverse relationship between MAO-A binding and mood,[13-15] the increase in MAO-A binding in the prefrontal and anterior cingulate cortices will be greater in those who experience more severe depression during acute cigarette withdrawal.

METHODS

STUDY PARTICIPANTS

Twenty-four healthy nonsmoking individuals (mean [SD] age, 33.2 [8.3]) and 24 individuals who smoke cigarettes but are otherwise healthy (mean [SD] age, 36 [6.7]) were recruited. Some of the nonsmoking participants (n=24) were described in an earlier study.[14] Participants were aged 18 to 50 years. Demographics are listed in **Table 1**. For each study participant, written consent was obtained after the procedures had been 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.

The severity of smoking was classified as moderate for those individuals smoking 15 to 24 cigarettes per day and as heavy for those smoking 25 cigarettes per day or more. To verify smoking status, scores on the Fagerström Test for Nicotine Dependence[36,37] and the Nicotine Dependence Syndrome Scale[38] were recorded. In addition, the exhaled carbon monoxide level (MicroSmokerlyzer; Bedfont Scientific Ltd, Kent, England) at the initial visit was taken, and a cutoff of 10 ppm was applied.[7] All participants were physically healthy and had no history of neurotoxin use. Women in perimenopause or menopause were excluded. Healthy participants were screened to rule out any Axis I disorders, and participants who smoke cigarettes were screened to rule out lifetime history of Axis I disorders other than cigarette abuse or dependence using the Structured Clinical Interview for *DSM-IV*.[39] All were screened to rule out borderline and antisocial personality disorder using the Structured Clinical Interview for *DSM-IV* for Axis II disorders.[40] All participants underwent a urine drug test at screening, and on each day they underwent a scan via [^{11}C]harmine PET. Those with positive results for other substances were excluded.

All study participants had no history of psychotropic medication use and had not taken over-the-counter medications for at least 1 month before scanning. Participants were required not to drink tea or coffee on the day of scanning. Because this latter criterion is difficult for those who drink a lot of coffee, only those who drink fewer than 3 cups of tea or coffee per day were enrolled. Given that some MAO-A inhibitor substances are found in some kinds of alcohol, participants were required not to drink any alcohol the day before and the day of scanning.

SCANNING DAY PROTOCOL

Healthy study participants underwent a single scan via [^{11}C]harmine PET. Cigarette-smoking study participants underwent 2 scans via [^{11}C]harmine PET, separated by a minimum of 1 month to allow healing from the arterial catheter insertion.

Scanning for cigarette-smoking individuals occurred during cigarette withdrawal or active smoking, and the order was randomized. During the day of active smoking, participants smoked on a regular schedule at a rate to match the number of cigarettes smoked daily. During the withdrawal day, participants stopped smoking cigarettes 8 hours before scanning via PET. Otherwise, the daily protocol was the same for both conditions. To verify compliance, participants were accompanied by a research coordinator for 8 hours before scanning via PET. During the time before scanning at 0, 2, 4, 6, and 8 hours before scanning, 10-cm visual analog scales (VASs) for mood (ie, happy-depressed), energy (ie, most-least), and anxiety (ie, relaxed-tense) and the Urge to Smoke Scale, an analog scale (range, 1-7) with 10 craving-related questions, were completed.[41] For the VAS, participants were instructed to draw a vertical line crossing the 10-cm linear scale at the point corresponding to the strength of their experience of the given dimension of the mood/energy state. As part of the instruction process, participants were asked to focus on their current internal state and to avoid thinking about recent life stressors or daily stressors. They also were asked to focus on their internal state at the moment of doing the VAS and not their state earlier in the day.

Before scanning via PET, plasma measurements of several MAO-A–binding substances (ie, harman, norharman, harmol, harmine, and 2-napthylamine) were measured using high-performance liquid chromatography with mass spectroscopy. To validate the assay for each substance, purchased standards were measured in duplicate to verify reliability and sensitivity. Harman and norharman are the most commonly reported MAO-A inhibitors found in cigarette smoke.[42] Harmol is often detected in plants that contain harman and norharman.[43] It was reported that harman sometimes may be metabolized in rodents to become harmine,[44] which has a high affinity for MAO-A (2 nM).[32] 2-Napthylamine is found in high concentrations in cigarette smoke and has a moderate affinity for MAO-A (52 μM).[11]

IMAGE ACQUISITION

A dose of 370 MBq of intravenous [^{11}C]harmine was administered as a bolus for each scan via PET. An automatic blood sampling system was used to measure arterial blood radioactivity continuously for the first 10 minutes. Manual samples were obtained at 2.5, 7.5, 15.0, 20.0, 30.0, 45.0, 60.0, and approximately 90.0 minutes after injection. The radioactivity in whole blood and plasma was measured, as described previously.[33] Frames were acquired as follows: 15 frames of 1 minute each, then 15 frames of 5 minutes each. The [^{11}C]harmine was of high radiochemical purity (98.91% [1%]; n=55) and high specific activity (1545.64 [820.55] mCi/μmol at the time of injection).

