



Full length article

Cigarette smoking is associated with amplified age-related volume loss in subcortical brain regions

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ABSTRACT

Background: Magnetic resonance imaging studies of cigarette smoking-related effects on human brain structure have primarily employed voxel-based morphometry, and the most consistently reported finding was smaller volumes or lower density in anterior frontal regions and the insula. Much less is known about the effects of smoking on subcortical regions. We compared smokers and non-smokers on regional subcortical volumes, and predicted that smokers demonstrate greater age-related volume loss across subcortical regions than non-smokers.

Methods: Non-smokers ($n = 43$) and smokers ($n = 40$), 22–70 years of age, completed a 4 T MRI study. Bilateral total subcortical lobar white matter (WM) and subcortical nuclei volumes were quantitated via FreeSurfer. In smokers, associations between smoking severity measures and subcortical volumes were examined.

Results: Smokers demonstrated greater age-related volume loss than non-smokers in the bilateral subcortical lobar WM, thalamus, and cerebellar cortex, as well as in the corpus callosum and subdivisions. In smokers, higher pack-years were associated with smaller volumes of the bilateral amygdala, nucleus accumbens, total corpus callosum and subcortical WM.

Conclusions: Results provide novel evidence that chronic smoking in adults is associated with accelerated age-related volume loss in subcortical WM and GM nuclei. Greater cigarette quantity/exposure was related to smaller volumes in regions that also showed greater age-related volume loss in smokers. Findings suggest smoking adversely affected the structural integrity of subcortical brain regions with increasing age and exposure. The greater age-related volume loss in smokers may have implications for cortical-subcortical structural and/or functional connectivity, and response to available smoking cessation interventions.

1. Introduction

The relationship between cigarette smoking and risk for cardiac, pulmonary and vascular disease as well as for multiple forms of cancer in humans is essentially incontrovertible (CDC, 2004). Moreover, considerable recent evidence now links smoking, in otherwise healthy individuals, to significant neurobiological and neurocognitive abnormalities that are not specifically attributable to the above diseases (Azizian et al., 2009; Durazzo et al., 2014a; Durazzo et al., 2010; Sharma and Brody, 2009). Macrostructural morphological abnormalities are the most consistently reported neurobiological consequence associated with chronic cigarette smoking (Durazzo et al., 2010; Pan et al., 2013; Sutherland et al., 2016). Most magnetic resonance (MR)

imaging studies investigating smoking-related changes in brain morphology focused on cortical gray matter (GM) volumes, and smaller volumes or lower density in anterior frontal regions and the insula were most consistently reported finding [see (Durazzo et al., 2010; Pan et al., 2013; Sutherland et al., 2016) for review]. Fewer studies described smoking-related effects on subcortical nuclei/region volumes. Older adult smokers (≥ 64 years of age) had decreased thalamic volume relative to non-smokers (Almeida et al., 2008). Young-to-middle aged otherwise healthy smokers, compared to non-smokers, demonstrated smaller volumes or lower density in the thalamus (Franklin et al., 2014; Liao et al., 2012), globus pallidus (Hanlon et al., 2016), and cerebellum (Brody et al., 2004; Franklin et al., 2014; Gallinat et al., 2006; Kuhn et al., 2012; Wetherill et al., 2015; Yu et al., 2011). Conversely, some

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studies indicated that young-to-middle-aged adult smokers had larger volumes than non-smokers in the putamen (Franklin et al., 2014; Wetherill et al., 2015; Yu et al., 2011). Subcortical neurobiological abnormalities may underlie compulsive consumption in substance use disorders, including nicotine dependence (see Franklin et al., 2014 and references cited therein). In the above studies, voxel-based morphometry compared GM density or volume between smokers and non-smokers. While this approach allowed testing for the effect of smoking status (i.e., smoker vs. non-smoker) collapsed across the age range of the participants, only one study (Franklin et al., 2014) specifically tested for, but did not observe, a smoking status by age interaction.

The risk for smoking-related diseases increases with years of smoking (CDC, 2004), which is inextricably related to age. In healthy adults, increasing age is associated with declines in multiple MR-based measures, including regional brain volumes (Crivello et al., 2014; Walhovd et al., 2011), brain metabolite levels (Chang et al., 2009), as well as neurocognition (Salthouse, 2000). In healthy participants 25–70 years of age, our previous MR imaging studies showed that smokers had both lower mean values and greater age-related declines in total hippocampal and hippocampal subregion volumes (Durazzo et al., 2013); we also observed similar age-related declines in anterior frontal brain metabolite (N-acetylaspartate and glutamate) concentrations (Durazzo et al., 2016c). In these studies, greater cigarette pack-years were related to smaller hippocampal volumes and lower metabolite levels. The greater age-related declines apparent in smokers suggest that chronic smoking amplified the effects of normal aging on hippocampal macrostructure and anterior frontal brain metabolite levels in our adult participants. Given that previous subcortical morphological studies did not specifically test for, or report, a smoking status by age interaction, it remains unclear if cigarette smoking is associated with more widespread subcortical volume loss with increasing age in adults. Such structural alterations may be clinically important because they could influence reward processing and response to smoking cessation interventions via alterations of structural and/or functional connectivity in frontolimbic and/or frontostriatal circuitry (Froeliger et al., 2015; Sutherland et al., 2016; Sweitzer et al., 2016). Based on our previous neuroimaging findings, we predicted that adult smokers demonstrate smaller mean volumes and greater age-related volume loss than non-smokers in the thalamus, cerebellum, brainstem, total subcortical lobar white matter, and basal ganglia nuclei. We predicted higher pack-years are related to smaller regional subcortical volumes in smokers.

