



Published in final edited form as:

Drug Alcohol Depend. 2017 August 01; 177: 228–236. doi:10.1016/j.drugalcdep.2017.04.012.

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

Timothy C. Durazzo^{a,b,*}, Dieter J. Meyerhoff^{c,d}, Karmen K. Yoder^e, and Donna E. Murray^{c,d}

^aDepartment of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, United States

^bMental Illness Research and Education Clinical Centers and Sierra-Pacific War Related Illness and Injury Study Center, VA Palo Alto Health Care System, United States

^cDepartment of Radiology and Biomedical Imaging, University of California, San Francisco, United States

^dCenter for Imaging of Neurodegenerative Diseases, San Francisco VA Medical Center, United States

^eIndiana University Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, United States

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.

*Corresponding author at: War Related Illness and Injury Study Centers, Mental Illness Research and Education Clinical Centers (151Y), VA Palo Alto Health Care System, 3801 Miranda Ave., Palo Alto, CA 94304, United States., tdurazzo@stanford.edu, timothy.durazzo@va.gov (T.C. Durazzo).

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.

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.

Keywords

Cigarette smoking; Magnetic resonance imaging; Brain volume; Subcortical; White matter; FreeSurfer

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 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 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) \times 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, $1.0 \times 1.0 \times 1.0$ mm³ 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 mm³) (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 sub-regions 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 ($\beta = -0.04$, $p = 0.020$). 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. 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 neurobio-logical 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, 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 sub-cortical 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 sub-cortical 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 below 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 sub-cortical 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).

Acknowledgements

This material is the result of work supported by the National Institute on Drug Abuse (DA24136 to TCD), the Department of Defense (W81XWH-05-2-0094 to TCD), and the National Institute on Alcohol and Alcoholism (AA10788 to DJM), and by resources and the use of facilities at the Palo Alto Veterans Administration Health Care System, Palo Alto, CA and San Francisco Veterans Administration Medical Center, San Francisco, CA. We thank Dr. Anderson Mon for assistance in MR data acquisition of early study participants. We also wish to extend our gratitude to the study participants, who made this research possible.

Role of funding sources

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.

References

- Abe C, Mon A, Hoefer ME, Durazzo TC, Pennington DL, Schmidt TP, Meyerhoff DJ, 2013 Metabolic abnormalities in lobar and subcortical brain regions of abstinent polysubstance users: magnetic resonance spectroscopic imaging. *Alcohol Alcohol* 48, 543–551. [PubMed: 23797281]
- Addicott M, Froeliger B, Kozink R, Van Wert D, Westman E, Rose J, McClernon F, 2014 Nicotine and non-nicotine smoking factors differentially modulate craving: withdrawal and cerebral blood flow as measured with arterial spin labeling. *Neuropsychopharmacology* 39, 2750–2759. [PubMed: 24820539]
- Addicott MA, Sweitzer MM, Froeliger B, Rose JE, McClernon FJ, 2015 Increased functional connectivity in an insula-based network is associated with improved smoking cessation outcomes. *Neuropsychopharmacology* 40, 2648–2656. [PubMed: 25895453]
- Almeida OP, Garrido GJ, Lautenschlager NT, Hulse GK, Jamrozik K, Flicker L, 2008 Smoking is associated with reduced cortical regional gray matter density in brain regions associated with incipient Alzheimer disease. *Am. J. Geriatr. Psychiatry* 16, 92–98. [PubMed: 18165464]
- Alonso JR, Cardellach F, Casademont J, Miro O, 2004 Reversible inhibition of mitochondrial complex IV activity in PBMC following acute smoking. *Eur. Respir. J* 23, 214–218. [PubMed: 14979494]
- Ambrose JA, Barua RS, 2004 The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J. Am. Coll. Cardiol* 43, 1731–1737. [PubMed: 15145091]
- Azizian A, Monterosso J, O'Neill J, London ED, 2009 *Magnetic Resonance Imaging Studies of Cigarette Smoking Nicotine Psychopharmacology*. Springer, Verlag Berlin Heidelberg, pp. 113–143.
- Beck AT, 1978 *Depression Inventory*. Center for Cognitive Therapy, Philadelphia.
- Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC, Bota RG, Bartzokis G, London ED, 2004 Differences between smokers and non-smokers in regional gray matter volumes and densities. *Biol. Psychiatry* 55, 77–84. [PubMed: 14706428]
- CDC, Services, U.S.D.o.H.a.H, 2004 *The Health Consequences of Smoking: A Report of the Surgeon General*. Centers for Disease Control and Prevention. . https://www.cdc.gov/tobacco/data_statistics/sgr/2004/.

