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Lower subcortical gray matter volume in both younger smokers and established smokers relative to non-smokers

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Abstract

Although established adult smokers with long histories of nicotine dependence have lower neural tissue volume than non-smokers, it is not clear if lower regional brain volume is also observed in younger, less established smokers. The primary goal of this study was to investigate neural tissue volume in a large group of smokers and non-smokers, with a secondary goal of measuring the impact of age on these effects. We used voxel-based morphometry to compare regional gray matter volume in 118 individuals (59 smokers, 59 age- and gender-matched non-smokers). Younger smokers had significantly lower gray matter volume in the left thalamus and the left amygdala than their non-smoking peers (family-wise error-corrected clusters, $P < 0.05$). There was no correlation between smoking use variables and tissue volume among younger smokers. Established smokers had significantly lower gray matter volume than age-matched non-smokers in the insula, parahippocampal gyrus and pallidum. Medial prefrontal cortex gray matter volume was negatively correlated with pack-years of smoking among the established smokers, but not the younger smokers. These data reveal that regional tissue volume differences are not limited exclusively to established smokers. Deficits in young adults indicate that cigarette smoking may either be deleterious to the thalamus and amygdala at an earlier age than previously reported, or that pre-existing differences in these areas may predispose individuals to the development of nicotine dependence.

Keywords

Gray matter; nicotine; smoking; voxel-based morphometry

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Authors Contribution

KH and CH were responsible for study concept and design. KH, JJ and MO acquired study and fMRI data. CH, XZ and MO conducted the data analysis. All authors contributed to the interpretation of the findings. KH, CH and MO drafted the article. JJ, MG and KB provided critical review and revision. CH made all final revisions. All authors have critically reviewed content and approved final version submitted for publication.

INTRODUCTION

Tobacco smoking remains the leading preventable cause of morbidity and mortality in the United States and 50% of long-term smokers, especially among those who began smoking in adolescence, will die from smoking-related diseases (USDHHS 2010). Smoking is now considered a pediatric epidemic with 25% of high school seniors smoking regularly. Eighty percent of these will likely continue to smoke as adults (USDHHS, 2012), joining the almost one out five adults in the United States who smoke cigarettes. While acute administration of nicotine may enhance attention and working memory, a growing body of evidence suggests chronic smoking adversely affects learning and memory (Swan & Lessov-Schlaggar 2007). Some studies (Paul *et al.* 2006; Stewart *et al.* 2006), but not all (Chen *et al.*, 2003), have found that cognitive deficits in chronic smokers are most prominent in established smokers.

Just as the adverse effects of smoking on general health are well known, there is a growing consensus that a long history of nicotine smoking is associated with alterations in neural tissue integrity throughout frontal–striatal–thalamic circuits involved in cognitive control and reward processing. Investigations of neural tissue integrity in established smokers consistently find lower gray matter volume and density in the prefrontal cortex (PFC) (Brody *et al.* 2004; Yu, Zhao & Lu 2011; Zhang *et al.* 2011; Liao *et al.* 2012) and the anterior cingulate cortex (Brody *et al.* 2004; Gallinat *et al.* 2006; Liao *et al.* 2012). PFC volume in these established smokers may be negatively correlated with pack-years of smoking (Zhang *et al.* 2011), a metric that incorporates both the average number of cigarettes smoked per day and the number of years spent as a smoker. However, beyond the frontal cortex, smokers also have lower tissue volume in subcortical structures including the cerebellum (Brody *et al.* 2004; Yu *et al.* 2011), thalamus (Liao *et al.* 2012) and insula, as well as the occipital cortex and the temporal lobe (Gallinat *et al.* 2006). A comparison of healthy established smokers (> 70 years of age) with matched non-smokers, revealed decreased gray matter volume bilaterally in the frontal cortex, thalamus, posterior cingulate cortex and the precuneus in the smoking group—areas associated with mild cognitive impairment and dementia (Almeida *et al.* 2008).

Although most neuro-imaging studies are conducted in established smokers who have been smoking for 10–30 years, smoking is typically initiated before the age of 20, after which intake quickly escalates. Developing nicotine dependence by an individual's second decade of life, may have particularly deleterious effects on the brain given that this is a time of active neural tissue myelination particularly in the PFC. The extent to which this loss of tissue volume is related to extended use, accelerated aging or vulnerability to becoming a smoker, however, is unclear. In a large study of brain morphometry and anisotropy in 48 nicotine-dependent smokers and 48 non-smokers by Zhang *et al.* (2011), pack-years of smoking was negatively correlated with PFC volume. This relationship, however, only existed in high pack-year smokers. They did not observe a difference in low pack-year smokers, nor did they find a significant correlation between pack-years and smoking severity score across their entire sample of high and low pack-year smokers.

