Published in final edited form as:

J Occup Environ Med. 2016 May ; 58(5): 436–443. doi:10.1097/JOM.0000000000000712.

Brain Anatomy in Latino Farmworkers Exposed to Pesticides and Nicotine

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Introduction

Migrant tobacco farmworkers in the US have consistently been shown to expect pesticide [1–5] and nicotine [6–8] exposure associated with their work. Nicotine and cholinesterase inhibiting (ChEI) pesticides, such as organophosphates and carbamates, both alter the function of the acetylcholine neurotransmitter system and have potential implications for brain health and disease in exposed individuals. While toxic exposures to nicotine [7, 9, 10] and ChEI pesticides [11–13] have known detrimental effects on the brain [14, 15], there is limited research evaluating long-term, low to moderate exposures as commonly experienced by migrant tobacco farmworkers.

Tobacco workers are exposed to nicotine through the manual manipulation of the tobacco plant. During the topping and priming of the tobacco plant, salivary nicotine levels approach those observed in regular smokers [16]. In extreme situations, tobacco workers can have systemic nicotine levels that become toxic and result in green tobacco sickness [7, 9, 10]. Increased systemic nicotine levels result in enhanced nicotinic acetylcholine receptor stimulation throughout the nervous system. While the primary cholinergic neurons are located in the basal forebrain and brain stem, projecting pathways that release acetylcholine are widely distributed throughout the brain. In fact, nicotinic acetylcholine receptors have been documented throughout the cerebral cortex, the basal ganglia, thalamus, and cerebellum (for recent review see [17]).

We are not aware of any literature evaluating the neurobiological consequences of long-term, low to moderate nicotine exposure other than the growing literature evaluating the

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The authors declare no conflicts of interest.

neurobiological effects of smoking. While tobacco has many potential compounds that affect the brain, nicotine is implicated in many of the findings. Acute exposure to nicotine through a medication or cigarette smoking is known to enhance attention and concentration [18–20]. Long-term smoking has been shown to be protective against Parkinson's disease (PD), a disease known to be associated with reduced brain dopamine [21–27]. The mechanism underlying this protective effect remains unknown but may be related to nicotine-induced dopamine release [28] or to reduced oxidative stress when nicotine binds free iron [29].

Despite the protective effect of smoking, there is substantial evidence that chronic smoking is associated with loss of brain tissue volume in multiple regions. Most consistently reported is a loss of gray matter in the lateral and medial frontal lobes [30–35]. Conflicting findings have been reported in the cerebellum and basal ganglia [32–38]. For example, in the basal ganglia there have been reports of total volume increases [38], increased [32] and decreased [34] gray matter volume, and increased white matter volume [36]. Further work is necessary to clarify these findings, but it is clear that smoking is associated with anatomical changes in the frontal lobes, basal ganglia, and cerebellum. Contributions from nicotine or other factors associated with smoking remain to be determined.

Exposure to ChEI pesticides increases acetylcholine levels throughout the body and enhances cholinergic neurotransmission in both nicotinic and muscarinic pathways. Current research on the cognitive effects of anti-cholinesterase pesticides contains conflicting findings [17]. Multiple studies have shown that chronic, low level ChEI pesticide exposure is associated with cognitive deficits [39–42]. However, other studies have shown either no cognitive effects [43, 44] or cognitive enhancement [45]. ChEI medications are commonly used to help alleviate the cognitive symptoms in dementia though the effectiveness remains debated [46, 47]. In normal healthy individuals, single and repeated doses of ChEI medications have also resulted in mixed outcomes, but enhanced cognition after mild sleep deprivation is consistently observed [48–50].

Although the cognitive outcomes remain equivocal, there is substantial evidence that long-term ChEI exposure increases the risk of developing neurodegenerative diseases, particularly PD [51]. It is interesting that pesticide exposure is positively associated with the development of PD, but smoking is inversely associated with the development of PD [24]. Evidence for other disorders such as Alzheimer's disease (AD) is more limited, but it has been suggested that anti-cholinesterase pesticide exposure is associated with increased risk of developing AD [12], and serum levels of persistent anti-cholinesterase pesticides have been shown to be higher in patients with AD than in control participants [52].