- Chang L, Jiang CS, Ernst T, 2009 Effects of age and sex on brain glutamate and other metabolites. *Magn. Reson. Imaging* 27, 142–145. [PubMed: 18687554]
- Cohen J, 1988 Statistical Power Analysis for the Behavioral Sciences. Lawrence Erlbaum Associates, Hillsdale, NJ.
- Crespo JA, Sturm K, Saria A, Zernig G, 2006 Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement. *J. Neurosci* 26, 6004–6010. [PubMed: 16738243]
- Crivello F, Tzourio-Mazoyer N, Tzourio C, Mazoyer B, 2014 Longitudinal assessment of global and regional rate of grey matter atrophy in 1,172 healthy older adults: modulation by sex and age. *PLoS One* 9, e114478. [PubMed: 25469789]
- Dale AM, Fischl B, Sereno MI, 1999 Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194. [PubMed: 9931268]
- Deveci S, Deveci F, Acik Y, Ozan A, 2004 The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. *Respir. Med* 98, 551–556. [PubMed: 15191041]
- Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ, 2004 Cigarette smoking exacerbates chronic alcohol-induced brain damage: a preliminary metabolite imaging study. *Alcohol. Clin. Exp. Res* 28, 1849–1860. [PubMed: 15608601]
- Durazzo TC, Meyerhoff DJ, Nixon SJ, 2010 Chronic cigarette smoking: implications for neurocognition and brain neurobiology. *Int. J. Environ. Res. Public Health* 7, 3760–3791. [PubMed: 21139859]
- Durazzo TC, Meyerhoff DJ, Nixon SJ, 2011a A comprehensive assessment of neurocognition in middle-aged chronic cigarette smokers. *Drug Alcohol Depend.* 122, 105–111. [PubMed: 21992872]
- Durazzo TC, Tosun D, Buckley S, Gazdzinski S, Mon A, Fryer SL, Meyerhoff DJ, 2011b Cortical thickness, surface area, and volume of the brain reward system in alcohol dependence: relationships to relapse and extended abstinence. *Alcohol. Clin. Exp. Res* 35, 1187–1200. [PubMed: 21410483]
- Durazzo TC, Meyerhoff DJ, Nixon SJ, 2013 Interactive effects of chronic cigarette smoking and age on hippocampal volumes. *Drug Alcohol Depend.* 133, 704–711. [PubMed: 24051060]
- Durazzo TC, Mattsson N, Weiner MW, 2014a Smoking and increased Alzheimer's disease risk: a review of potential mechanisms. *Alzheimers Dementia* 10, S122–S145.
- Durazzo TC, Mattsson N, Weiner MW, Korecka M, Trojanowski JQ, Shaw LM, 2014b History of cigarette smoking in cognitively-normal elders is associated with elevated cerebrospinal fluid biomarkers of oxidative stress. *Drug Alcohol Depend.* 142, 262–268. [PubMed: 25037769]
- Durazzo TC, Mon A, Pennington D, Abe C, Gazdzinski S, Meyerhoff DJ, 2014c Interactive effects of chronic cigarette smoking and age on brain volumes in controls and alcohol-dependent individuals in early abstinence. *Addict. Biol* 19, 132–143. [PubMed: 22943795]
- Durazzo TC, Meyerhoff DJ, Murray DE, 2015 Comparison of regional brain perfusion levels in chronically smoking and non-smoking adults. *Int. J. Environ. Res. Public Health* 12, 8198–8213. [PubMed: 26193290]
- Durazzo TC, Korecka M, Trojanowski JQ, Weiner MW, R OH, Ashford JW, Shaw LM, 2016a Active cigarette smoking in cognitively-normal elders and probable Alzheimer's disease is associated with elevated cerebrospinal fluid oxidative stress biomarkers. *J. Alzheimers Dis.* 54, 99–107. [PubMed: 27472882]
- Durazzo TC, Mattsson N, Weiner MW, 2016b Interaction of cigarette smoking history with APOE genotype and age on amyloid level glucose metabolism, and neurocognition in cognitively normal elders. *Nicotine Tob. Res* 18, 204–211. [PubMed: 25847292]
- Durazzo TC, Meyerhoff DJ, Mon A, Abe C, Gazdzinski S, Murray DE, 2016c Chronic cigarette smoking in healthy middle-aged individuals is associated with decreased regional brain N-acetylaspartate and glutamate levels. *Biol. Psychiatry* 79, 481–488. [PubMed: 25979621]
- First MB, Spitzer RL, Gibbon M, Williams JBW, 1998 Structured Clinical Interview for DSM-IV Axis I Disorders – Patient Edition (SCID-I/P, Version 2.0, 8/98 Revision). Biometrics Research Department, New York, NY.