Given that very few studies have a large enough sample size to directly compare younger and established smokers to age-matched non-smokers, it is difficult to determine whether structural abnormalities observed in established populations are also present in younger smokers. These younger smokers typically have shorter smoking histories despite similar smoking severity scores [as determined by the Fagerstrom Test of Nicotine Dependence (FTND)]. In one of the few studies of younger smokers, Gallinat *et al.* (2006) found that relatively young smokers (in their 20s and early 30s) had significantly lower gray matter volume in the anterior cingulate, orbitofrontal, occipital and temporal cortices than matched non-smokers. Additionally, there was a significant correlation between the number of pack-years as a smoker and lower tissue volume in the frontal and temporal lobes as well as in the cerebellum. These data suggest that there are differences in neural tissue volume among younger smokers with relatively short cigarette-use histories relative to their age-matched non-smoking peers.

The primary goal of this study was to determine whether, in a large sample of male and female nicotine-dependent smokers ranging in age from 20 to 49, we could replicate findings from smaller studies that have demonstrated lower gray matter volume in cohorts of established adult smokers. Our secondary goal was to determine if these previously observed neural tissue volume deficits were present in a cohort of young nicotine-dependent individuals relative to their age-matched non-smoking peers. Understanding the time course of these changes has important public health and treatment implications and will aid in the delineation of when these changes begin to appear.

METHODS

Participants

Data was collected from a total of 122 individuals (62 smokers, 60 non-smokers) between the ages of 20 and 49. These individuals were recruited through various sources including posted flyers, newspaper ads and Internet advertisement. Participants were recruited from the greater metropolitan area of Charleston, South Carolina and Lexington, Kentucky. Study procedures were conducted at the Medical University of South Carolina (78% of participants) and the University of Kentucky (22%). All study procedures were performed in accordance with Good Clinical Practice Guidelines and the Declaration of Helsinki with approval from the Medical University of South Carolina and the University of Kentucky Institutional Review Boards. The final sample included 118 individuals. The imaging data from four individuals (all smokers) were discarded because of ghosting artifacts. Equivalent magnetic resonance imaging (MRI) scanners and MPRAGE image acquisition parameters were used at both sites. *Post hoc* imaging analysis revealed no significant differences in the bias field or average Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) template from individuals consented at the two sites. There was no significant difference in age or gender of the individuals enrolled at the two sites.

Interested individuals contacted the research center and were prescreened by telephone for initial inclusion criteria (e.g. between the ages of 18–55, no history of head trauma, no current neurologic or psychiatric diagnosis, no history of migraine headaches, no metal in their head or neck, no history of claustrophobia, not currently pregnant or intending to

become pregnant). After providing informed consent, individuals were screened for psychiatric conditions and substance use disorders (Sheehan *et al.* 1998), received a brief medical history, a urine drug screen and a urine pregnancy screen (for women only). Participants were excluded for both current or a lifetime history of substance or alcohol dependence (other than nicotine for the smokers), current substance abuse (other than nicotine for the smokers), any other current psychiatric condition, a history or present medical illness known to effect brain vasculature or structure (e.g. diabetes, stroke, traumatic brain injury, hypertension), or the use of psychoactive medication. Nicotine dependence was assessed in the smokers using the FTND (Fagerstrom 1978; Heatherton *et al.* 1991). To be eligible to participate, all smokers had to meet the criteria for dependence and could not currently be using any non-cigarette tobacco products, nicotine replacement therapy, bupropion or varenicline. All participants were also given the Beck Depression Inventory (Beck, Steer & Brown 1996), and the Brief Symptoms Inventory (Piersma, Reaume & Boes 1994). All non-smokers had to deny a history of regular cigarette use, but otherwise meet the same criteria as the smokers. The non-smokers were not excluded if they had fewer than 10 lifetime uses of cigarettes. There was no difference in age ($t = 1.32$, $P = .19$), gender ($\chi^2 = 3.13$, $P = .08$) or race ($\chi^2 = 0.34$, $P = 0.56$) between the smokers and non-smokers.