Exposure to ChEI compounds is also implicated in Gulf War Illness [53–55], a syndrome that is characterized by multiple neurological deficits. Individuals with Gulf War Illness have global brain atrophy exceeding that observed in veterans who are not believed to have been exposed to ChEI pesticides [56]. Findings of regional gray matter loss are non-specific and have been reported in occipital, parietal, and frontal lobes as well as in the cerebellum and hippocampus [54, 57]. White matter has been less studied with reports of increases and decreases in tissue integrity [57–59] and decreased or no change in white matter volume [54, 56].

Despite some uncertainty that remains concerning neurobiological consequences of exposure to compounds that alter cholinergic transmission, it is vital that further studies evaluate brain changes in populations exposed to both nicotine and cholinesterase inhibitors. The current study was designed to measure neuroanatomical differences between migrant Latino tobacco farmworkers and Latino residents in North Carolina who had not worked in farming or other occupations with high likelihood of pesticide and nicotine exposure during the prior 3 years. The main objective was to use magnetic resonance imaging (MRI) and voxel-based morphometry (VBM) to compare brain anatomy between the farmworkers and non-farmworkers. It was hypothesized that farmworkers would have decreased frontal lobe gray matter signal compared to non-farmworkers. It was also hypothesized that group differences would be detected in the basal ganglia and cerebellum gray matter, but the conflicting findings in the literature left open the potential for increased or decreased signal in the farmworkers. Blood cholinesterase activities and urine cotinine (a nicotine metabolite) levels were included in the analyses to identify associations between anatomical differences and exposure.

Methods

Participants

The analysis reported is based on data collected from a subsample of study participants as part of an ongoing community-based participatory research program, "Pesticide Exposure & Neurological Outcomes for Latinos: PACE4." All participants were Latino men aged 30–70 at time of entry into the parent study with most reporting Spanish as their primary language. The ongoing PACE4 Study follows a cohort (initial size $n=447$) of Latino migrant farmworkers and Latino immigrant non-farmworkers. All farmworkers were recruited from counties located in the east central region of North Carolina. Farmworkers had to be currently employed as agricultural laborers and have worked in agriculture for at least three years. Our ethnographic experience indicates that all of these farmworkers participate in tobacco farming during some portion of the agricultural season. Non-farmworkers were recruited from the Forsyth County area located in the west central part of the state and could not have worked in occupations routinely associated with potential pesticide exposure, such as farm work, forestry, grounds keeping, lawn maintenance, or pest control within the last three years. Any potential participants told by a health care professional that they had diabetes were excluded from the parent study. The study involved the collection of blood, urine, and surveys, as well as the performance of neurologic/cognitive tests at various time points during the 2012, 2013, and 2014 North Carolina agricultural seasons.

This ancillary study was performed during the 2013 agricultural season on 51 farmworkers and 30 non-farmworkers that included the collection of brain MRI scans. In addition, blood cholinesterase activity and urine cotinine levels were determined from samples collected that same summer. From the total 81 participants recruited for this ancillary study, 74 participants had complete data (brain imaging, at least one blood sample, and one urine sample), 4 participants had no blood sample, 2 had no urine sample, and 1 participant was missing both. The final analyses were restricted to these 74 participants (48 farmworkers and 26 non-farmworkers). The Institutional Review Board of Wake Forest School of Medicine

approved the study. All participants gave written informed consent to participate in the main study and were consented again for participation in the MRI study. Individuals were compensated for participating.

Image acquisition

MRI scans were collected between May and November 2013. All imaging was performed on a Siemens 3T Skyra with a 32-channel head coil. High-resolution (0.98 mm × 0.98 mm × 1.0 mm) T1-weighted structural scans were acquired in the sagittal plane using a single-shot 3D MPRAGE GRAPPA2 sequence (acquisition time = 5 minutes and 30 seconds, TR = 2.3 seconds, TE = 2.99 ms, 192 slices).

Voxel-based morphometry (VBM)

The current study utilized both modulated and unmodulated VBM because they differ in sensitivity to changes in gray volume and tissue contrast. It has recently been demonstrated that VBM is sensitive to changes in brain tissue contrast because the tissue segmentation step is based on differences in contrast between gray and white matter [60–62]. Of particular interest is the fact that heavy metal accumulation, such as iron, results in decreased gray matter signal [63, 64], particularly in the frontal lobes and basal ganglia.