2. Methods

2.1. Participants

Eighty-three healthy, community-dwelling participants [43 non-smokers (eight females) and 40 smokers (five females)] were recruited via electronic billboards and word-of-mouth. Participants were between the ages of 22 and 70 (see Table 1 for demographics). Participants provided written informed consent according to the Declaration of Helsinki, and all procedures were approved by the University of California San Francisco and the San Francisco VA Medical Center.

Detailed inclusion/exclusion criteria are fully described elsewhere (Durazzo et al., 2011a). In summary, participants were screened for history of neurologic (e.g., seizure disorder, neurodegenerative disorder, demyelinating disorder, closed head trauma with loss of consciousness), general medical (e.g., hypertension, myocardial infarction, Type-1 or 2 diabetes, cerebrovascular accident, any form of cancer), and psychiatric (i.e., mood, thought, anxiety, substance/alcohol use disorders) conditions known or suspected to influence neurocognition and/or brain neurobiology. All females were pre-menopausal, by self-report. All non-smoking participants never smoked, or smoked less than 40 cigarettes during their lifetime and used no cigarette/tobacco products for 10 years prior to study. All smoking participants were actively smoking at the time of assessment, smoked at least 10 cigarettes per day

Table 1

Group Demographics, Alcohol and Cigarette Use Histories, Mood Measures, and Intracranial Volume (ICV).

Measure; min–max	Non-smokers (n = 43)	Smokers (n = 40)
Age (years)	43 (13) 22–70	47 (11) 22–64
Education (years)	16 (2) 12–20	15 (2) 12–20
Male (%)	81	87
Caucasian (%)	60	70
Lifetime average drinks/month	19 (11) 1–54	26 (14) 3–56*
FTND	NA	5 (2) 2–8
Pack-years	NA	26 (16) 0.5–63
Age onset of smoking	NA	16 (6) 13–24
BDI	3 (3) 0–13	6 (3) 0–13*
STAI	32 (7) 21–46	34 (9) 20–56
Intracranial volume (cc)	1409 (201) 972–1738	1477 (208) 828–1750

Note. BDI: Beck Depression Inventory. FTND: Fagerstrom Tolerance Test for Nicotine Dependence. Min: minimum. max: maximum. NA: not applicable. STAI: State – trait Anxiety Inventory – Trait. *Smokers > Non-smokers, $p < 0.05$. Mean (SD).

for 5 years or more, and had no periods of smoking cessation greater than 1 month in the 5 years prior to study, with no concurrent use of other tobacco products. No smoker was engaged in any pharmacological/behavioral smoking cessation program.

2.2. Medical, psychiatric, substance, alcohol consumption assessment

Participants completed the screening section of the Structured Clinical Interview for DSM-IV Axis I disorders, Patient Edition, Version 2.0 [SCID-I/P; (First et al., 1998)], as well as an in-house questionnaire designed to screen for medical, psychiatric, neurological and developmental conditions that may affect neurocognition or neurobiology [see (Durazzo et al., 2004)]. Participants completed standardized questionnaires assessing lifetime alcohol consumption [Lifetime Drinking History, LDH; (Skinner and Sheu, 1982; Sobell et al., 1988)] and substance use [in-house questionnaire assessing substance type, quantity and frequency of use (Abe et al., 2013)]. From the LDH, we derived average number of drinks per month over lifetime (one drink defined as containing 13.6 g of pure ethanol). Participants also completed self-report measures of depressive [Beck Depression Inventory, BDI; (Beck, 1978)] and anxiety symptomatology [State-Trait Anxiety Inventory, form Y-2, STAI; (Spielberger et al., 1977)]. Smokers completed a measure of nicotine dependence level [Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991)], self-reported the number of cigarettes currently smoked per day, and the number of years of smoking over lifetime. Pack-years [(number of cigarettes per day/20) x total number of years of smoking] were calculated for smokers. Comparable frequencies of smokers and non-smokers (30%) reported intermittent “recreational” use (i.e., ≤ 3 episodes/month) of cannabis or cocaine during late adolescence or early adulthood. Prior to assessment, participants’ urine was tested for five common illicit substances (i.e., THC, opiates, PCP, cocaine, amphetamines), and participants were breathalyzed for recent ethanol consumption. No participant was positive for the above common illicit substances or ethanol at the time of assessment.

2.3. Magnetic resonance imaging (MRI) acquisition and processing

MRI data were acquired on a 4.0 T Bruker MedSpec system using an 8-channel transmit-receive head coil (Siemens, Erlangen, Germany). A Magnetization Prepared Rapid Gradient (TR/TE/TI = 2300/3/950 ms, 7° flip angle, $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ resolution) sequence was used to acquire 3D sagittal T1-weighted images for morphological analyses. The publicly available FreeSurfer (v5.1) segmentation and cortical surface reconstruction methods were used to obtain regional, bilateral cortical, subcortical GM and total subcortical lobar white matter (WM)

volume, and total intracranial volume (ICV) (all in mm^3) (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 2004, 1999). All segmented subcortical and parcellated cortical T1-weighted images were visually inspected by one of the authors (TCD) for accuracy; any errors in segmentation/parcellation were manually edited, reprocessed and again inspected as previously described (Durazzo et al., 2014c). The final segmented subcortical and parcellated cortical volumes passed all quality requirements (Durazzo et al., 2011b). The subcortical regions of interest (ROIs) interrogated were the total bilateral subcortical lobar WM, bilateral thalamus, caudate, putamen, pallidum, amygdala, nucleus accumbens, cerebellar cortex and cerebellar WM. Midline ROIs included the brain stem as well as the total corpus callosum volume and corpus callosum subregions (anterior, mid-anterior, central, mid-posterior, and posterior). Individual ROI volumes were scaled to their corresponding ICV and reported as the percentage of ICV.