A secondary goal of this investigation was to determine whether tissue volume abnormalities were limited to established smokers or if they were present in younger smokers with shorter histories of use. Demographic and drug use variables for these groups (established smokers and younger smokers) are presented in Table 1. Age and length of smoking were correlated in our sample (as expected). The number of years of smoking was not normally distributed. Consequently, we performed group-based analyses of the imaging data rather than attempting a non-parametric correlation analyses. Based on the natural distribution of the ages and length of smoking history, we divided the group into a cohort of younger individuals [$n = 59$; non-smokers ($n = 29$), smokers ($n = 30$; 20–29 years old with a smoking history of less than 10 years)] and a cohort of established individuals [$n = 59$, non-smokers ($n = 31$), established smokers ($n = 28$; 35–49 with a smoking history of more than 10 years). By design, there was a significant difference [$t(56) = 13.85$, $P < 0.001$] in age and years smoked [$t(56) = 10.61$, $P < 0.001$] between the younger smokers and the established smokers. There were no significant differences between the younger smokers and the established smokers on (1) the number of cigarettes smoked per day [15.25 for the younger smokers versus 17.23 for the established smokers; $t(56) = 1.09$, $P = 0.28$]; (2) the level of nicotine dependence [FTND: 4.25 for the younger smokers versus 4.85 for the established smokers; $t(56) = 1.02$, $P = 0.32$]; or (3) the age of initiation of regular smoking [16.23 for the younger smokers versus 17.69 for the established smokers; $t(56) = 1.68$, $P = 0.11$]. There were no significant differences in gender, race or years of education between the younger smokers and established smokers.

Voxel-based morphometry data acquisition and analysis

Participants were allowed to smoke until 1 hour before the MRI scanning session. Exhaled carbon monoxide levels (< 10 ppm) were measured with a MicroSmokelyzer (Bedfont Scientific Ltd., Kent, United Kingdom) to confirm recent smoking among the members of

the smoking group ($\chi = 4.46$). High-resolution T1-weighted anatomical images were acquired for each participant on a 3T Siemens TIM Trio MRI scanner (Siemens, Erlangen, Germany) at both universities (TR = 1750 ms, TE = 4 ms, voxel dimensions $1.0 \times 1.0 \times 1.0$ mm, 160 slices, full brain and cerebellar coverage, no gap). *Post hoc* analyses comparing the quality of the data acquired from each site included homogeneity of variance test assessing the mean signal and regional signal variation within the native and DARTEL modulated images. There were no significant differences between the contrast distribution or bias fields on the scans from the different sites. Data were analyzed at the Medical University of South Carolina using statistical parametric mapping (SPM) software (SPM8, Wellcome Department of Cognitive Neurology, London, UK) running on MATLAB 7.0.4 (The Mathworks, Natick, MA, USA).

The voxel-based morphometry data were preprocessed using DARTEL 10, a unified segmentation/normalization framework that may achieve greater inter-subject registration than previous segmentation and normalization procedures. The data were preprocessed using a series of standard steps: (1) segmentation into gray matter, white matter and cerebrospinal fluid (CSF) through the unified model; (2) creation of a study-specific gray matter and white matter template through DARTEL; (3) affine registration of the individual data to the DARTEL template and subsequently to standardized tissue probability maps in Montreal Neurological Institute (MNI) space; (4) spatial normalization of the modulated, coregistered images to standardized MNI space; (5) smoothing of the modulated, coregistered, normalized images (8 mm full width at half max isotropic Gaussian kernel).

Statistical analyses—In order to account for the effects of aging on the structure of the brain (Milton *et al.* 1991; Davis *et al.* 2009; Madden *et al.* 2009), age was included as covariate in all statistical models. Voxel-based statistics were calculated for all smokers versus non-smokers (primary aim), for younger smokers versus younger non-smokers (secondary aim), and for established smokers versus non-smokers (secondary aim) using the voxel-based morphometry toolbox in SPM8. The relationship between smoking parameters (e.g. length of smoking, pack-years of smoking, FTND score) and tissue volume was also investigated. For all analyses, a whole brain uncorrected threshold was first applied to the data ($P < 0.01$, $k = 100$ voxels). Clusters that were significant at this level were then subjected to a cluster-corrected family-wise error threshold ($P < 0.01$).

RESULTS

Primary analysis

All smokers versus all non-smokers—There were several cortical and subcortical regions in which the smokers had significantly lower gray matter densities than non-smokers (Table 2, Fig. 1). The cortical regions with lower gray matter volume included the right insular cortex, the left and right frontal poles, the left and right frontal orbital cortices, and the right frontal gyrus. The subcortical regions with lower gray matter volume included the left and right amygdala, the left thalamus, the right putamen, and the left parahippocampal gyrus. Relative to controls, smokers had significantly higher gray matter volume in the left occipital cortex and the right lingual gyrus. There was no significant correlation between

gray matter volume and FTND score, length of nicotine use (years), or pack-years of smoking when all smokers (younger and established) were included in the model.