- Fischl B, Dale AM, 2000 Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U. S. A* 97, 11050–11055. [PubMed: 10984517]
- Fischl B, Sereno MI, Dale AM, 1999 Cortical surface-based analysis. II: Inflation flattening, and a surface-based coordinate system. *Neuroimage* 9, 195–207. [PubMed: 9931269]
- Fischl B, Destrieux C, Halgren E, Segonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM, 2004 Automatic parcellation of the human cerebral cortex. *Cereb. Cortex* 14, 11–22. [PubMed: 14654453]
- Franklin TR, Wetherill RR, Jagannathan K, Johnson B, Mumma J, Hager N, Rao H, Childress AR, 2014 The effects of chronic cigarette smoking on gray matter volume: influence of sex. *PLoS One* 9, e104102. [PubMed: 25090480]
- Froeliger B, McConnell PA, Stankeviciute N, McClure EA, Kalivas PW, Gray KM, 2015 The effects of N-Acetylcysteine on frontostriatal resting-state functional connectivity, withdrawal symptoms and smoking abstinence A double-blind, placebo-controlled fMRI pilot study. *Drug Alcohol Depend.* 156, 234–242. [PubMed: 26454838]
- Gallinat J, Meisenzahl E, Jacobsen LK, Kalus P, Bierbrauer J, Kienast T, Witthaus H, Leopold K, Seifert F, Schubert F, Staedtgen M, 2006 Smoking and structural brain deficits: a volumetric MR investigation. *Eur. J. Neurosci* 24, 1744–1750. [PubMed: 17004938]
- Halliwel B, 2006 Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* 97, 1634–1658. [PubMed: 16805774]
- Hanlon CA, Owens MM, Joseph JE, Zhu X, George MS, Brady KT, Hartwell KJ, 2016 Lower subcortical gray matter volume in both younger smokers and established smokers relative to non-smokers. *Addict. Biol* 21, 185–195. [PubMed: 25125263]
- Kuhn S, Romanowski A, Schilling C, Mobascher A, Warbrick T, Winterer G, Gallinat J, 2012 Brain grey matter deficits in smokers: focus on the cerebellum. *Brain Struct. Funct* 217, 517–522. [PubMed: 21909705]
- Liao Y, Tang J, Liu T, Chen X, Hao W, 2012 Differences between smokers and non-smokers in regional gray matter volumes: a voxel-based morphometry study. *Addict. Biol* 17, 977–980. [PubMed: 20731627]
- Mazzone P, Tierney W, Hossain M, Puvenna V, Janigro D, Cucullo L, 2010 Pathophysiological impact of cigarette smoke exposure on the cerebrovascular system with a focus on the blood-brain barrier: expanding the awareness of smoking toxicity in an underappreciated area. *Int. J. Environ. Res. Public Health* 7, 4111–4126. [PubMed: 21317997]
- McKee SA, Falba T, O'Malley SS, Sindelar J, O'Connor PG, 2007 Smoking status as a clinical indicator for alcohol misuse in US adults. *Arch. Intern. Med* 167, 716–721. [PubMed: 17420431]
- Mertens JR, Weisner C, Ray GT, Fireman B, Walsh K, 2005 Hazardous drinkers and drug users in HMO primary care Prevalence, medical conditions, and costs. *Alcohol Clin. Exp. Res* 29, 989–998. [PubMed: 15976525]
- Mineur YS, Fote GM, Blakeman S, Cahuzac EL, Newbold SA, Picciotto MR, 2016 Multiple nicotinic acetylcholine receptor subtypes in the mouse amygdala regulate affective behaviors and response to social stress. *Neuropsychopharmacology* 41, 1579–1587. [PubMed: 26471256]
- Mon A, Durazzo TC, Gazdzinski S, Hutchison KE, Pennington D, Meyerhoff DJ, 2013 Brain-Derived Neurotrophic Factor (BDNF) genotype is associated with lobar gray and white matter volume recovery in abstinent alcohol dependent individuals. *Genes Brain Behav.* 12, 98–107. [PubMed: 22989210]
- Pan P, Shi H, Zhong J, Xiao P, Shen Y, Wu L, Song Y, He G, 2013 Chronic smoking and brain gray matter changes: evidence from meta-analysis of voxel-based morphometry studies. *Neurological Sci.* 34, 813–817.
- Richter P, Werner J, Heerlein A, Kraus A, Sauer H, 1998 On the validity of the beck depression inventory. *Rev. Psychopathol* 31, 160–168.
- Salmon AB, Richardson A, Perez VI, 2010 Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radic. Biol. Med* 48, 642–655. [PubMed: 20036736]
- Salthouse TA, 2000 Aging and measures of processing speed. *Biol. Psychol* 54, 35–54. [PubMed: 11035219]