The images via PET 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 the manner described previously.[14]

IMAGE ANALYSIS

For the region of interest (ROI) method, each participant underwent magnetic resonance imaging (GE Signa 1.5-T scanner; fast spoiled gradient echo, T1-weighted image; *x*, *y*, *z* voxel dimensions, 0.78, 0.78, and 1.5 mm; GE Medical Systems, Milwaukee, Wisconsin). The ROIs were determined on magnetic resonance images that were coregistered to each summed image obtained via [^{11}C]harmine PET using a mutual information algorithm.[45] The ROIs were determined using a semiautomated method in which regions on a template magnetic resonance image are transposed onto the individual image via a series of transformation and deformation parameters that match the template image to the coregistered image,[46,47] followed by selection of gray matter voxels within the ROI.[48,49] The location of the ROI was verified by visual assessment of the ROI on the coregistered and summated image obtained via [^{11}C]harmine PET.

The ROIs selected included those for which abnormal function, neurochemistry, or MAO-A binding has been implicated in mood regulation and/or mood disorders.[13-19] The ROIs sampled included the whole prefrontal cortex, the anterior cingulate cortex (ie, Brodmann areas 24, part of 32), the dorsal putamen, the ventral striatum,[50] the thalamus, the anterior temporal cortex (ie, Brodmann areas 38, part of 20, 21, and 22), the midbrain, and the hippocampus.

One can measure MAO-A V_T via [^{11}C]harmine PET. The MAO-A V_T represents the total tissue binding of [^{11}C]harmine at equilibrium, of which 85% is specifically binding to MAO-A. 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 for radiotracer passage across the blood-brain barrier and k_3 and k_4 describe the radioligand transfer between the free and nonspecific compartment and the specific binding compartment. Among different groups, K_1/k_2 is similar (for further details, see Ginovart et al[33]). One may validly and reliably measure V_T with an unconstrained 2-tissue com-