Given that hypotheses were focused on regions sensitive to iron accumulation, analyses were performed using both modulated VBM and unmodulated VBM. Modulated VBM is most sensitive to tissue volume as the expansions and contractions of brain tissue required to warp brains to a standard template (Jacobian determinants) are used to scale the segmented tissue images [65]. Statistical comparisons are intended to identify differences in the magnitude of tissue warping. Unmodulated VBM directly compares the warped segmented tissue images and does not use the expansion and contractions parameters in the Jacobian determinants. Thus, this method is highly sensitive to differences in tissue contrast and less sensitive to differences in tissue volume. Heavy metal accumulation that alters tissue contrast will present as a decrease in the gray matter signal when using unmodulated VBM [61].

The standard procedures for VBM were implemented in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; <http://www.fil.ion.ucl.ac.uk/spm>) using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) algorithm and the default settings [66]. The T1-weighted scan from each subject was independently coregistered to MNI space using a 6 parameter affine transformation. After coregistration, the T1-weighted scans were segmented into gray matter, white matter, and cerebrospinal fluid probability maps. SPM DARTEL was implemented on the entire cohort. This method utilizes segmented gray and white matter images to create an average template, iteratively aligning each subject's image as the template is refined. At the completion of this process, the segmented images from each subject were aligned with the final template. The magnitude of tissue deformation necessary was captured in the Jacobian determinates from the deformation fields. The Jacobian determinates were used to scale the segmented images, producing modulated images to quantify the volume of tissue in each voxel. Both modulated and unmodulated images were generated for each study participant. The study template was then normalized to MNI space, and these warping parameters were applied to the modulated

and unmodulated subject images. All images were smoothed using a 10 mm³ Gaussian smoothing kernel.

Cholinesterase Assay

Total blood cholinesterase, butyrylcholinesterase, and acetylcholinesterase activity were measured from blood samples that were collected 6/2/2013 – 10/20/2013. For the current analyses, the average value across the summer was used for each participant. The vast majority of participants had 3 or 4 samples over the summer but five individuals (4 non-farmworkers, 1 farmworker) had two samples and a single participant (farmworker) had one sample. Cholinesterase activities were assayed with a modification of the radiometric method of Johnson and Russell [67] as described in the Supplemental Digital Content and population values are reported in Supplemental Digital Content Table S1.

Cotinine Assay

Urine cotinine levels were measured and used in the image analyses as a covariate. Urine samples that were collected between 6/2/2013 – 10/20/2013 were assayed for cotinine content. The assay was performed by Salimetrics LLC using standard procedures. Details are presented in the Supplemental Digital Content and population values are reported in Supplemental Digital Content Table S1.

Statistical analyses

Demographic variables were evaluated using SAS 9.4 (SAS Institute, Cary, NC). Descriptive statistics (count, percent) were calculated for participant characteristics of interest for farmworkers and non-farmworkers. Chi-square or Fisher's exact tests were used as appropriate to test the association between the participant characteristics and farmworker status. All image analyses were performed in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). The primary voxel-wise group comparison was performed using a regression analysis that included age and total intracranial volume (TIV) as covariates. Additional regression analyses were performed that included additional covariates for cholinesterase activity, urine cotinine levels, and smoking history. Statistical significance for all analyses were set at $p < 0.005$ and corrected for multiple comparisons at $p = 0.05$ using a cluster extent correction [68].

Results

Participants

The study population groups were well balanced for age and education and smoking history (Table 1). The percentage of individuals in each age group was comparable between groups across 3 age ranges (30–34, 35–44, and greater than 45). The non-farmworkers tended to have a larger portion that completed high school, while the farmworkers has a greater proportion that had 7th–11th grade educations. However, these differences did not reach statistical significance. Although there were no significant differences in smoking history, there were trends for stronger smoking histories in the farmworkers. Mexico was the country of origin for all of the farmworkers and 58% of the non-farmworkers.