Secondary analysis

Younger versus established smokers—Construction of a general linear model with age group (younger, established) and smoking status (smoker, non-smoker) as variables revealed significant main effects smoking status multiple cortical and subcortical regions, including the medial and lateral PFC, insula/parahippocampal gyrus and the thalamus (consistent with the primary analysis). There was also a main effect of age group in the right insula. That is, the established smokers had significantly lower gray matter volume. The interaction between age group and smoking status revealed no clusters of over 100 voxels ($P < 0.01$ uncorrected) that exceeded a family-wise error correction of $P < 0.01$ at the cluster level. The main effects of age group were explicitly explored in the subsequent pairwise comparisons.

Younger smokers versus non-smokers—The young smokers had significantly lower gray matter volume than the younger non-smokers in the left amygdala [$t = 6.03$, cluster size = 601, $P = 0.002$, MNI (X,Y,Z): -21, 2, -18] and the left thalamus ($t = 5.75$, cluster size = 234, $P < 0.000$, MNI: -4, -25, 1) (Table 3, Fig. 2a). There were no areas in which the younger smokers had significantly higher gray matter volume than the younger non-smokers. Among the younger smokers, there was no significant correlation between gray matter volume and length of nicotine use (years), FTND score, nor pack-years of smoking.

Established adult smokers versus non-smokers—The established smokers had significantly lower gray matter volume than the non-smokers in the left insula/parahippocampal gyrus/pallidum ($t = 6.18$, cluster size = 609, $P < 0.001$, MNI: -24, 3, -21). A large cluster in the right insula, nearly symmetric to the left insula cluster, also had lower gray matter volume in established smokers, but it did not meet the predefined 100 voxel limit for cluster significance ($t = 5.41$, cluster size = 97, $P < 0.001$, MNI: 38, 0, -3) (Table 4, Fig. 2b). The established smokers also had significantly higher gray matter volume than the non-smokers in the Brodmann 18 area of the occipital cortex, a brain region involved in secondary visual processing. As with the younger smokers, there was not a significant correlation between length of nicotine use or FTND score and gray matter volume. There was, however, a significant negative correlation between pack-years of smoking and tissue volume in the medial PFC (Brodmann area 10; -7, 37, 3; cluster size = 122) among the established smokers.

Younger versus established smokers—There were no regions in which the younger smokers had significantly lower gray matter than the established smokers. With age as a controlled factor in the model, the only region in which the established smokers had significantly lower gray matter volume than the younger smokers was the right insula/pallidum ($t = 5.65$, cluster size = 140, MNI: 38, -9, 7).

DISCUSSION

Despite strong public health efforts to curb smoking rates, each day, at least 3800 adolescents try their first cigarette and 33% of teenagers that are daily smokers will eventually die of a smoking-related condition (USDHHS, 2012). Nicotine is one of the most addictive drugs available with individuals often smoking through their entire lives despite multiple quit attempts. Although over 80% of adult smokers begin smoking during adolescence (USDHHS, 2012), a time in which the brain is still maturing, very little is known about alterations in brain structure that may occur in young smokers. The purpose of this study was to determine whether alterations in neural tissue volume associated with chronic smoking are present in both young adults and more advanced smokers. There are three primary conclusions from these data: (1) consistent with prior research overall, smokers have lower gray matter volume in multiple in cortical and subcortical regions; (2) young adult smokers have lower volume in the left thalamus and amygdala relative to age-matched non-smoking controls; and (3) established smokers have significantly lower volume in the insula than age-matched non-smokers. Given the role of the insula in chronic smoking, these results suggest that these established smokers may represent a subgroup of smokers that were unable to quit and may be a predictor of long-term use. Additionally, the presence of lower gray matter volume in the younger smokers suggests that nicotine either effects neural tissue volume quickly or that these are pre-existing abnormalities that predispose these individuals to smoking and nicotine dependence.

Lower thalamic and amygdala volumes in younger smokers

To our knowledge, this is the first study to demonstrate a difference in the volume of the thalamus and amygdala between smokers and non-smokers in their 20s. All of the smokers in the sample between the ages of 20 and 29 met the criteria for nicotine dependence. As a group, they had smoked an average of 6.6 years. Both the younger and established smoking groups began smoking at approximately 16 years old. This is consistent with previous research indicating that approximately 90% of smokers start smoking before the age of 18. Brody *et al.*'s (2004) reported gray matter differences between smokers and non-smokers in their late 30s with a range between 21 and 65 years of age. Gallinat *et al.* (2006) examined a younger population and found significant decreases in the gray matter volume in frontal lobe, occipital cortex, thalamus, precuneus and cuneus between a small group of 22 smokers and 23 never-smokers, average age 30.8 and 30.3, respectively.