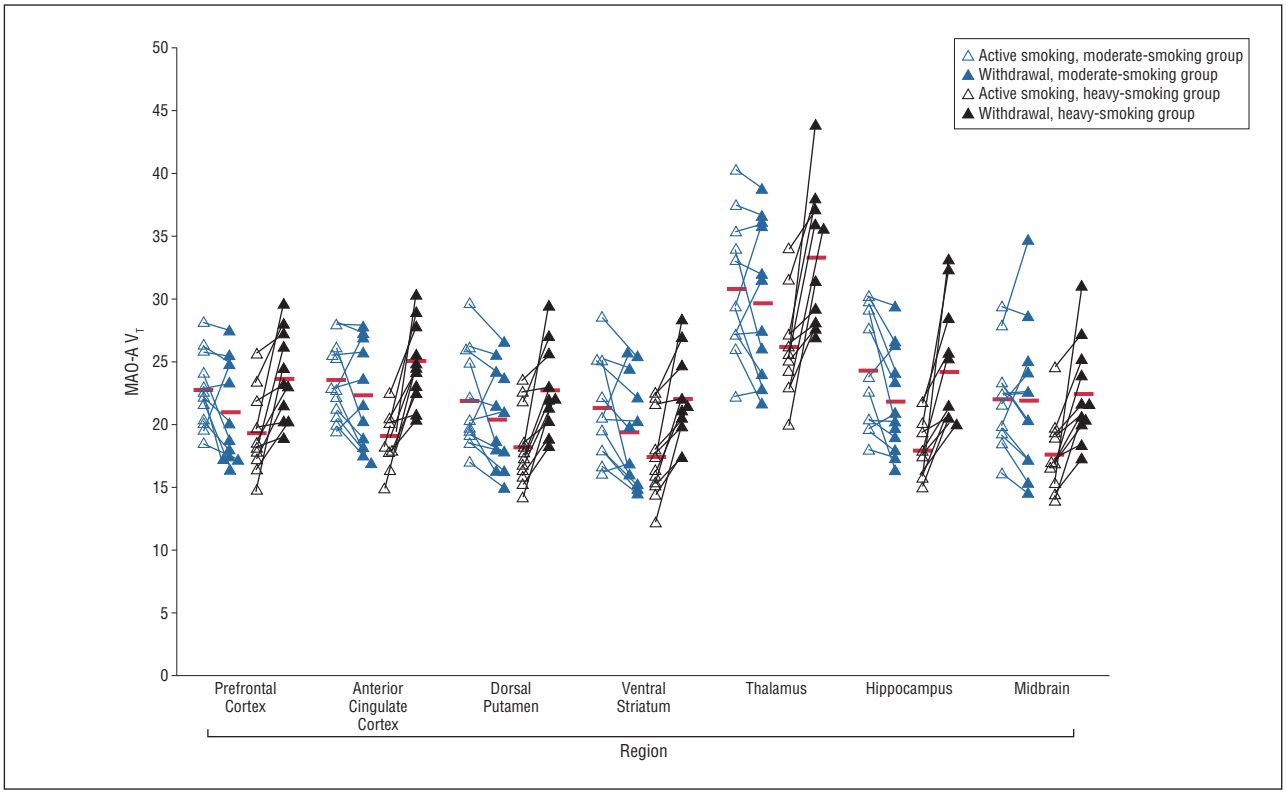

Figure 1. Monoamine oxidase A (MAO-A) binding in cigarette-smoking individuals during active smoking and withdrawal. Repeated-measures multivariate analysis of variance (MANOVA) found a highly significant effect between smoking severity and change in MAO-A V_T (an index of MAO-A density) in the prefrontal cortex and anterior cingulate cortex regions ($F_{1,22}$=25.58, P<.001). Repeated-measures MANOVA also found a significant effect between smoking severity and change in MAO-A V_T for all the regions assayed ($F_{1,22}$=28.24, P<.001). The effect was confirmed in each region with a repeated-measures analysis of variance (ANOVA) (interaction between smoking severity and change in MAO-A V_T, repeated-measures ANOVA, $F_{1,22}$=11.16-28.87, P=.003 to P<.001). P<.001 for all regions except the midbrain (P=.003).

partment model or the Logan model with arterial sampling (for which the underestimate of V_T is negligible); we applied the latter technique in our study.[33] This method has been described in greater detail elsewhere.[14,33]

STATISTICAL ANALYSIS

We performed 4 main analyses. The first was a repeated-measures multivariate analysis of variance (MANOVA) applied to determine the effect of condition (ie, active smoking vs withdrawal) and smoking severity (ie, heavy vs moderate) on prefrontal and anterior cingulate cortex MAO-A V_T in the entire cigarette-smoking group. The second was a MANOVA to assess the group effect (ie, withdrawal from smoking vs health) on prefrontal and anterior cingulate cortex MAO-A V_T in the heavy-smoking and healthy nonsmoking groups. The third was a repeated-measures multivariate analysis of covariance (MANCOVA) in the heavy–cigarette-smoking group with harman plasma level as the covariate and conditions of active smoking and withdrawal on prefrontal and anterior cingulate cortex MAO-A V_T. The fourth was a repeated-measures MANCOVA in the heavy–cigarette-smoking group with change in the depression VAS as the covariate and conditions of active smoking and withdrawal on prefrontal and anterior cingulate cortex MAO-A V_T. For the fourth analysis, the covariate reflecting change in the depression VAS was determined as follows: the change score on the depression VAS for each protocol day was defined as the VAS score before scanning minus the VAS score at t=0. The difference in the change score between active smoking and withdrawal days was the covariate applied in the fourth main analysis.

RESULTS

CIGARETTE-SMOKING HABITS OF THE CIGARETTE-SMOKING GROUP

Twelve individuals comprised the moderate-smoking subgroup and 12 comprised the heavy-smoking subgroup. The mean (SD) number of cigarettes smoked per day was 20.89 (–2.42) and 40.00 (–7.69), respectively, in these groups. Exhaled carbon monoxide levels were 25.70 (–10.74) and 6.58 (3.78) ppm during active-smoking days and 38.00 (–13.64) and 12.00 (–7.62) ppm during withdrawal days in the moderate- and heavy-smoking groups, respectively. In our sample, the ratio of cigarette smoking in men to women was 7:1, which was consistent with the sex frequencies of cigarette smoking for moderate and heavy levels among the ethnicities represented in Toronto.[2,51]

DIFFERENCE IN MAO-A V_T BETWEEN THE ACTIVE SMOKING STATE AND WITHDRAWAL

The predominant change was an elevation in MAO-A V_T during withdrawal in the heavy-smoking subgroup not present in the moderate-smoking subgroup (**Figure 1** and **Table 2**). The magnitude of this change was 23.7% and 33.3% in the prefrontal and anterior cingulate cortices, respectively. Repeated-

Table 2. Mean (SD) MAO-A V_T in Healthy Control and Cigarette-Smoking Individuals in Withdrawal and Active Smoking Conditions[a]

Area	Healthy Control Group	Moderate-Smoking Group		Heavy-Smoking Group	
		Withdrawal	Active Smoking	Withdrawal	Active Smoking
PFC	18.88 (3.78)	20.95 (3.98)	22.69 (2.94)	23.59 (3.54)[b]	19.32 (3.05)
ACC	19.94 (4.03)	22.28 (4.01)	23.50 (3.00)	25.04 (3.54)[b]	19.05 (2.81)
Dorsal putamen	18.60 (3.49)	20.38 (3.92)	21.81 (3.94)	22.69 (3.27)[c]	18.16 (2.97)
Ventral striatum	19.04 (3.80)	19.32 (4.27)	21.29 (3.99)	22.00 (3.28)[d]	17.42 (3.24)
Thalamus	27.57 (5.92)	29.62 (6.23)	30.73 (5.30)	33.24 (5.44)[e]	26.09 (3.77)
Hippocampus	21.78 (3.63)	21.79 (4.15)	24.30 (4.77)	24.15 (4.82)[f]	17.90 (2.37)[c]
Midbrain	19.07 (3.90)	21.86 (5.83)	22.00 (3.78)	22.38 (3.90)[d]	17.58 (2.84)

Abbreviations: ACC, anterior cingulate cortex; MAO-A, monoamine oxidase A; PFC, prefrontal cortex; V_T, an index of MAO-A density.