Brain Imaging

VBM analyses were performed to directly identify whole brain anatomical differences in gray matter between the farmworkers and the non-farmworkers. The modulated VBM analyses did not reveal any significant group differences that survived correction for multiple comparisons. All further analyses focused on the unmodulated data since the findings from modulated VBM did not reach statistical significance.

The unmodulated VBM results revealed multiple group differences. The farmworkers had higher gray matter signal in the right basal ganglia and in the cerebellum (Figure 1). The regional differences in the basal ganglia ($p = 0.005$) were predominantly localized to the putamen. The group differences in the cerebellum were located in the inferior aspect of the left posterior lobe ($p = 0.001$) and in the anterior lobe of the cerebellar vermis ($p = 0.013$). The non-farmworkers had greater gray matter signal in the right ventro- and dorsolateral prefrontal cortex ($p = 0.012$ and 0.027 , respectively), the left medial prefrontal cortex ($p = 0.001$), and the right lateral temporal lobe ($p = 0.028$). Table 2 provides details of the location, size, and statistical significance of each region that exhibited group differences in tissue signal. In addition to these group differences that reached statistical significance, it is important to note that farmworkers also had greater gray matter signal in the left putamen and right cerebellum that did not survive correction for multiple comparisons. These regions are also included in Table 2.

The inclusion of total blood cholinesterase activity averaged over the summer as a covariate in the analysis did not have remarkable effects on group differences (Supplemental Digital Content Table S2). The regions that exhibited significant group differences in gray matter signal were not meaningfully changed in size or statistical significance. The locations of the peaks of significance were identical or within a few millimeters of the original findings. The only notable difference was in the region in the left putamen, which was double in size when including cholinesterase in the analysis; however, it still failed to achieve statistical significance. Analyses were also performed using average summer butyrylcholinesterase and acetylcholinesterase activity as covariates. As with total cholinesterase, there were no meaningful differences between the main analysis and these additional analyses (Supplemental Digital Content Table S3 and S4).

The inclusion of urine cotinine levels in the analysis resulted in considerable changes in the size of the regional differences and the inclusion of new significant regions that were not observed in the original analysis (Figure 2 and Table 3). For the regions that exhibited greater gray matter signal in the farmworkers, the right putamen was influenced the least, and was reduced from 891 voxels in the original analysis to 778 voxels. The group difference in the cerebellar vermis was reduced from 669 voxels to 484. The findings in the left cerebellum were substantially reduced from 1293 voxels down to 530 voxels, but this difference remained significant ($p = 0.025$). The trend in the left putamen remained evident, but the trend initially observed in the right cerebellum was totally abolished.

For the areas that exhibited greater signal in the non-farmworkers, the most substantial change was an increase in the size of the region in the right ventrolateral prefrontal cortex from the original size of 705 voxels to 1404 voxels. The right dorsolateral cortex difference

also nearly doubled in size. The left medial prefrontal region was reduced from 1346 voxels to 960 and the lateral temporal region was statistically abolished with only 4 voxels remaining. It is interesting to note that the right medial prefrontal cortex ($p = 0.025$) and the right inferior temporal ($p = 0.05$) regions exhibited significantly greater signal in the non-farmworkers with the inclusion of cotinine as a covariate, but these regions were not observed in the original group comparisons.

Given that cotinine is a nicotine metabolite, it was important to consider the possibility that smoking history could be contributing to the findings. A larger proportion of the farmworkers had smoking histories compared to the non-farmworkers; however, this difference did not achieve significance. To ensure that nicotine exposure due to smoking history was not driving the findings, it was included as an additional covariate in the analysis. There were no meaningful changes in the results with the addition of smoking history to the cotinine analysis (Supplemental Digital Content Table S5). We also performed an analysis comparing only the individuals that reported no history of smoking. The finding of higher gray matter signal in the right putamen and cerebellar vermis of farmworkers remained significant (Supplemental Digital Content Figure S1). The region in the left cerebellum was fragmented into many small regions that no longer achieved significance. Overall, smoking status does not appear to be driving the main study findings.