There are at least two potential explanations for observing this low gray matter in the thalamus of these younger smokers. One hypothesis is that the abnormalities in the thalamus may have existed even before they have begun smoking and potentially be a predisposing factor or marker of vulnerability to nicotine dependence. A recent study by Liu *et al.* (2013) demonstrated that adolescents with prenatal exposure to tobacco showed a trend toward smaller volumes in the pallidum and the thalamus than their peers who were not exposed to prenatal tobacco. Additionally, a smaller thalamus volume in these adolescents was related to higher levels of impulsivity, a factor known to contribute to smoking initiation. Although we did not collect data on parental smoking habits in this study, parental smoking habits are a significant predictor of adolescent smoking. In a recent multi-generational study of

smoking patterns among 214 parents and their 314 children, Vuolo & Staff (2013) demonstrated that children of parents who either currently smoked or had previously smoked, were nearly three times as likely to smoke as children whose parents had never smoked. In the present study, there was no correlation between pack-years of smoking and tissue volume in any area, further supporting the hypothesis that these differences may exist before the initiation of smoking. Furthermore, there was no difference in gray matter volume in these areas when established and younger smokers are compared directly.

Another interpretation is that the thalamus may be particularly sensitive to the neurotoxic effects of cigarette smoking. There are numerous volatile compounds in cigarette smoke, which have the potential to effect the healthy and integrity of brain tissue through their effects on vasculature and inflammation. Cigarette smoking, for example, is well known to increase arterial wall stiffness (Failla *et al.* 1997), impair endothelial-dependent arterial dilatation (Lekakis *et al.* 1998) and impair micro-vascular function (Black *et al.* 2001). These factors are all important in the pathogenesis of atherosclerosis. The thalamus derives most of its arterial blood supply from perforating blood vessels from the base of the brain, terminal branches from the anterior choroidal artery, posteromedial central arteries and posterior communicating artery (Schuenke, Schulte & Schumacher 2007). Areas of the brain supplied by terminal small blood vessels (such as the medial dorsal thalamus) may also be more susceptible to subtle vascular damage from smoking.

Additionally, the thalamus has a very high concentration of nicotinic receptors (Perry *et al.* 1989; Xuereb *et al.* 1990; Spurdin *et al.* 1997). Although the nicotine itself is unlikely to be the cause of a neurotoxic event, it is possible that a high density of nicotine receptors in a given brain region may increase the likelihood that, when this region is activated, it is more vulnerable to the compounds contributing to oxidative damage or inflammation. Brody *et al.* (2006) have demonstrated that cigarette smoking is associated with 88% receptor occupancy of the nicotinic acetylcholine receptors in the thalamus. Additionally, previous animal research has found that nicotine and its metabolites accumulate in the brain reaching as much as four times the concentration found in the blood (Ghosh *et al.* 2001). Furthermore pre-clinical research has demonstrated neurotoxic effects of nicotine with even brief periods of intermittent or continuous nicotine exposure during adolescence (Abreu-Villaca *et al.* 2003). The acute administration of nicotine impairs vascular function possibly via sympathetic activation and inhibition of nitric oxide synthase (Black *et al.* 2001). Additionally, cotinine, a major metabolite of nicotine, is known to have adverse effects on microcirculation via vasoconstriction and recently has been shown to impair healing and increase neuronal degeneration following a spinal cord injury (Dalgic *et al.* 2013). Again, although the pathogenesis of changes in tissue volume in the thalamus may not be directly related to the nicotine itself, the activation of these receptors may increase the likelihood that they are affected by the other compounds in tobacco smoke. Essentially, the nicotinic receptor rich environment of the thalamus may be more vulnerable to these adverse effects of tobacco smoke.

In addition to lower thalamus volume, the young smokers in this study had lower volume in the left amygdala relative to their non-smoking peers. To our knowledge, this is the first study to demonstrate lower amygdala volume in smokers. This is likely driven by the

inclusion of a large cohort of young smokers in our study compared with previous studies. When the total sample was divided into a group of younger smokers and older smokers for example, lower amygdala volume was present in the younger smoking group but not the older smoking group. The lack of a difference in the established smokers is consistent with prior studies in this area (Brody *et al.* 2004; Gallinat *et al.* 2006; Yu *et al.* 2011; Zhang *et al.* 2011). Although it is not clear why this volume deficit would be reserved for the younger population, it is possible that any pre-existing differences between younger smokers and non-smokers fade over time. The amygdala volume tends to be fairly stable through aging (Good *et al.* 2001), however, so the presence of a difference in young smokers and the absence of a difference in established smokers is likely not due exclusively to aging in the older sample. Although longitudinal studies in this area have not been performed, it is possible that the younger smokers with a smaller amygdala volume will either (1) not continue to be smokers, suggesting resilience or survivor effect, or (2) that they are at a higher risk for other psychiatric diseases, which would exclude them from typical studies of established smokers. Further, longitudinal research on young smokers or at risk youth is needed to understand the relevance of these findings may have to the escalation, maintenance or potential smoking cessation success in these younger smokers.