- Sankoh AJ, Huque MF, Dubey SD, 1997 Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Stat. Med* 16, 2529–2542. [PubMed: 9403954]
- Sharma A, Brody A, 2009 *In Vivo Brain Imaging of Human Exposure to Nicotine and Tobacco.*, Nicotine Psychopharmacology. Springer, Verlag Berlin Heidelberg, pp. 145–171.
- Skinner HA, Sheu WJ, 1982 Reliability of alcohol use indices. the lifetime drinking history and the MAST. *J. Stud. Alcohol* 43, 1157–1170. [PubMed: 7182675]
- Smith KJ, Kapoor R, Felts PA, 1999 Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol.* 9, 69–92. [PubMed: 9989453]
- Sobell LC, Sobell MB, Riley DM, Schuller R, Pavan DS, Cancilla A, Klajner F, Leo GI, 1988 The reliability of alcohol abusers' self-reports of drinking and life events that occurred in the distant past. *J. Stud. Alcohol* 49, 225–232. [PubMed: 3374136]
- Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA, 1977 *Self-Evaluation Questionnaire.* Consulting Psychologist Press, Palo Alto, CA.
- Sutherland MT, Riedel MC, Flannery JS, Yanes JA, Fox PT, Stein EA, Laird AR, 2016 Chronic cigarette smoking is linked with structural alterations in brain regions showing acute nicotinic drug-induced functional modulations. *Behav. Brain Funct* 12, 16. [PubMed: 27251183]
- Swan GE, Lessov-Schlaggar CN, 2007 The effects of tobacco smoke and nicotine on cognition and the brain. *Neuropsychol. Rev* 17, 259–273. [PubMed: 17690985]
- Sweitzer MM, Geier CF, Addicott MA, Denlinger R, Raiff BR, Dallery J, McClernon FJ, Donny EC, 2016 Smoking abstinence-induced changes in resting state functional connectivity with ventral striatum predict lapse during a quit attempt. *Neuropsychopharmacology.* 10.1038/npp.2016.56.
- Valavanidis A, Vlachogianni T, Fiotakis K, 2009 Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int. J. Environ. Res. Public Health* 6, 445–462. [PubMed: 19440393]
- WHO, 2015 *WHO Global Report on Trends in Prevalence of Tobacco Smoking 2015.* World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.
- Walhovd KB, Westlye LT, Amlie I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Fjell AM, 2011 Consistent neuroanatomical age-related volume differences across multiple samples. *Neurobiol. Aging* 32, 916–932. [PubMed: 19570593]
- Wang X, Michaelis EK, 2010 Selective neuronal vulnerability to oxidative stress in the brain. *Front. Aging Neurosci* 2, 12. [PubMed: 20552050]
- Wetherill RR, Jagannathan K, Hager N, Childress AR, Rao H, Franklin TR, 2015 Cannabis, cigarettes, and their co-Occurring Use: disentangling differences in gray matter volume. *Int. J. Neuropsychopharmacol* 18 10.1093/ijnp/pyv061.
- Yu R, Zhao L, Lu L, 2011 Regional grey and white matter changes in heavy male smokers. *PLoS One* 6, e27440. [PubMed: 22076160]
- Zimniak P, 2011 Relationship of electrophilic stress to aging. *Free Radic. Biol. Med* 51, 1087–1105. [PubMed: 21708248]