2.4. Statistical analyses

2.4.1. Demographic and clinical variables

Demographic and clinical variables were compared between smokers and non-smokers with *t*-tests and Fisher's Exact Test, where indicated.

2.4.2. Primary analyses

To test our hypothesis of greater age-related subcortical volume loss in smokers versus non-smokers, we employed generalized linear modeling (GENLIN), and specifically tested for a smoking status (smoker vs. non-smoker) by age interaction. In preliminary analyses comparing smokers and non-smokers, similar magnitude group differences were observed for the left and right hemisphere of bilateral regions/nuclei; therefore, results for the summed volumes of bilateral ROIs are presented. Additionally, group differences across corpus callosum subregions were highly consistent; thus results for the total corpus callosum volume are only reported. Dependent measures were ROI volumes (percent of ICV), and covariates included BDI and average lifetime drinks/month (smokers and non-smokers were different on these measures; see Table 1 and 3.1 below). Significant univariate effects for smoking status were followed-up with *t*-tests (two-tailed). Although we predicted *a priori* that smokers exhibit smaller subcortical volumes, we adopted a conservative approach and corrected the *t*-test alpha level ($p = 0.05$) for multiplicity of tests with a modified Bonferroni method (Sankoh et al., 1997), based on 11 ROIs and the inter-correlations among ROIs for all participants ($r = 0.63$). This produced an adjusted two-tailed alpha level of $p < 0.022$ for post-hoc *t*-tests for each ROI. Interactions between smoking status and age were considered significant at $p < 0.05$. Effect sizes for statistically significant differences in mean volume between smokers and non-smokers were calculated with Cohen's *d* (Cohen, 1988).

2.4.3. Exploratory analyses

Statistically significant smoking status \times age interactions were further explored via a median split on age, which divided the sample into four groups: younger non-smokers and smokers (22–45 years of age; mean age of 35 years), and older non-smokers and smokers (46–70 years of age; mean age of 55 years). Younger non-smokers ($n = 24$) and younger smokers ($n = 17$) were not different on any clinical or demographic variable. Older non-smokers ($n = 19$) and older smokers ($n = 23$) were equivalent on clinical and demographic variables, except that older smokers had fewer years of education.

Two exploratory analyses were conducted on the basis of the age median split for ROIs showing a significant smoking status \times age interaction in the Primary Analyses: 1) Slopes of volume across age were statistically compared among the four groups via GENLIN, and group differences on slopes were considered statistically significant at $p < 0.05$. 2) Mean ROI volumes were compared across the four groups. Main effects, interactions and pairwise *t*-tests (two-tailed;

providing comparisons of mean volumes among the four groups) were considered statistically significant at $p < 0.05$. Comparisons of slopes and mean volumes between younger smokers and non-smokers were adjusted for age because of the established relationship between age and regional brain volumes. Comparisons of slopes and mean volumes among older smokers and non-smokers were adjusted for age and education; comparisons of slopes and mean volumes between younger and older smokers were adjusted for pack-years.

2.4.4. FTND score

Associations between the 11 ROI volumes and cigarette pack-years (measure of exposure magnitude) and FTND score (measure of nicotine dependence level) were examined with linear regression; part (semi-partial) correlations were reported, adjusting for age and lifetime average drinks/month. Although we predicted that higher pack-years in smokers are inversely related to all subcortical volumes, we corrected the alpha level ($p = 0.05$) for the part correlations for multiplicity of tests with the above described modified Bonferroni procedure; a two-tailed $p < 0.022$ was considered statistically significant.

3. Results

3.1. Participant demographics and clinical variables

Smokers and non-smokers were equivalent on age, sex, percent of Caucasians, level of anxiety symptomatology and education (see Table 1). Smokers had significantly higher BDI scores, and consumed more average drinks per month over lifetime (all $p < 0.05$). Although statistically different between groups, the average BDI score for both groups was in the normal range (i.e., < 10) and well below the cutoff for mild depressive symptomatology (Richter et al., 1998). Participant alcohol consumption did not approach a hazardous level [see (McKee et al., 2007; Mertens et al., 2005)].

3.2. Primary analyses: main effects and interactions for smoking status and age

Significant smoking status \times age interactions were observed for volumes of the total subcortical WM [$\chi^2(1) = 4.40$, $p = 0.036$], total corpus callosum [$\chi^2(1) = 4.50$, $p = 0.034$], thalamus [$\chi^2(1) = 4.29$, $p = 0.038$], and cerebellar cortex [$\chi^2(1) = 4.61$, $p = 0.032$]; in these regions, smokers showed significantly greater volume loss with increasing age than non-smokers (see Figs. 1 and 2). Smokers also showed trends for greater age-related volume loss in the brainstem ($p = 0.08$) and caudate ($p = 0.10$). Except for the pallidum, cerebellar WM and brain stem, age was inversely related to volumes in all other ROIs (all $p < 0.01$). Greater lifetime average drinks/month, although low, was associated with smaller total corpus callosum volume ($p = 0.018$). No main effects were observed for smoking status in any ROI (all $p > 0.12$). Findings were essentially unchanged when female participants were removed from the analyses. The greater volume loss in smokers in the above regions was similar in the left and right hemispheres in bilateral ROIs (data not shown).