The basis for these lower gray matter volumes in the thalamus and amygdala of young adult smokers could have important neurobiologic implications. The medial dorsal thalamus for example, receives afferent projections directly from the olfactory cortex and the amygdala, and sends projections to the medial PFC. As a result, the medial dorsal thalamus plays a pivotal role in memory, attention and planning. Notably, the data from the present study suggest that there may be a specific deficit in the well-established neurocircuit between the left amygdala and the medial dorsal thalamus in young adults who have been smoking for less than a decade.

Left-sided laterality in gray matter disruptions among smokers

It is worth mentioning that the alterations in amygdala and thalamic gray matter density among our young smokers were all on the left side. Although the established smokers had lower volume in the vicinity of both the left and the right insula, there remained a left-sided bias in the deficits. Although the issue of laterality is often not discussed in addiction literature, it is well known that the left and right hemispheres provide unique contributions to cognitive and reward processing. Previous functional MRI studies of reward have demonstrated a strong left-sided bias to activation within cortical and subcortical areas involved in reward processing (Thut *et al.* 1997; Koepp *et al.* 1998; Delgado *et al.* 2000). These functional MRI data support an emerging consistency within addiction literature that deficits in brain volume among smokers are often biased toward left-sided cortical and subcortical targets involved in reward processing.

Among the largest studies that have assessed brain tissue density in smokers, deficits in gray matter density are often in the left hemisphere. Brody *et al.* (2004) demonstrated that the smokers had lower gray matter densities in the left (but not the right) anterior cingulate cortex. Liao *et al.* (2012) demonstrated that smokers had significantly lower density in the left thalamus, left medial PFC and left anterior cingulate cortex, with no differences in the

right hemisphere. Gallinat *et al.* (2006) demonstrated lower gray matter volume in the right thalamus and cingulate, but the majority of structural differences were on the left side (including the PFC, parahippocampal gyrus and posterior cingulate). Zhang *et al.* (2011) demonstrated that smokers had significantly lower left (but not right) PFC density. This left-sided deficit was negatively correlated with pack-years of smoking. They also found that the left insula (but not the right) had significantly higher density in smokers than non-smokers. Extending this literature to treatment, Froeliger *et al.* (2010) recently demonstrated that treatment-seeking smokers that were able to remain abstinent had significantly greater left-sided putamen volumes (and right occipital cortex volumes) than individuals that relapsed. In the present investigation, alterations within the younger smokers were all on the left side (amygdala and the thalamus). Although the neurobiologic basis for this bias toward left-sided deficits in gray matter volume among smokers is not clear, the consistency of these results and their potential relationship to length of use and abstinence warrants further directed investigation.

Lower parahippocampal gyrus and insula volumes in established smokers

Decreased volume in the left parahippocampal gyrus was found between smokers and non-smokers when all individuals were included in the analysis. The results of each group independently, however, demonstrate that this decrease in parahippocampal volume is only present when the established smokers are compared with the established non-smokers—suggesting that parahippocampal deficits are only present after an extended history of tobacco use. The parahippocampal gyrus plays an important role in memory encoding and retrieval, particularly recognition of scenes (Aguirre *et al.* 1996). Damage in this region may account for visual search deficits found in middle-age smokers who continue to smoke throughout their 40s (Richards *et al.* 2003). Given its extreme ventral position on the brain and the high percentage of surface area that is surrounded by CSF (relative to other brain regions), the smaller volume in this area may be related to a global structural atrophy rather than a neurotransmitter or neural network specific degradation. Additionally, although none of the smokers in this study had a history of alcohol dependence or current alcohol abuse, they likely have longer alcohol use histories than the younger smokers. Although atrophy in the gray matter in this area is typically associated with chronic heavy alcohol users (see Bühler & Mann 2011) it is possible that the alterations in gray matter volume in this study, particularly in the areas surrounded by CSF are related to accumulated alcohol intake.

In addition to reduced density in the left parahippocampal gyrus, the established smokers had significantly lower gray matter volume in the left and right insula. At first this seems very counterintuitive given the work by Naqvi & Bechara (2009), which demonstrated that cigarette smokers *lost* the urge to smoke following a lesion to the insula. Additionally, Zhang *et al.* (2011) demonstrated that smokers had higher density in the left insula than non-smokers. Similar to the findings in this study, Franklin *et al.* (2002) found decreased gray matter concentration in the insula of cocaine-dependent individuals (many of whom were smokers) compared with controls. Additionally, as with the parahippocampal gyrus, the left and right anterior insula are positioned on the ventral surface of the brain and surrounded by CSF in both the ventral and medial aspects. Consequently, when interpreting the results of

these analyses, especially in older adults, we should consider the role that extended smoking history has to global structural atrophy in areas on ventral and medial aspects of the cortex.