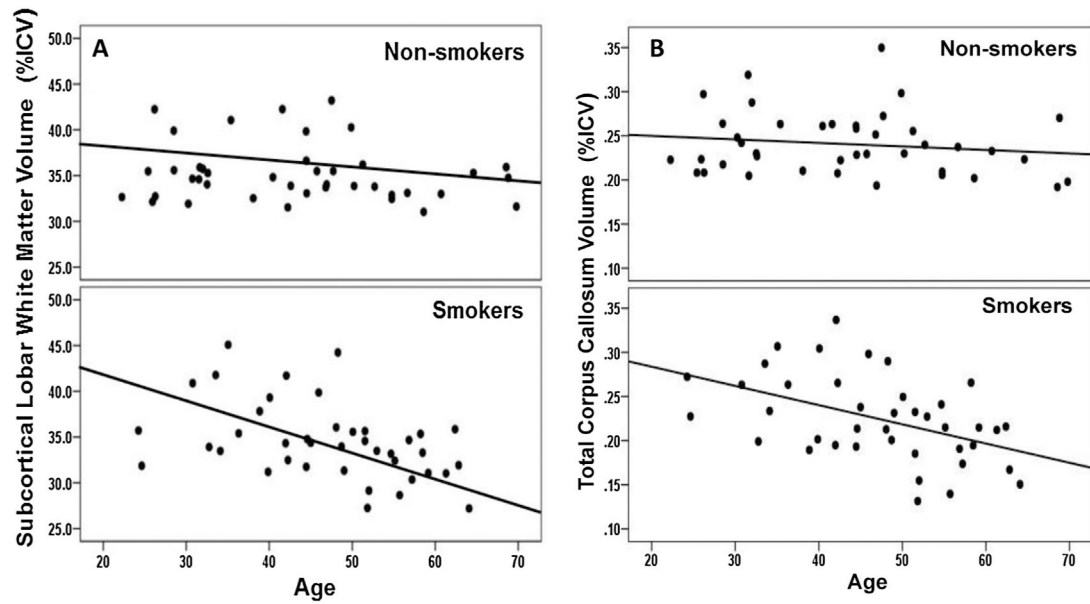


Fig. 1.

(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.