[a] For the multivariate analysis of variance comparisons with the healthy group with positive results, both of which are in the heavy-smoking group; post hoc, individual analysis of variance results also are presented comparing MAO-A V_T levels between healthy and cigarette-smoking individuals.

[b] $P=.001$.

[c] $P<.005$.

[d] $P<.05$.

[e] $P<.01$.

[f] $P=.10$.

measures MANOVA found a highly significant interaction between smoking severity (ie, moderate or heavy smoking) and condition (ie, measurement of MAO-A V_T during active smoking and withdrawal) in the prefrontal cortex and anterior cingulate cortex regions ($F_{1,22}=25.58$, $P<.001$). Repeated-measures ANOVA confirmed similar results in the individual regions of an interaction between smoking severity and condition (ie, active smoking and withdrawal) on MAO-A V_T, the prefrontal cortex ($F_{1,22}=20.9$, $P<.001$), and the anterior cingulate cortex ($F_{1,22}=28.09$, $P<.001$). Repeated-measures MANOVA also found a significant interaction between smoking severity and condition (ie, active smoking or withdrawal) on MAO-A V_T for all the regions assayed ($F_{1,22}=28.24$, $P<.001$), and in these additional regions, the findings were confirmed with a repeated-measures ANOVA in each region (interaction between smoking severity and condition, repeated-measures ANOVA, $F_{1,22}=11.16$-28.87, $P=.003$ to $P<.001$).

Repeated-measures MANOVA found no significant effect of condition on the prefrontal and anterior cingulate cortex MAO-A V_T ($F_{1,11}=3.195$, $P=.10$) within the moderate-smoking group. Similarly, no effect of condition was present on MAO-A V_T within all the brain regions (repeated-measures MANOVA, $F_{1,11}=3.292$, $P=.10$).

DIFFERENCE IN MAO-A V_T BETWEEN THE WITHDRAWAL-FROM-HEAVY-SMOKING AND HEALTHY NONSMOKING STATES

Prefrontal and anterior cingulate cortex MAO-A V_T levels were significantly greater in the withdrawal state of those in the heavy-smoking subgroup compared with healthy nonsmoking controls (MANOVA, $F_{2,33}=6.72$, $P=.004$). This finding was confirmed in each region (independent t test, $t_{34}=3.61$ and 3.72, $P=.001$ and $P=.001$, respectively). The magnitudes were 25.0% and 25.6% greater in the withdrawal state from heavy cigarette smoking compared with healthy nonsmoking individuals. Greater MAO-A V_T during withdrawal from heavy cigarette smoking compared with healthy nonsmoking controls also was found in all the brain regions assayed (MANOVA, $F_{7,28}=3.25$, $P=.01$). Most other individual regions also were significantly different (independent t test, $t_{34}=3.39$-1.66, $P=.002$-$.10$, respectively) (**Figure 2**).

RELATIONSHIP BETWEEN β-CARBOLINE LEVELS AND CHANGE IN MAO-A V_T IN THE HEAVY-SMOKING GROUP

Harman and norharman levels were detectable (ie, >5 pg/mL) in all 12 study participants in the heavy–cigarette-smoking group during the active-smoking state, but levels of harmine, harmol, and 2-napthylamine were not detectable (ie, ≥5 pg/mL) during the active-smoking state. The reduction in harman levels between active-smoking and withdrawal states significantly covaried with the change in prefrontal and anterior cingulate cortex MAO-A V_T (repeated-measures MANCOVA, $F_{1,10}=9.97$, $P=.01$), which was confirmed by correlations within the individual regions ($r=0.71$, $P=.009$, and $r=0.68$, $P=.02$, respectively) (**Figure 3**). The reduction in norharman between active smoking and withdrawal states did not significantly covary with the change in prefrontal and anterior cingulate cortex MAO-A V_T (MANCOVA, $F_{1,10}=1.67$, $P=.23$).

RELATIONSHIP BETWEEN MOOD CHANGES, RATING SCALES OF STATE, AND CHANGE IN MAO-A V_T IN HEAVY-SMOKING GROUP

The change toward depression on the VAS was found in the withdrawal day and the active-smoking day. The difference in this change score significantly covaried with the increase in MAO-A V_T within the prefrontal cortex and anterior cingulate cortex in participants in the heavy-smoking subgroup (repeated-measures MANCOVA, $F_{1,10}=11.91$, $P=.006$; prefrontal cortex: $r=0.74$, $P=.006$; anterior cingulate cortex: $r=0.70$, $P=.01$) (**Figure 4**). A similar analysis for the other VAS scores was not significant (energy: MANCOVA, $F_{1,10}=2.57$, $P=.14$; anxiety: MANCOVA, $F_{1,10}=0.01$, $P=.92$).