3.3. Exploratory analyses comparing younger and older groups

3.3.1. Comparisons of slopes of volume across younger smokers and non-smokers, as well as older smokers and non-smokers were conducted with GENLIN for the subcortical WM, total corpus callosum, thalamus, and cerebellar cortex, regions in which smokers showed greater age-related volume loss in our Primary Analyses. Older smokers showed greater age-related volume loss than younger non-smokers in the subcortical WM ($\beta = -0.39$, $p = 0.021$), total corpus callosum ($\beta = -0.05$, $p = 0.014$), and cerebellar cortex ($\beta = -0.11$, $p = 0.046$). Older non-smokers had greater age-related volume loss than younger non-smokers in the total corpus callosum ($\beta = -0.04$,

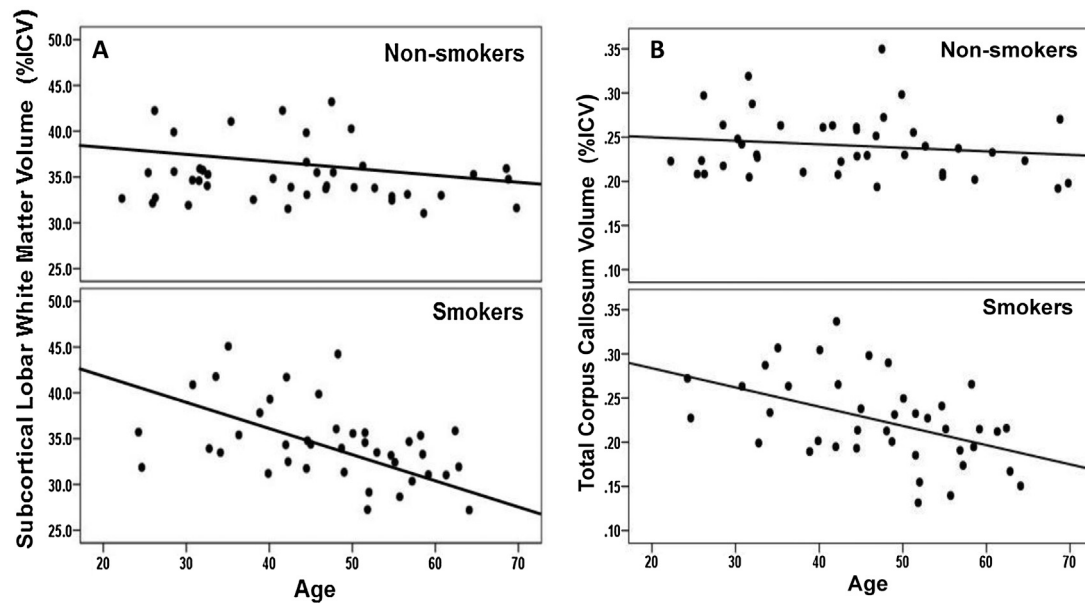


Fig. 1. (A) Subcortical lobar white matter volume across age for Non-smokers and Smokers. (B) Total corpus callosum volume across age for Non-smokers and Smokers.

$p = 0.020$). No other group differences were observed (see Figs. 3–6). Both older smokers and non-smokers showed moderate strength associations between age and subcortical WM, total corpus callosum, and thalamus volumes, while both younger non-smokers and smokers demonstrated very weak associations between age and volumes of all ROIs.

3.3.2. Smoking status (smokers vs. non-smokers) \times age group (older vs. younger participants) interaction GENLIN models were conducted for the subcortical WM, total corpus callosum, thalamus, and cerebellar cortex, where smokers showed greater age-related volume loss in the Primary Analyses. Main effects of age group (younger vs. older) were observed for the total corpus callosum [$\chi^2(1) = 4.24$, $p = 0.039$], thalamus [$\chi^2(1) = 9.43$, $p = 0.002$], cerebellar cortex [$\chi^2(1) = 13.25$, $p < 0.001$] and a trend for subcortical WM [$\chi^2(1) = 3.71$, $p = 0.054$], where the younger participants had larger volumes than the older participants. No significant main effects were observed for smoking status (all $p > 0.051$). Trends for a smoking status \times age group interaction were observed for the total corpus callosum ($p = 0.055$) and

thalamus ($p = 0.062$), which were driven by smaller mean volumes in older smokers. Pairwise t -tests indicated older smokers had smaller volumes than younger and older non-smokers in the above four ROIs, and smaller volumes than younger smokers in the total corpus callosum, thalamus, and cerebellar cortex (all $p < 0.05$). No other significant mean volume differences were observed (see Table 2). In comparisons between older smokers and older non-smokers, greater age was related to smaller subcortical WM, total corpus callosum, and thalamic volumes (all $p < 0.01$), but education was not associated with any volume (all $p > 0.50$). Age was not related to volumes in comparisons between younger smokers and younger non-smokers (all $p > 0.40$).