Consistent with most of the studies in this area, the majority of differences between smokers and non-smokers suggest that smokers have *lower* gray matter volumes than non-smokers. As a departure from this pattern, however, the smokers in this study had higher levels of gray matter volume in the left and right occipital cortex/cuneus. Although the center of mass of these clusters are slightly shifted such that one is in the lingual gyrus and the other is Brodmann area 18, both regions are located in the extreme posterior and ventral portions of the modulated gray matter masks. The secondary analysis revealed that established smokers had significantly elevated gray matter volume in this area relative to the younger smokers. It is unclear why the smokers would have elevated tissue volume in this area, especially when other voxel-based morphometry studies have found a significant decrease in occipital cortex volume among smokers (Gallinat *et al.* 2006). It is well known that nicotine has positive effects on visuospatial attention (Parrott & Craig 1992). Lawrence, Ross & Stein (2002) demonstrated that transdermal nicotine replacement in mildly abstinent smokers improved visuospatial task performance for example. Furthermore, nicotine increased occipital cortex BOLD activity independent of the task being performed. Although we are not aware of any studies that have empirically tested the relationship between chronic nicotine administration and cell proliferation in the occipital cortex, it is plausible that through increasing visuospatial attention, nicotine induces an increase in dendritic arborization or axonal branching in these visual association areas.

Limitations

As with the majority of studies in clinical populations, this cross-sectional study is not able to clearly determine the etiology of the structural abnormalities. The reported differences in younger smokers may be the direct result of cigarette smoking or they may reflect pre-existing differences that predispose to the development of nicotine dependence. This could include both genetic and/or environmental explanations. Additionally several limitations of this preliminary study should be noted. The data were collected on two Siemens TIM Trio MRI scanners at different locations, which despite being the same model and using the same acquisition sequence, may have introduced some variance into the data. Differences in cumulative alcohol exposure between the younger and established smokers could also contribute to differences between the two groups. Chronic alcohol dependence is well known to have adverse effects on tissue volume in multiple brain regions including the parietal and temporal gray matter, thalami, and frontal and parietal white matter, with widespread sulks enlargement (Gazdzinski *et al.* 2005). However, individuals with either current or lifetime history of alcohol dependence and current alcohol abuse were excluded from the study, thus minimizing this potential confound. To control for any effect of age including the difference in mean age between the established smoking and non-smoking group, age was utilized as a covariate in all of models. Although the groups were not significantly different in their distribution of men and women, given that gender is associated with differences in amygdala size, future research may want to specifically investigate this interaction within the younger smokers. The study was limited by lack of smokers over 50 years of age and adolescent nicotine-dependent smokers. This is likely

related to the age distribution of smokers, and as the studies included neuro-imaging, established individuals were more likely to be excluded because of co-morbid medical conditions and taking medications that could potentially affect brain function. Finally, it is worth mentioning that while several other voxel-based morphometry studies in cigarette smokers have found changes in the cerebellum (Brody *et al.* 2004; Gallinat *et al.* 2006; Yu *et al.* 2011), these were not present in our sample. This may reflect the size of our sample, the use of DARTEL algorithms, fairly strict statistical thresholds or the age distribution of the sample. It is also possible that a lack of a positive result in this area is due to the challenges of accurately coregistering the cerebellum (a structure surrounded by CSF and near the edge of the field of view) across a large sample of individuals. We manually checked the data for artifacts and segmentation irregularities; however, it is possible that anatomical variance in the sample was too large to find a consistent result.

In summary, the study found significant reductions in gray matter volume in the left thalamus and amygdala in young adults who have been smoking for less than a decade. The results indicate that smoking may have a more deleterious effect on brain structure than previously reported. It is unclear if these differences predate smoking or if these are alterations in brain volume emerged during the first decade of smoking. Future studies are necessary to determine whether lower tissue density in the thalamus and amygdala of these younger smokers influences future smoking behavior or their vulnerability to using escalation and initiation of drug use. Through these efforts it may be possible to identify brain -based biomarkers that contribute to adolescents' vulnerability to nicotine dependence. Further research in adolescent and emerging adult populations will be important to further explore these findings.

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Figure 1.