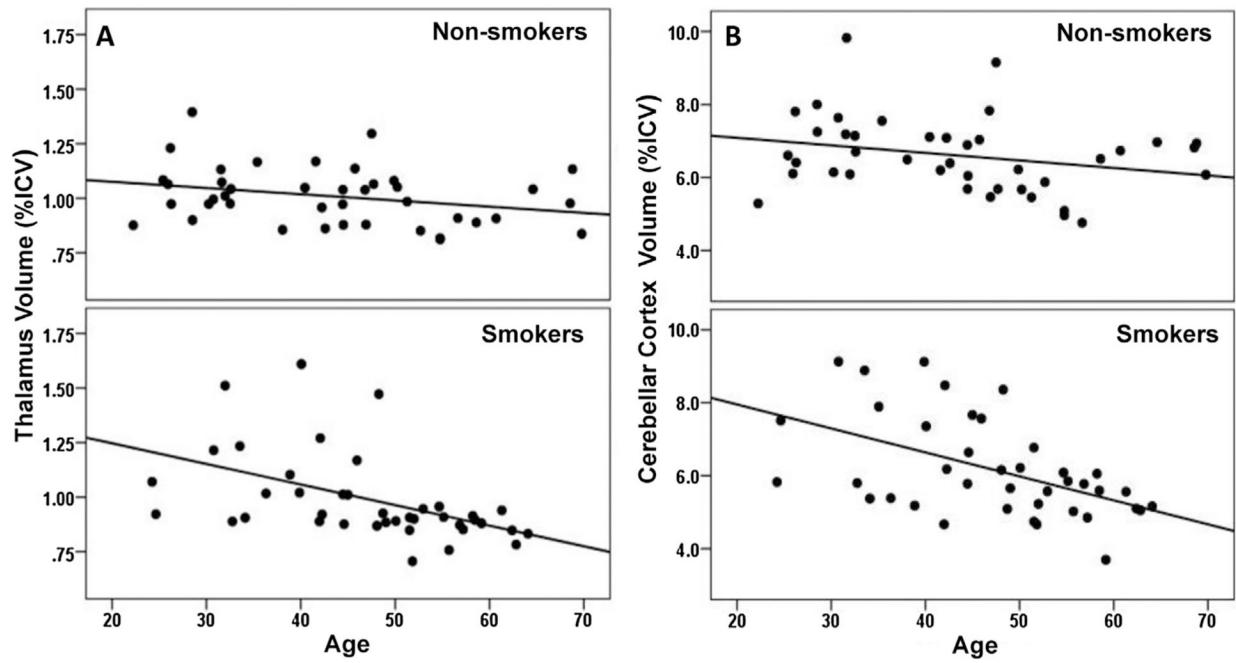


Fig. 2.

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

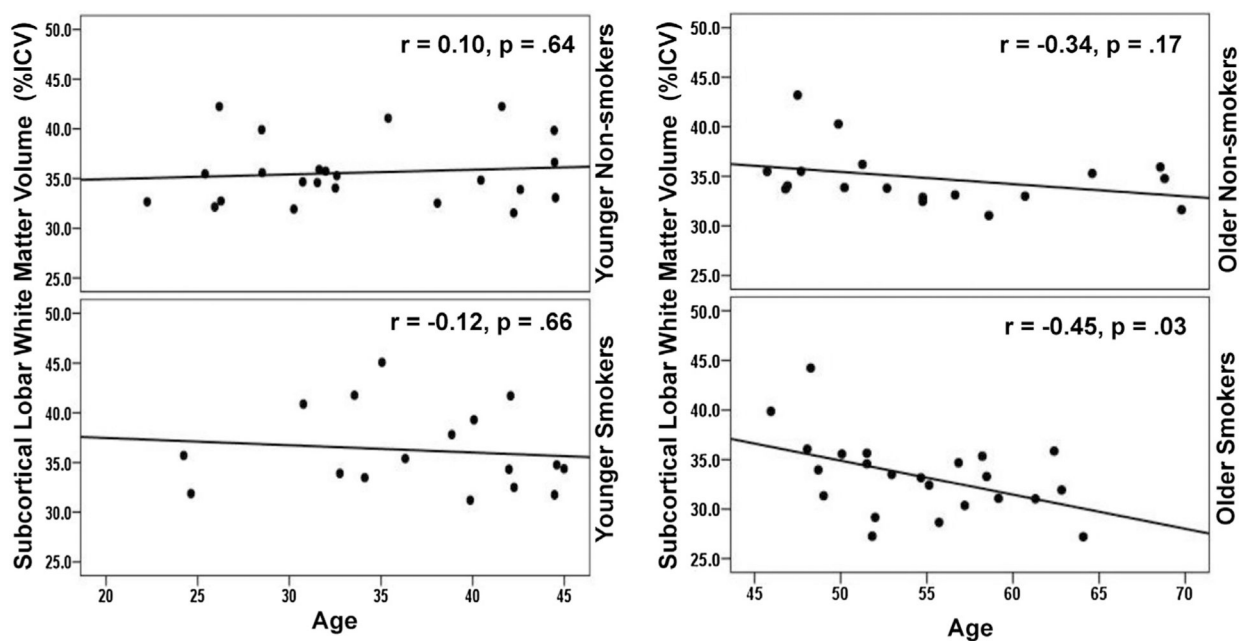


Fig. 3.