Discussion

The present study was designed to determine whether there are any neuroanatomical differences between Latino male tobacco farmworkers and a control population of Latino males that do not work in farming. The driving hypothesis behind this work was that occupational exposure to pesticides and nicotine associated with farming would have effects on brain anatomy. The analysis of brain tissue volume (modulated VBM) did not reveal any significant differences between groups. However, the analysis of gray matter signal (unmodulated VBM) revealed that the farmworkers had increased signal, compared to non-farmworkers, in the right putamen, left cerebellum, and the cerebellar vermis. There were also statistical trends, of biological significance, suggesting gray matter signal increases in the left putamen and right cerebellum in the farmworkers. The non-farmworkers had increased gray matter signal in the right prefrontal cortex, the medial prefrontal cortex, and the lateral aspect of the right temporal lobe.

One of the pronounced findings from this study was that there were no group differences for modulated VBM but there were substantial significant findings for unmodulated VBM. Recent studies have clearly shown that changes in tissue iron content can have dramatic effects on VBM results due to tissue contrast changes [61, 62]. Specifically, it has been demonstrated that iron accumulation in the brain can result in reduced gray matter signal. Thus, increased iron in gray matter results in decreased gray matter signal when assessed using VBM. Iron is known to accumulate in brain tissue with aging [69] and is thought to be associated with oxidative stress and neurodegeneration [70, 71]. The basal ganglia and the cerebellum are particularly susceptible to iron deposition [72]. The medial and lateral frontal lobes have been shown to exhibit reduced gray matter signal in VBM analyses in older adults, a finding also attributed to iron accumulation [62].

While the exact cause of the group differences in gray matter signal could not be identified using the current study design, urine cotinine levels accounted for at least a portion of the group difference. It is possible that nicotine exposure in the farmworkers may be related to reductions in brain iron deposition in the putamen and cerebellum. This is consistent with the data indicating that nicotine is protective against PD [21–27], and that PD patients have increased brain iron accumulation [73–75]. Multiple mechanisms have been proposed for antioxidant effects of nicotine including reduced glutamate release [76], direct binding of iron [77, 78], as well as nicotine-induced increases in ascorbate in the basal ganglia and nucleus accumbens [79]. However, nicotine has also been reported to enhance oxidative stress [80].

There are no data reporting the effects of long-term, low level nicotine exposure on brain anatomy in humans other than in tobacco smokers. It has been shown that smokers, compared to nonsmokers, have greater gray matter in the bilateral putamen, cerebellar vermis, and parahippocampal gyrus [32]. The putamen findings were highly consistent with the present study. There has also been a report of actual increase in the volume of the putamen in smokers [81]. However, there have also been reports of decreased gray matter [34] and increased white matter [36] in the putamen of smokers. Several studies have shown that chronic smoking is associated with reduced gray matter in the medial frontal cortex [31, 32]. There is also evidence that smokers have reduced gray matter in the ventral and dorsal lateral frontal cortex in regions comparable to the findings shown here [33, 35, 82]. Studies that report group differences in the cerebellum between smokers and non-smokers indicate that gray matter is reduced in the smokers, particularly in the posterior lobe [33, 35–37].

The conflicting findings within the literature on the effects of smoking on brain anatomy and between the literature and the current study may be explained by multiple factors. First, the methodology to perform VBM has changed considerably over the past 15 years as improved methods have been developed [66]. Results can differ substantially between various VBM methods [83, 84]. It has been recommended that caution be used when interpreting VBM analyses in studies where brain iron concentrations may differ between groups [62]. Given that smoking not only increases nicotine levels but also has the potential to increase oxidative stress, some findings in the smoking literature may be due to changes in brain iron. Recent work has also suggested that acute changes in blood flow can mimic tissue volume changes using VBM [85] and nicotine is known to alter cerebral blood flow [86, 87].

Although the present study did not identify a relationship between brain anatomy and blood cholinesterase, it is premature to rule out ChEI pesticide exposure as a contributing factor. First, blood cholinesterase activity was from the summer that MRI scans were collected. Earlier exposures that were not measured may have had lasting effects on brain anatomy. Second, human blood cholinesterase values are highly variable across time, such that inhibition from low level pesticide exposure may be difficult to detect at the individual level [5]. Moreover, correlations between blood cholinesterase activity and acetylcholinesterase activity in tissues (e.g., brain) can be affected by many factors including time after exposure, red blood cell density, liver function and others [88–90].