Brain tissue volume in nicotine-dependent smokers versus age-matched non-smoking controls. The data in this figure include the brain regions with significantly higher (red color map) or lower (blue color map) gray matter volume in smokers ($n=58$) relative to controls ($n=60$) (voxel level: $P < 0.01$, 100 voxel minimum, uncorrected; cluster level: family-wise error-corrected $P < 0.01$; SPM8, voxel-based morphometry toolbox, data displayed using MRIcron software <http://www.mccauslandcenter.sc.edu/mricro/mricron>)

Figure 2.

Brain tissue volume in younger smokers and established smokers relative to their age-matched controls. The data in this figure include the brain regions with (a) significantly higher (red color map) or lower (blue color map) gray matter volume in younger smokers ($n = 30$) relative to younger non-smokers ($n = 29$) ($P < 0.01$, family-wise error-corrected clusters). (b) Significantly higher (red color map) or lower (blue color map) gray matter volume in established smokers ($n=28$) relative to controls ($n= 31$) (voxel level: $P < 0.01$, 100 voxel minimum, uncorrected; cluster level: family-wise error-corrected $P < 0.01$; SPM8, voxel-based morphometry toolbox, data displayed using MRICron software <http://www.mccauslandcenter.sc.edu/mricro/mricron>)

Table 1

Participant demographics and smoking use variables.

	<u>Nicotine-dependent smokers</u>		<u>Non-smoking controls</u>	
	Younger	Established	Younger	Established
Sample size	30	28	29	31
Age (mean)	23.9	40.04	23.0	36.0
Age (range)	20–29	30–49	20–29	30–49
Gender (m/f)	21/12	15/13	14/15	21/12
Race (% Caucasian)	85	75	86	73
Education (years)	20.1 ± 0.3	21.9 ± 0.6	20.7 ± 0.3	22.3 ± 0.4
Cigarettes per day	15.3 ± 6.5	17.2 ± 8.0	NA	NA
Years at this level*	6.6 ± 3.6	22.9 ± 6.7	NA	NA
Pack-years*	5.2 ± 0.4	19.8 ± 3.5	NA	NA
Age of initiation	16.2 ± 1.0	16.2 ± 1.5	NA	NA
FTND (mean ± SD)	4.3 ± 2.3	4.9 ± 2.8	NA	NA

* $P < 0.05$.

FTND = Fagerstrom Test of Nicotine Dependence; NA = not applicable; SD = standard deviation.

Table 2

Gray matter volume in all smokers relative to all non-smokers.

Smokers relative to non-smokers	Significant clusters ^a	MNI coordinates	Maximum *	Cluster size
Lower tissue volume	L Insula, parahippocampal gyrus, amygdala	-22 2 -20	8.7	2586
	L Medial and orbital frontal cortex	-10 56 -15	5.89	707
	L Thalamus	-7 -25 1	7.43	983
Higher tissue volume	R Insula, parahippocampal gyrus, amygdala	33 5 -20	6.28	360
	R Frontal pole	30 51 -11	6.92	278
	R Putamen	33 -15 -5	6.13	408
	L Occipital cortex/cuneus	-48 -82 10	6.83	610
	R Lingual gyrus	8 -66 6	5.88	422

* t value at the voxel level.

^a Family-wise error-corrected clusters listed ($P < 0.01$ at cluster level). Based on Harvard–Oxford Cortical Subcortical Structural Atlases. All data were initially passed through an uncorrected $P < 0.01$, minimum 100 voxel threshold. Montreal Neurological Institute.

Table 3
Gray matter volume in younger smokers relative to non-smokers (2nd decade of life).

Younger smokers versus non-smokers		Significant clusters ^a	MNI coordinates		Maximum [*]	Cluster size
Lower signal intensity	L	Amygdala	-21	2	-18 6.03	601
	L	Thalamus	-4	-25	1 5.75	234
Higher signal intensity	-	No significant clusters	-	-	- -	-

^{*} *t* value at the voxel level.
^a Family-wise error-corrected clusters listed (*P* < 0.01 at cluster level). All data were initially passed through an uncorrected *P* < 0.01, minimum 100 voxel threshold.

Table 4

Gray matter volume in established smokers relative to non-smokers.

Established smokers versus non-smokers	Significant clusters ^a	MINI coordinates	Maximum [*]	Cluster size
Lower signal intensity	L	Insula/parahippocampus/pallidum	-24 3 -21	6.18 609
	R	Insula	38 0 -3	5.41 97 ^b
Higher signal intensity	R	Cuneus (Brodmann 18)	9 -64 4	5.78 181

^{*} *t* value at the voxel level.

^a Family-wise error-corrected clusters listed ($P < 0.01$ at cluster level).

^b Did not meet the 100 voxel limit for significance. All data were initially passed through an uncorrected $P < 0.01$, minimum 100 voxel threshold.