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

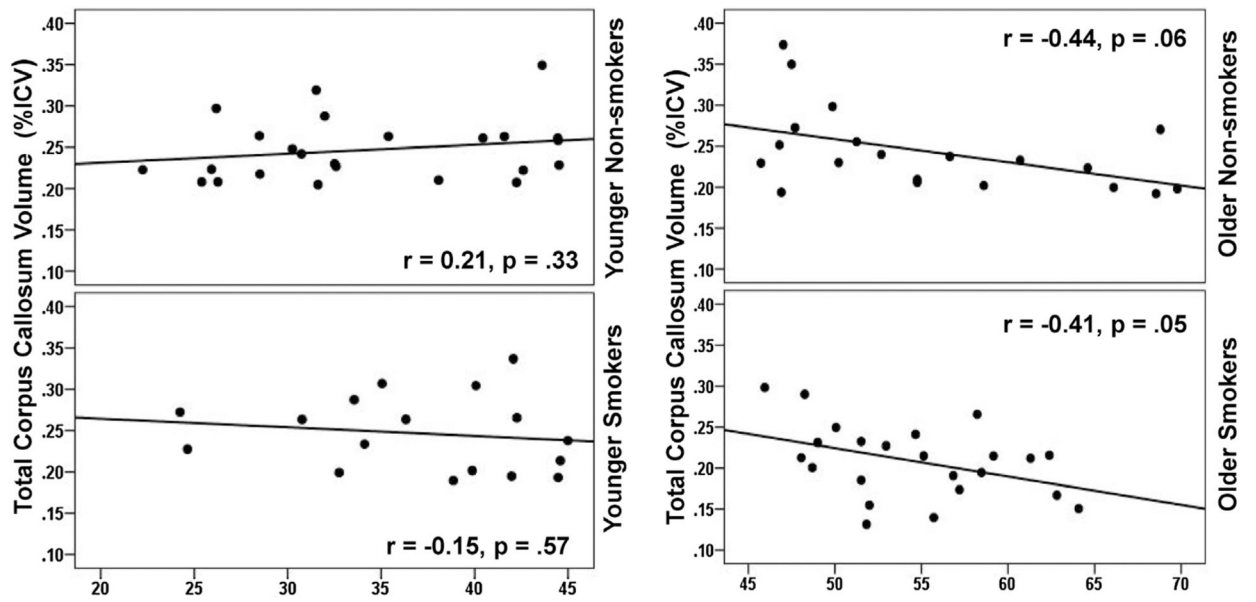


Fig. 4.