3.4. Associations of pack-Years and FTND with regional subcortical volumes in smokers

In smokers, higher pack-years showed significant negative associations of moderate strength with the volumes for total amygdala (see

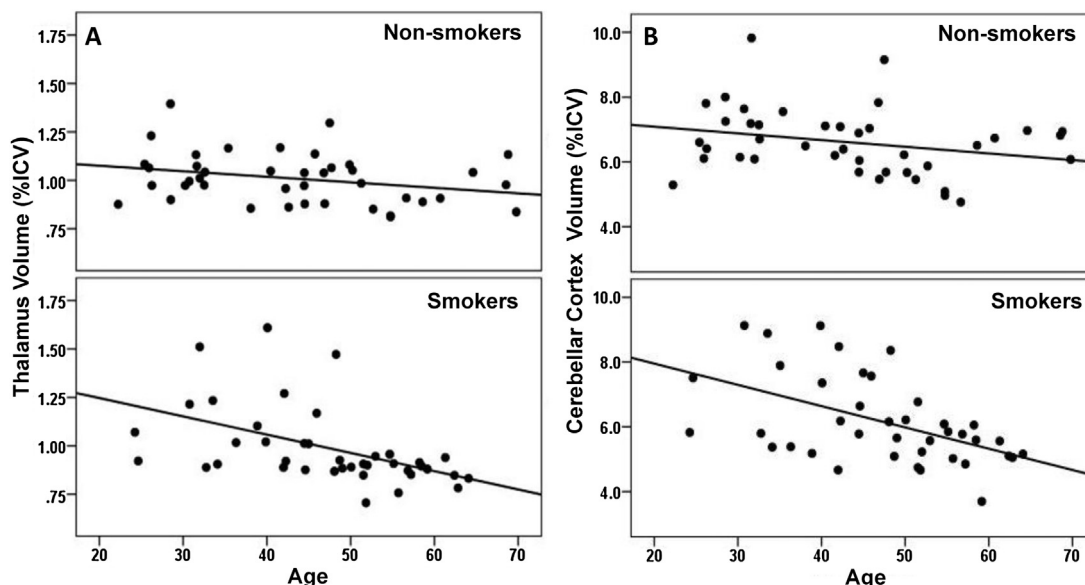


Fig. 2. (A) Thalamus volume across age for Non-smokers and Smokers. (B) Cerebellar cortex volume across age for Non-smokers and Smokers.

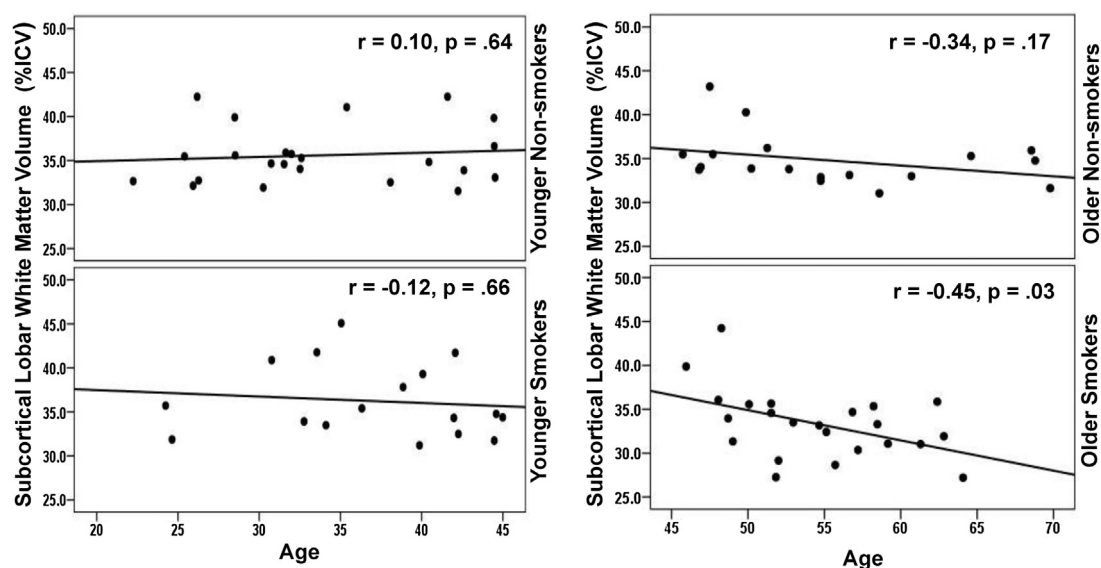


Fig. 3. Subcortical lobar white matter volume across age for Younger and Older groups.

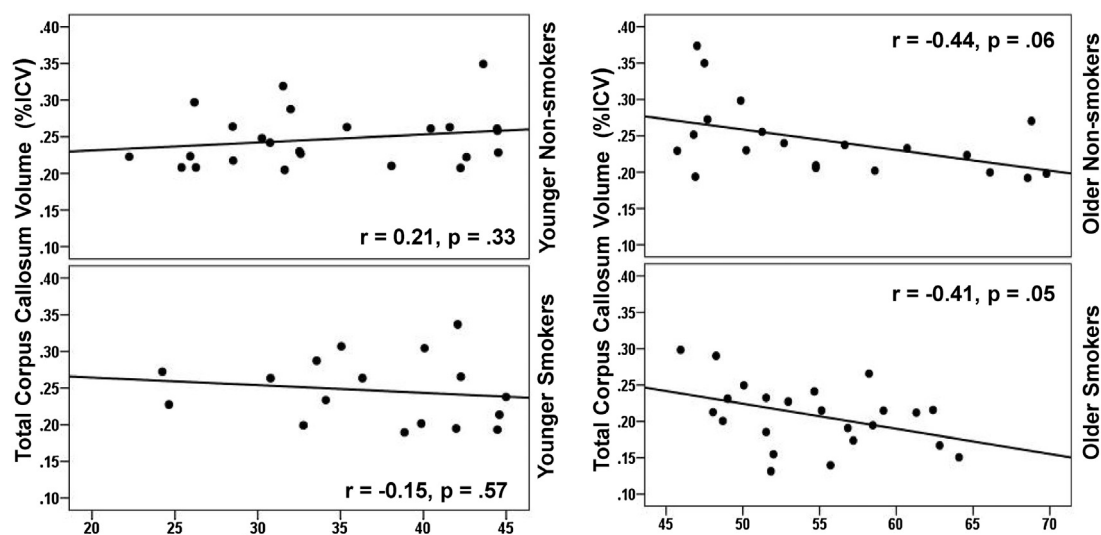


Fig. 4. Total corpus callosum volume across age for Younger and Older groups.