There is no literature examining the effects of chronic, low-level pesticide exposure on human brain anatomy. The literature on the cognitive effects of pesticide exposure is equivocal with negative [39, 40, 91], neutral [43, 44] and positive [45] effects being reported. Despite these conflicting findings, there is growing evidence that long-term exposure to low doses of organophosphates increase the odds of developing PD [92] even in smokers [24]. Possible mechanisms include increased oxidative stress [51], impaired axonal transport [93], and neuronal cell death [94]. Interestingly, in rodents, nicotine actually increases the metabolism of ChEI pesticides and reduces brain acetylcholinesterase inhibition [95]. It is intriguing to consider the possibility that in farmworkers the pesticides and nicotine could interact to reduce the toxic effects of pesticide exposure. The negative findings in the current study are quite preliminary, and there is a need to further examine the relationship between ChEI pesticide exposure and brain anatomy.

Limitations

Given the cross-sectional nature of the study, it is not possible to determine the cause of differences in brain anatomy between Latino farmworkers and non-farmworkers. While the study populations were comparable for demographic variables, there remains the possibility that unmeasured variables, such as environmental, educational, or economic differences, are contributors to the findings. While significant group differences were observed indicating sufficient statistical power, the sample size was relatively small, possibly limiting the reliability of the covariate analyses. Future studies should increase the sample size and compare migrant Latino tobacco farmworkers to migrant Latino farmworkers that have never worked in tobacco.

While there is now convergent evidence that VBM analyses are sensitive to tissue iron concentrations, the method does not quantitatively assess tissue iron. The current study did not acquire brain images that could be used to quantify iron. Nevertheless, similar findings due to iron deposition in the putamen and cerebellum have been observed in older adults [61, 62]. It will be important for future studies to acquire quantitative iron images and anatomical images that are less sensitive to iron levels [62]. Such studies will shed further light on the neurobiology underlying the findings reported here.

The inclusion of blood cholinesterase activity and urinary cotinine levels in the brain analyses were not able to fully account for the population differences observed. Urinary cotinine is a measure of acute exposure to nicotine. It is possible that assessments of long-term or chronic exposure to nicotine would be more informative. The cholinesterase activity levels were taken from blood, and such measures may not correlate well with brain cholinesterase activity, e.g., brain tissue can produce more enzyme molecules following exposure while red blood cells do not. Such issues are difficult to address without large-scale longitudinal studies, but attention to these issues is vital for future research.

Conclusions

The current study clearly demonstrated Latino tobacco farmworkers had greater gray matter signal in the putamen and cerebellum compared to non-farmworkers. Although further research is warranted due to study limitations, the findings suggest that nicotine exposure in

the farmworkers is associated with reduced iron accumulation in the basal ganglia and cerebellum. Portions of the medial and lateral frontal lobes exhibited the opposite group difference, consistent with either increased iron or atrophy in the farmworkers. Pesticide exposure was not associated with the neuroanatomical changes in this study. Further studies directed at understanding the neurobiology underlying the current findings would have substantive health implications for tobacco farmworkers exposed to nicotine and ChEI pesticides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the participation of community partners North Carolina Farmworkers Project, Benson, NC, and El Buen Pastor Latino Community Services, Winston-Salem, NC

This project was funded by NEIHS grants ES008739 and ES008739-16S1. The sponsor had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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Figure 1.

Significant differences in gray matter signal between farmworkers and non-farmworkers. Hot colored regions represent areas where the farmworkers had greater signal than the non-farmworkers. Cool colored regions show where the non-farmworkers had greater signal than the farmworkers. Images are axial slices located at MNI coordinates shown on the sagittal localizer image to the right. Color bars represent statistical T-scores. All regions are significant at $p < 0.05$ corrected for multiple comparisons. The right side of the brain is on the right side of the image in this and subsequent images.

Figure 2.

Group differences in gray matter signal after the inclusion of urine cotinine levels as a covariate. Hot colored regions represent areas where the farmworkers had greater signal than the non-farmworkers. The regions in the cerebellum were substantially reduced in extent. Cool colored regions show where the non-farmworkers had greater signal than the farmworkers. Most of these group differences were expanded in extent with the inclusion of cotinine in the analysis. Images are axial slices located at MNI coordinates shown on the sagittal localizer image to the right. Color bars represent statistical T-scores. All regions significant at $p < 0.05$ corrected for multiple comparisons.