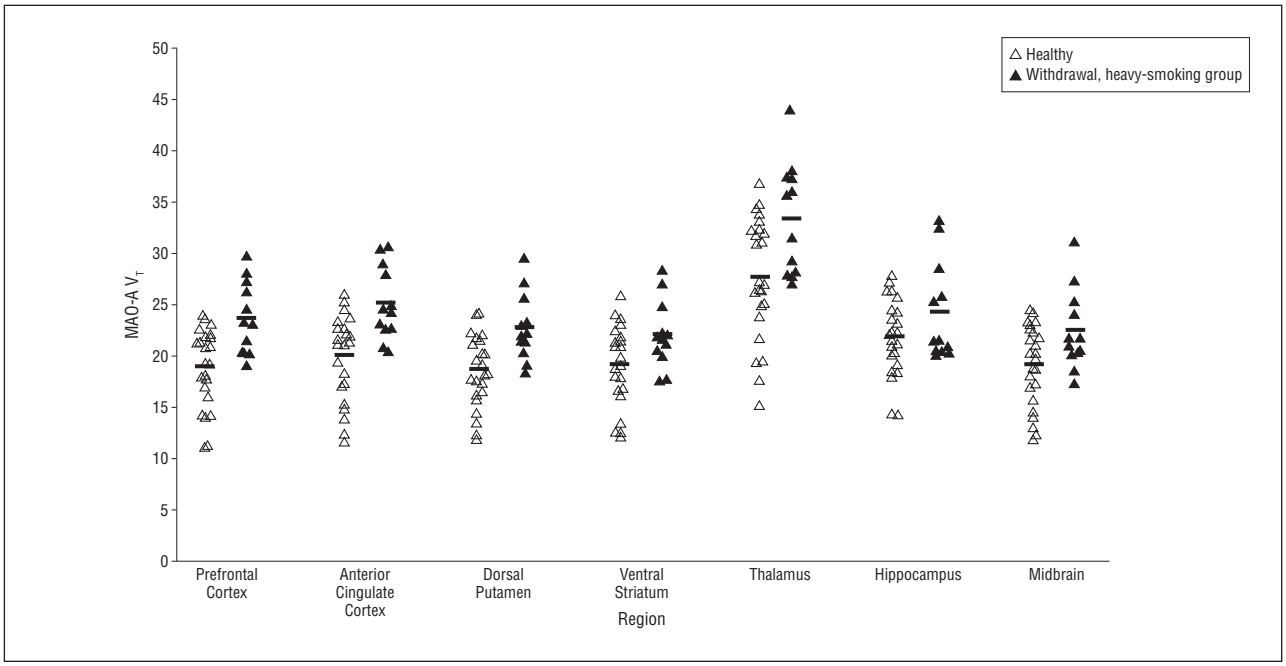

Figure 2. Greater monoamine oxidase A (MAO-A) binding during acute cigarette withdrawal in the heavy-smoking group compared with healthy nonsmoking control individuals. The MAO-A V_T (an index of MAO-A density) was significantly greater in the prefrontal and anterior cingulate cortices in the withdrawal state of those in the heavy-smoking subgroup compared with healthy controls (multivariate analysis of variance, $F_{2,33}$=6.72, P=.004). Most other individual regions also were significantly different (independent t test). P=.001 for the prefrontal cortex, P=.001 for the anterior cingulate cortex, P=.002 for the dorsal putamen, P=.009 for the thalamus, P=.03 for the ventral striatum, P=.02 for the midbrain, and P=.10 for the hippocampus.