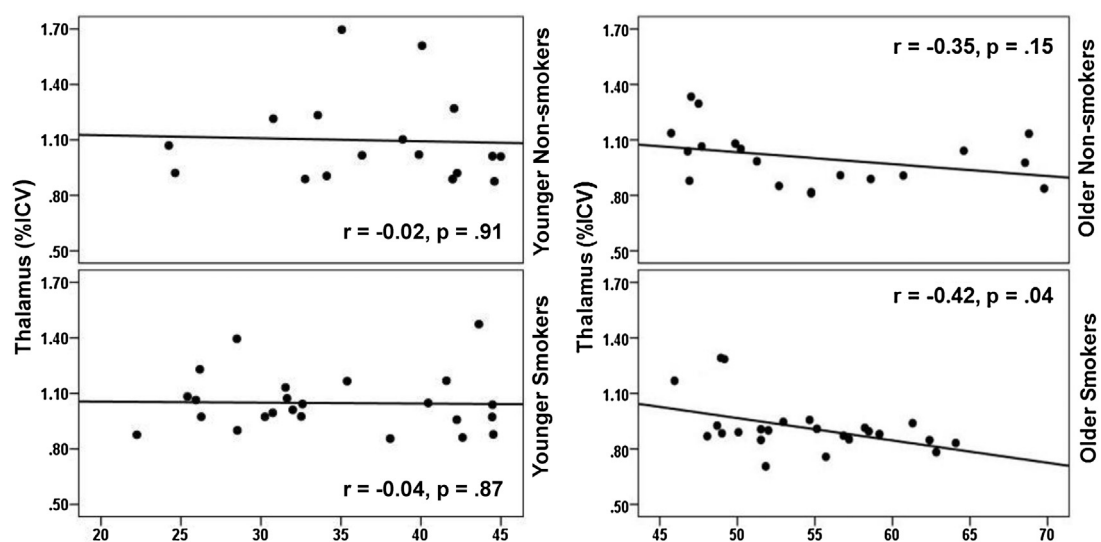


Fig. 5. Thalamus volume across age for Younger and Older groups.

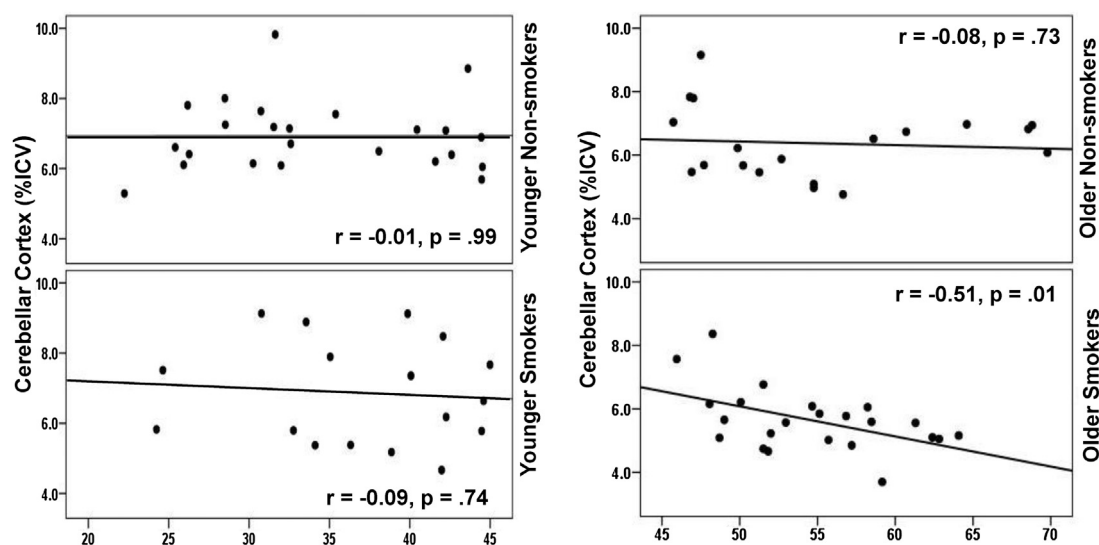


Fig. 6. Cerebellar cortex volume across age for Younger and Older groups.

Fig. 7), nucleus accumbens, total corpus callosum and subcortical WM after adjusting for age and lifetime average drinks/month (see Table 3); the magnitudes were generally similar for bilateral structures (data not shown). A higher FTND score was associated with lower total corpus callosum volume ($r = -0.37$, $p = 0.007$) after adjusting for age and lifetime average drinks/month in smokers; no other significant associations between FTND and subcortical volumes were observed. The magnitudes and directions of the associations between pack-years, FTND and volumes across ROIs for younger and older smokers were largely equivalent.

4. Discussion

The primary findings of this 4 T quantitative MRI study were: 1) Otherwise healthy adult smokers demonstrated greater age-related volume loss than non-smokers in the bilateral and total (sum of left and right hemisphere) subcortical WM, thalamus, and cerebellar cortex, as well as the total corpus callosum; 2) Older smokers showed significantly smaller volumes than younger and older non-smokers in the subcortical WM, corpus callosum, thalamus and cerebellar cortex, as well as smaller volumes than younger smokers in the corpus callosum, thalamus, and cerebellar cortex; 3) In smokers, greater pack-years were associated with smaller amygdala, nucleus accumbens, corpus callosum and subcortical WM volumes.