Table 1

Study population characteristics.

Farmworkers (n=48)					Non-farmworkers (n=26)				
Participant Characteristics	n	Percent	n	Percent	n	Percent	n	Percent	p-value ^a
Age									0.6685
30–34 years	18	37.5	8	30.8					
35–44 years	18	37.5	9	34.6					
45+ years	12	25.0	9	34.6					
Education									0.0654
0–6 grade	17	35.4	7	26.9					
7–11 grade	24	50.0	9	34.6					
12 grade or more	7	14.6	10	38.5					
Country of birth									<0.0001
Mexico	48	100	15	57.7					
Other			11	42.3					
Occupation									N/A
Farmworker	48	100							
Construction			7	26.9					
Production			6	23.1					
Food preparation/restaurant			3	11.5					
Maintenance/cleaning			3	11.5					
Sales			1	3.8					

Farmworkers (n=48)		Non-farmworkers (n=26)			
Participant Characteristics	n	Percent	n	Percent	p-value ^a
Mechanic			2		7.7
Other			1		3.8
Unemployed			3		11.5
Pack years smoked at baseline ^b					0.1328
0 years	22	45.8	19		73.1
< 1 year	15	31.3	5		19.2
1–4 years	8	16.7	1		3.8
9–25 years	3	6.3	1		3.8

^a p-value for the comparison of farmworkers to non-farmworkers

^b Pack years smoked at baseline (pack years = # of packs/day * # of years smoked)

^a p-value for the comparison of farmworkers to non-farmworkers

^b Pack years smoked at baseline (pack years = # of packs/day * # of years smoked)

Table 2
Regional differences in gray matter controlling for age and total intracranial volume.

Statistical Contrast	Region	Size (# of voxels)	p-value ^a	T score	MNI coordinates (X, Y, Z) ^b
FW > NFW	Right putamen	891	0.005	4.54	28, 6, 1
	Left cerebellum	1293	0.001	4.48	-27, -60, -48
	Cerebellar vermis	669	0.013	3.59	-4, -37, -15
	Left putamen	102	0.292	4.02	-21, -10, 4
	Right cerebellum	325	0.070	3.85	21, -58, -48
FW < NFW	Right ventrolateral prefrontal	705	0.012	4.46	51, 15, 19
	Right dorsolateral prefrontal	517	0.027	3.82	26, 30, 45
	Right medial prefrontal	367	0.056	3.26	-4, 48, -21
	Left medial prefrontal	1346	0.001	5.44	-9, 56, 1
	Right temporal	503	0.028	3.29	70, -28, -12
	Right inferior temporal	14	0.721	2.82	45, -64, -18

^ap-value for comparing farmworkers to non-farmworkers.
^bMNI (Montreal Neurological Institute) coordinates for peak voxel location.
FW = farmworker NFW = non-farmworker

Table 3
Gray matter differences controlling for age, total intracranial volume, and urine cotinine.

Statistical Contrast	Region	Size (# of voxels)	p-value ^a	T score	MNI coordinates (X, Y, Z) ^b
FW > NFW	Right putamen	778	0.009	4.52	28, 6, 3
	Left cerebellum	530	0.025	3.79	-28, -60, -48
	Cerebellar vermis	484	0.031	3.41	-4, -37, -14
	Left putamen	176	0.171	4.31	-21, -10, 4
	Right cerebellum	n/a	n/a	n/a	n/a
FW < NFW	Right ventrolateral prefrontal	1404	0.001	4.32	52, 14, 16
	Right dorsolateral prefrontal	976	0.004	4.39	24, 32, 45
	Right medial prefrontal	531	0.025	4.07	14, 57, -9
	Left medial prefrontal	960	0.004	5.13	-14, 56, -3
	Right temporal	4	0.868	2.70	69, -30, -12
	Right inferior temporal	388	0.050	3.37	52, -55, -23

^ap-value for comparing farmworkers to non-farmworkers.
^bMNI (Montreal Neurological Institute) coordinates for peak voxel location.
FW = farmworker NFW = non-farmworker