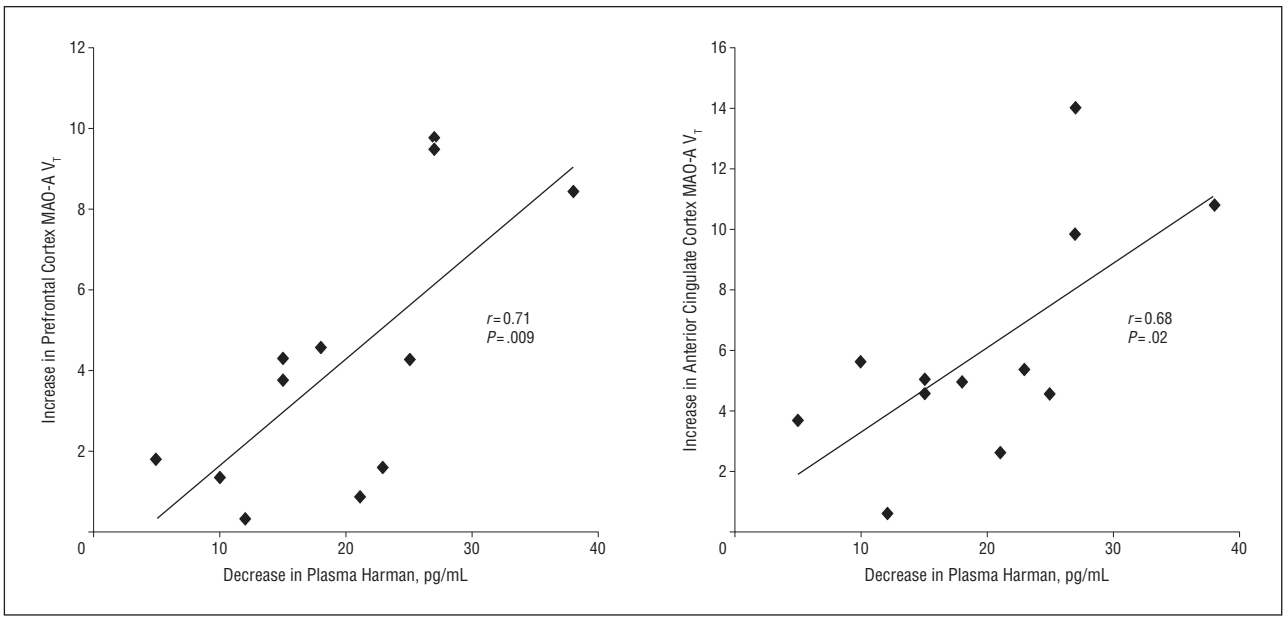

Figure 3. During withdrawal, the increase in monoamine oxidase A (MAO-A) V_T (an index of MAO-A density) covaried strongly with the decline in plasma harman (repeated-measures multivariate analysis of covariance, $F_{1,10}$=9.97, P=.01; harman was the covariate, and MAO-A V_T in the prefrontal and anterior cingulate cortices in active smoking and withdrawal were the dependent variables). Increase in MAO-A V_T is calculated from the MAO-A V_T during withdrawal minus MAO-A V_T during active smoking. The r and P values presented for the individual regions were calculated from the Pearson correlation coefficient.

COMMENT

Ours is the first study, to our knowledge, to measure brain MAO-A binding in acute cigarette withdrawal. Prefrontal and anterior cingulate cortex MAO-A V_T levels increased during withdrawal in the heavy-smoking group but not in the moderate-smoking group. In those who smoked heavily, prefrontal and anterior cingulate cortex MAO-A V_T levels increased during withdrawal such that the levels of MAO-A V_T were greater than those in healthy controls. The magnitude of increase in MAO-A V_T in the prefrontal and anterior cingulate cortices during withdrawal was significantly correlated with the shift in VAS scores toward depressed mood in those who smoke heavily. The increase in MAO-A V_T level also correlated with the decline in the MAO-A–binding substance har-