The greater age-related volume loss in smokers was pronounced in the total lobar WM and corpus callosum. The thalamus and cerebellar cortex were the only subcortical GM ROIs that showed statistically

significant greater age-related volume loss in smokers. Comparisons of slopes of volumes across age between younger and older participants suggested the greater age-related volume loss observed in smokers in the above regions was primarily driven by significantly larger volume loss with age in older smokers relative to younger non-smokers. Correspondingly, older smokers were the only group to show significant associations between age and subcortical WM, corpus callosum, thalamus and cerebellar cortex volume. There were no significant differences among smokers and non-smokers, as a whole, on any ROI volume. However, older smokers had smaller total lobar WM, corpus callosum, thalamus and cerebellar cortex volumes than younger non-smokers, younger smokers and older non-smokers; the largest magnitude differences were between younger non-smokers and older smokers, reflecting the interacting effects of age and smoking status on these regions. No significant differences were observed between younger non-smokers, younger smokers and older non-smokers; the effect sizes from these comparisons were generally weak, indicating the lack of differences between these groups was not a function of inadequate power related to group size.

A central mechanism hypothesized to be related to the neurobiological abnormalities observed in smokers is increased brain oxidative stress (OxS) that is promoted by elevated free radical species and decreased endogenous antioxidant levels (Durazzo et al., 2014a; Swan and Lessov-Schlaggar, 2007). The gas and particulate phases of cigarette smoke have extremely high concentrations of short-and-long-lived free radical species and other oxidizing agents (Ambrose and Barua, 2004; Valavanidis et al., 2009). In addition to increased free radical levels,

Table 2
Comparisons of Regional Volumes (% of intracranial volume) between Younger and Older Smokers and Non-Smokers.

Measure	YoungNS n = 24	YoungS n = 17	OlderNS n = 19	OlderS n = 23	Effect Size (Cohen's d)					
					YoungNS vs. YoungS	YoungNS vs. OlderNS	YoungNS vs. OlderS	YoungS vs. OlderNS	YoungS vs. OlderS	OlderNS vs. OlderS
Subcortical white matter	36.19 (4.04)	35.17 (3.71)	35.62 (3.70)	33.58 (3.70)	0.26	0.15	0.75*	0.12	0.43	0.55*
Total corpus callosum	0.247 (0.039)	0.239 (0.037)	0.246 (0.042)	0.218 (0.042)	0.02	0.03	0.72*	0.18	0.53*	0.67*
Thalamus	1.05 (0.19)	1.06 (0.19)	1.00 (0.13)	0.93 (0.15)	0.21	0.29	0.72*	0.35	0.53*	0.51*
Cerebellar cortex	6.93 (1.22)	6.74 (1.25)	6.38 (0.99)	5.74 (1.23)	0.15	0.50	0.97**	0.32	0.81**	0.58*

Note. Mean (standard deviation); YoungNS: younger non-smokers; YoungS: younger smokers; OlderNS: older non-smokers OlderS: older smokers; * $p < 0.05$, ** $p < 0.01$ for pairwise t -tests (two-tailed).

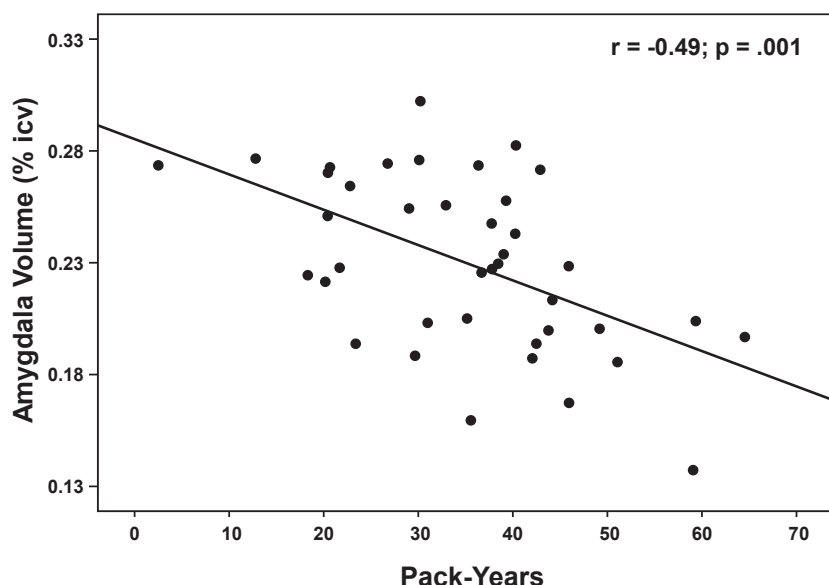


Fig. 7. Association of amygdala volume with pack-years.

Table 3
Associations between Regional Volumes and Pack-Years in Smokers.