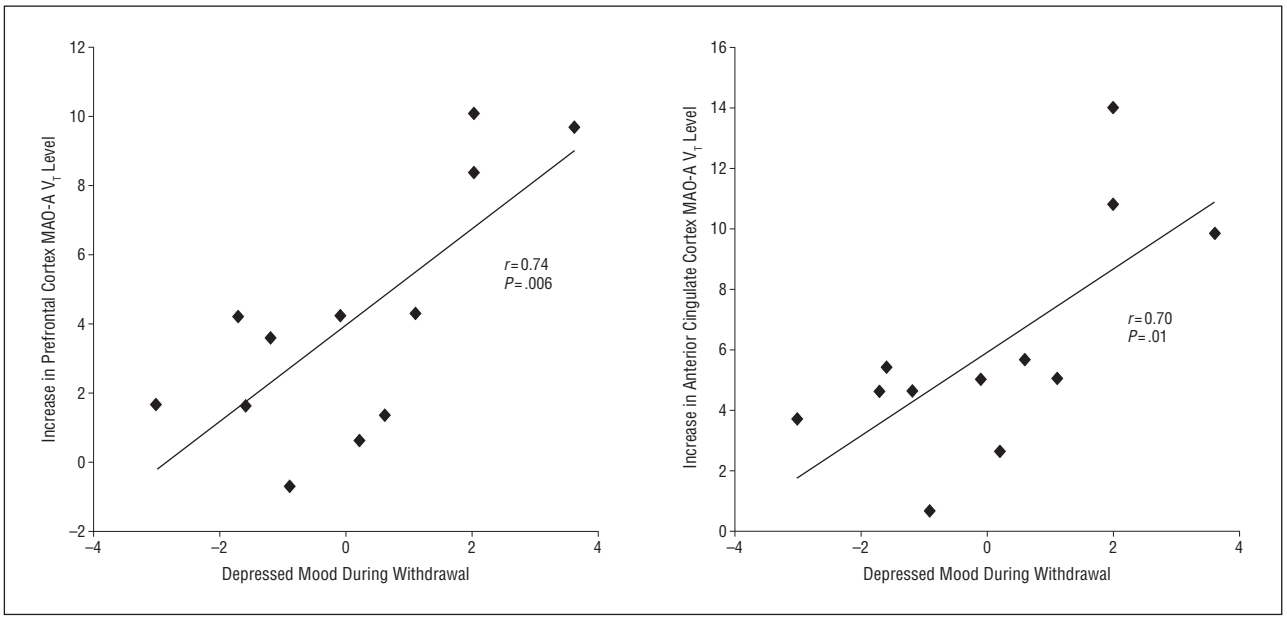

Figure 4. Correlation between the increase in monoamine oxidase A (MAO-A) V_T (an index of MAO-A density) and shift to depressed mood between active smoking and withdrawal in heavy-smoking individuals. The change toward depressed mood on the visual analog scale (VAS) was found in the withdrawal day and the active-smoking day. The change in the VAS score represents the difference in change score between active-smoking and withdrawal days. The change score on the VAS for each protocol day was defined as the VAS score before scanning minus the VAS score at $t=0$. The increase in MAO-A V_T is calculated from the MAO-A V_T during withdrawal minus the MAO-A V_T during active smoking. The difference in this change score covaried significantly with the increase in MAO-A V_T in the prefrontal and anterior cingulate cortices in individuals in the heavy-smoking subgroup (repeated-measures multiple analysis of covariance, $F_{1,10}=11.91$, $P=.006$; difference in change score covariate, MAO-A V_T in active smoking and withdrawal dependent variable).

man in those who smoke heavily. Plasma levels of 3 other MAO-A–binding substances were extremely low. These results have significant implications for quitting heavy smoking and for understanding what has previously appeared to be a paradoxical association of cigarette smoking with major depressive disorder and suicide. Understanding the neurobiology of heavy cigarette smoking is important because those who smoke heavily are much more likely to have major depressive disorder and to experience medical complications resulting from cigarette smoking.[52,53]

The increase in prefrontal and anterior cingulate cortex MAO-A V_T during withdrawal from heavy smoking was robust and highly significant. The process of a rapid change in available binding is important because substances with greater addictiveness are more rapidly removed from target sites.[7,54] A plausible explanation for the MAO-A V_T changes during cigarette withdrawal from heavy smoking is that rapid loss of harman during acute cigarette withdrawal lead to an increase in available regional MAO-A binding. The relationship between the increase in MAO-A V_T and greater depression may be explained by the inverse relationship between MAO-A levels and metabolism rate of monoamines because abrupt decreases in serotonin, norepinephrine, and dopamine are consistently associated with depressed mood,[26-29] and elevated MAO-A V_T levels, particularly in the prefrontal and anterior cingulate cortices, are consistently associated with states that generate low mood, such as major depressive episodes, postpartum blues, and predisposition to recurrence of major depressive episodes.[13-15] Subregions of the prefrontal cortex and anterior cingulate cortex participate in interpreting experiences optimistically or pessimistically, including recalled and anticipated experiences.[55-57] Alterations in extracellular serotonin modulate this optimism and pessimism, and markers associated with ongoing decreased serotonin release in these regions are associated with greater pessimism.[58-60] Subregions of the prefrontal cortex and/or anterior cingulate cortex also participate in the recall of emotional material and the interpretation of the valence of perceptual stimuli.[16] Manipulations of extracellular norepinephrine levels influence these latter functions such that relatively lower norepinephrine is associated with a bias of these functions toward negative valence.[61,62] Thus, excessive metabolism of serotonin and norepinephrine in these regions may affect many cognitive processes implicated in generating depressed mood.

It is intriguing that the MAO-A V_T level was greater in the withdrawal condition of the heavy-smoking group compared with healthy nonsmoking controls. Possible explanations are that people who smoke heavily have a preexisting trait or condition associated with greater MAO-A levels or that long-term, heavy cigarette smoking is associated with greater production and reduced degradation of MAO-A protein (although the MAO-A site can be transiently occupied by MAO-A–binding substances). A preexisting difference between groups in personality is unlikely to explain greater MAO-A V_T during withdrawal in the cigarette-smoking participants because this potential bias should be related to a reduction in MAO-A binding in the cigarette-smoking group. Personality traits of greater neuroticism and lower deliberation are associated with cigarette smoking,[63] but greater neuroticism (particularly aggression) and lower deliberation also are associated with reduced MAO-A binding.[64,65] In the present study, levels of neuroticism and personality facets of angry-hostility or deliberation were

similar between those who smoked cigarettes and those who did not. Relative levels of these traits were not reported in the cross-sectional studies comparing MAO-A binding in cigarette smoking to health[12,66,67] because the relationship between personality traits and MAO-A binding was discovered recently.[64,65] The question of whether MAO-binding substances may increase production of MAO-A binding has been investigated minimally; however, ethanol administration (which may be associated with greater plasma levels of harman in humans[68]) was associated with greater levels of monoamine oxidase B (MAO-B) protein.[69] Future studies will need to address whether substances such as harman or norharman influence synthesis or removal of MAO-A.