Region	r	p-value
Amygdala	−0.49	0.001
Nucleus Accumbens	−0.42	0.004
Total Corpus Callosum	−0.38	0.006
Subcortical Lobar White Matter	−0.35	0.016
Caudate	−0.32	0.028
Putamen	−0.26	0.049
Thalamus	−0.25	0.09
Brain Stem	−0.21	0.19
Pallidum	−0.12	0.48
Cerebellar Gray Matter	−0.07	0.65
Cerebellar White Matter	−0.02	0.91

Note. r-values are part (semi-partial) correlations adjusted for age and lifetime average drinks/month; $p < 0.022$ is statistically significant.

smoking is associated with markedly elevated carboxyhemoglobin levels (Deveci et al., 2004), altered mitochondrial respiratory chain function (Alonso et al., 2004), and induction of proinflammatory cytokine release by peripheral and central nervous system glial cells (Mazzone et al., 2010), which combine to further escalate cerebral OxS. It is well established that OxS is directly associated with damage to membrane lipids, proteins, carbohydrates, DNA and RNA of neuronal, glial, and vascular tissue of the brain [see (Durazzo et al., 2014a) and references therein]. Oligodendrocytes (the myelin-producing cells of the brain), granular neurons of the cerebellar cortex, and neurons in several hippocampal subregions are highly susceptible to OxS (Smith et al., 1999; Wang and Michaelis, 2010). While not universally accepted [see (Salmon et al., 2010)], increasing OxS burden with aging is suggested to be a fundamental mechanism contributing to neurodegeneration in normal aging (Halliwell, 2006; Zimniak, 2011). We previously observed that current and former cigarette smoking in cognitively normal elders (Durazzo et al., 2016a, 2014b) and current smoking in those with probable Alzheimer disease (Durazzo et al., 2016a) are associated with significantly elevated cerebrospinal fluid isoprostane concentration, which is a biomarker of increased central nervous system OxS. In these groups of elders (76 ± 6 years of age), higher isoprostane concentration was related to smaller hippocampal volume. Consistent with this relationship, young-to-older adult smokers showed greater age-related volume loss than non-smokers in the total hippocampus and several hippocampal subregions (Durazzo et al., 2013). In the current study, adult smokers, as a whole, exhibited greater

age-related volume loss in the lobar WM and across the entire corpus callosum; however, the results of the exploratory analyses suggested this effect was largely driven by the older smokers (46–70 years of age). Collectively, our findings in young-to-elder adults suggest the chronic OxS imposed by cigarette smoking may interact with the OxS associated with normal aging, which may amplify degeneration in subcortical brain regions that are either highly vulnerable to OxS (e.g., lobar subcortical WM, hippocampus) and/or have a high metabolic activity (e.g., cerebellar cortex, hippocampus, and thalamus).

Greater cigarette pack-years in smokers were associated with smaller amygdala, nucleus accumbens, total corpus callosum and subcortical lobar WM volumes, while higher level of nicotine dependence was only related to smaller corpus callosum volume. This pattern suggests that greater amount/duration of cigarette exposure rather than nicotine dependence level was related to smaller tissue volume in most subcortical regions, consistent with findings for cortical volumes/density (Durazzo et al., 2010; Pan et al., 2013; Sutherland et al., 2016). Subregions of the amygdala (Mineur et al., 2016) and nucleus accumbens (Crespo et al., 2006) have a high density of cholinergic receptors, but it is not clear if the chronic upregulation and decreased sensitivity of nicotinic receptors associated with nicotine dependence is related to the morphological integrity of these tissue. Additionally, as the FTND is an ordinal metric of limited range, it may not be as robust a predictor as pack-years.

This study has limitations that may affect the generalizability of our findings. Although smokers demonstrated greater age-related volume loss in several ROIs, a longitudinal design is required to verify the findings of this cross-sectional study. The formation of the older and younger groups was based on a median split of the participants' age range, yielding groups above and ≤ 45 years of age. Consequently, our operationalization of older and younger groups, and the corresponding volumetric findings, should be considered preliminary. Undocumented premorbid/comorbid group differences in lifestyle or subclinical biomedical conditions (e.g., diet/nutrition, exercise, subclinical pulmonary or cardiovascular dysfunction) and/or genetic polymorphisms (Mon et al., 2013) may have influenced the results. Since this study excluded individuals with clinically significant smoking-related morbidity, it is possible that the age-related effects were underestimated in this healthy cohort (Durazzo et al., 2014a). Additionally, given the subcortical lobar WM was not further divided into major lobes, further investigation needs to examine any regional specificity of age-related WM volume loss in smokers. Finally, the small number of females precluded assessment of sex effects.

5. Conclusions

The study results provide novel evidence that cigarette smoking is associated with accelerated age-related volume loss in multiple subcortical brain regions. The findings also suggested the smoking-related effects on subcortical WM and GM regions/nuclei were most apparent in the older age group, starting at 46 years of age in this cohort. These data offer further insight into the potential neurobiological substrates related to the neuropsychological abnormalities observed in cigarette smokers across adulthood (Durazzo et al., 2016b, 2010), and may have implications for cortical-subcortical structural and functional connectivity in smokers with increasing age. Why are these findings and continued research on the neurobiological consequences of smoking clinically relevant? The fundamental reasons are that over 1 billion people worldwide are cigarette smokers, and smoking-related diseases kill at least 6 million individuals annually (WHO, 2015). Understanding the effects of chronic smoking on brain micro-and-macrostructural integrity, biochemistry, functional and structural connectivity, as well as their functional correlates is required to inform the development of more efficacious smoking cessation interventions (Addicott et al., 2014, 2015; Durazzo et al., 2014a, 2016c, 2015).

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The study sponsors (National Institutes of Health and Department of Defense) had no role 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 paper for publication.

Contributors

Dr. Durazzo was responsible for study concept and design, participant screening, MR data acquisition, all MR data processing, all statistical analyses, data interpretation and writing the manuscript. Dr. Murray was involved in MR data acquisition. Drs. Meyerhoff, Murray, and Yoder were involved in data interpretation and manuscript preparation/editing. All authors approved the final version of the manuscript.

Conflict of interest

No conflict declared.

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