We prefer an occupancy explanation to account for the rapid change in MAO-A V_T within individuals because 2 brain-penetrant substances that bind to MAO-A, harman and norharman, were detected in plasma in the active-smoking state and were rapidly removed from plasma during withdrawal. In addition, the changes in plasma levels of harman correlated significantly with the change in MAO-A V_T in the prefrontal and anterior cingulate cortices (as well as most other brain regions). To our knowledge, ours is the first study to measure plasma levels of a series of MAO-A–binding substances in people who smoke cigarettes, unconfounded by coffee or alcohol consumption.[68] Because plasma levels of harmine, harmol, and naphthylamine were below the threshold of 5 pg/mL, they were viewed as unlikely candidates for an MAO-A occupancy model. Given the low levels of other suspected MAO-A–binding substances, the fairly high affinity of harman for MAO-A (for harman, Ki [equilibrium dissociation for a competitor] = 220 nM, and for norharman, Ki = 2200 nM[35]), and the significant correlation of plasma harman levels with change in MAO-A binding, reductions in harman best explain the greater MAO-A V_T levels during withdrawal from heavy cigarette smoking.

Greater rates of completed suicide and major depressive disorder are associated with cigarette smoking, the former occurring even when controlling for comorbidity of psychiatric illnesses.[52,53,70,71] This relationship is perplexing because, in the past, it was believed that cigarettes had an antidepressant effect[23] by steadily occupying MAO-A. Our study resolves this paradox by demonstrating that binding to MAO-A is transient. Greater MAO-A V_T in the prefrontal and anterior cingulate cortices during acute cigarette withdrawal and associated depressed mood is relevant to suicide research. Depressed mood is associated with greater risk of death by suicide, and in a retrospective study of 100 individuals who completed suicide, Barraclough et al[72] reported that 5 deaths were associated with a recent change in cigarette smoking. A retrospective study investigates a related issue, namely, comparing suicide rates during active treatment for quitting vs placebo, and then assessing the link to suicide; however, it did not focus on the early quit period.[73] Future investigations of the relationship between suicide and cigarette smoking should consider the early quit period in those who smoke heavily and focus on individuals with major depressive disorder.

The rapid increase in MAO-A V_T levels during acute cigarette withdrawal has significant implications for the clinical use of MAO-A inhibitors to assist quitting. This rapid increase in MAO-A V_T is targetable with an MAO-A inhibitor such as moclobemide. The results of the present study predict that people who smoke cigarettes heavily should benefit from treatment with an MAO-A inhibitor early in withdrawal. We are aware of 1 such study. In 88 nondepressed individuals, Berlin et al[74] reported that treatment with the MAO-A inhibitor moclobemide was associated with significantly greater abstinence rates at 1 week through 6 months compared with placebo. Interestingly, 60% of the participants in this trial smoked fairly heavily, at a rate of more than 30 cigarettes per day. It is also possible that better quit rates may be obtained in the future with substitution therapy for multiple targets with consideration for the timing of withdrawal effects. For example, during cigarette withdrawal, the occupancy of $\alpha_4\beta_2$ receptors persists for more than a day, and the occupancy of MAO-B persists for at least 11 hours.[8,75]

Our study has limitations typical of studies involving PET. The measure of MAO-A, an index of MAO-A density called MAO-A V_T, reflects total binding and is computationally efficient, highly stable, and the least variable measure of [^{11}C]harmine binding. However, approximately 15% of this measure reflects free and nonspecific binding, so it is assumed that free and nonspecific binding does not differ tremendously between conditions.[33] An elevation in MAO-A V_T also may reflect greater affinity of MAO-A, although this would not change our interpretation because greater affinity of MAO-A for monoamines would be expected to contribute to monoamine loss. We performed 4 main analyses in this study, so it could be argued that the significances for the main analyses should be multiplied by 4; however, each of these uncorrected significances were sufficiently low, so the results still hold even when considering the 4 main analyses.

In summary, ours is the first study, to our knowledge, of brain MAO-A binding during early cigarette withdrawal. To better explain the complexity of acute cigarette withdrawal after heavy smoking, we argue for adding a new model to previously established mechanisms, focusing on the rapid change in available MAO-A binding. During the withdrawal state in individuals who smoke heavily, MAO-A binding increases rapidly in affect modulating brain regions, such as the prefrontal cortex and anterior cingulate cortex, with MAO-A binding exceeding levels observed in healthy nonsmoking individuals. A greater increase in MAO-A V_T in the prefrontal and anterior cingulate cortices may contribute to a shift toward depressed mood given the correlation between the 2 measures, namely, the role of MAO-A in metabolizing monoamines[23,24] and the inverse relationship between MAO-A V_T and mood.[13-15] The increase in MAO-A V_T during acute withdrawal is explained best by a reduction in harman because plasma levels of this MAO-A–binding substance were associated with the change in MAO-A V_T between active smoking and withdrawal. This study also resolves the paradox of how cigarette smoking can be associated with major depressive disorder and suicide if persistent, antidepressant-like MAO-A inhibition is present. Our answer is that the MAO-A inhibition observed is not like the effect of antidepressants because the in-

hibition is highly transient and MAO-A V_T increases during early withdrawal. This finding suggests that the early withdrawal period should be an area of focus in future investigations of suicide in heavy cigarette smoking.[70,71] The increase in MAO-A V_T during acute withdrawal also argues in support of additional clinical trials of MAO-A inhibitors for the earliest stages of quitting heavy cigarette smoking, during which relapse rates are highest.[74]

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