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

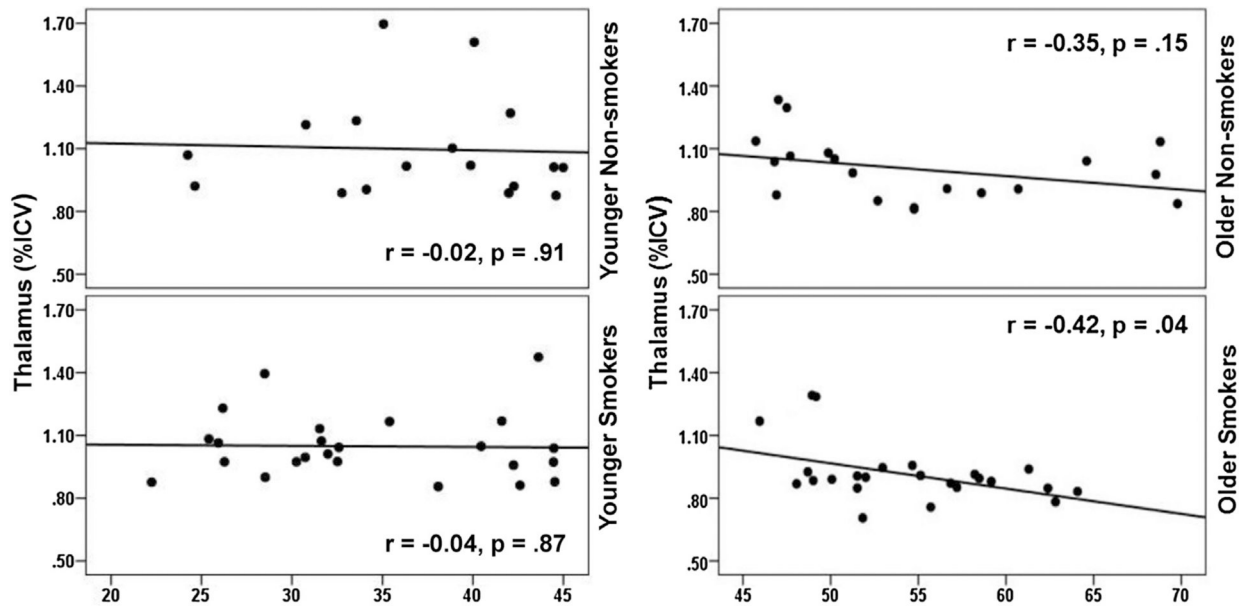


Fig. 5.
Thalamus volume across age for Younger and Older groups.

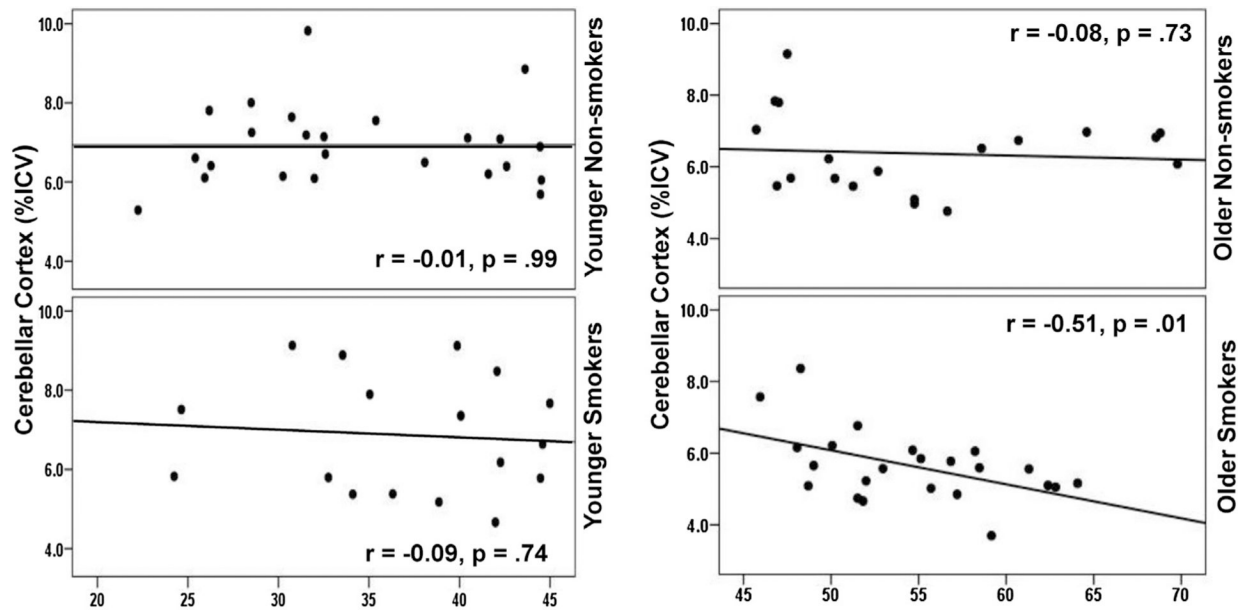


Fig. 6.
Cerebellar cortex volume across age for Younger and Older groups.

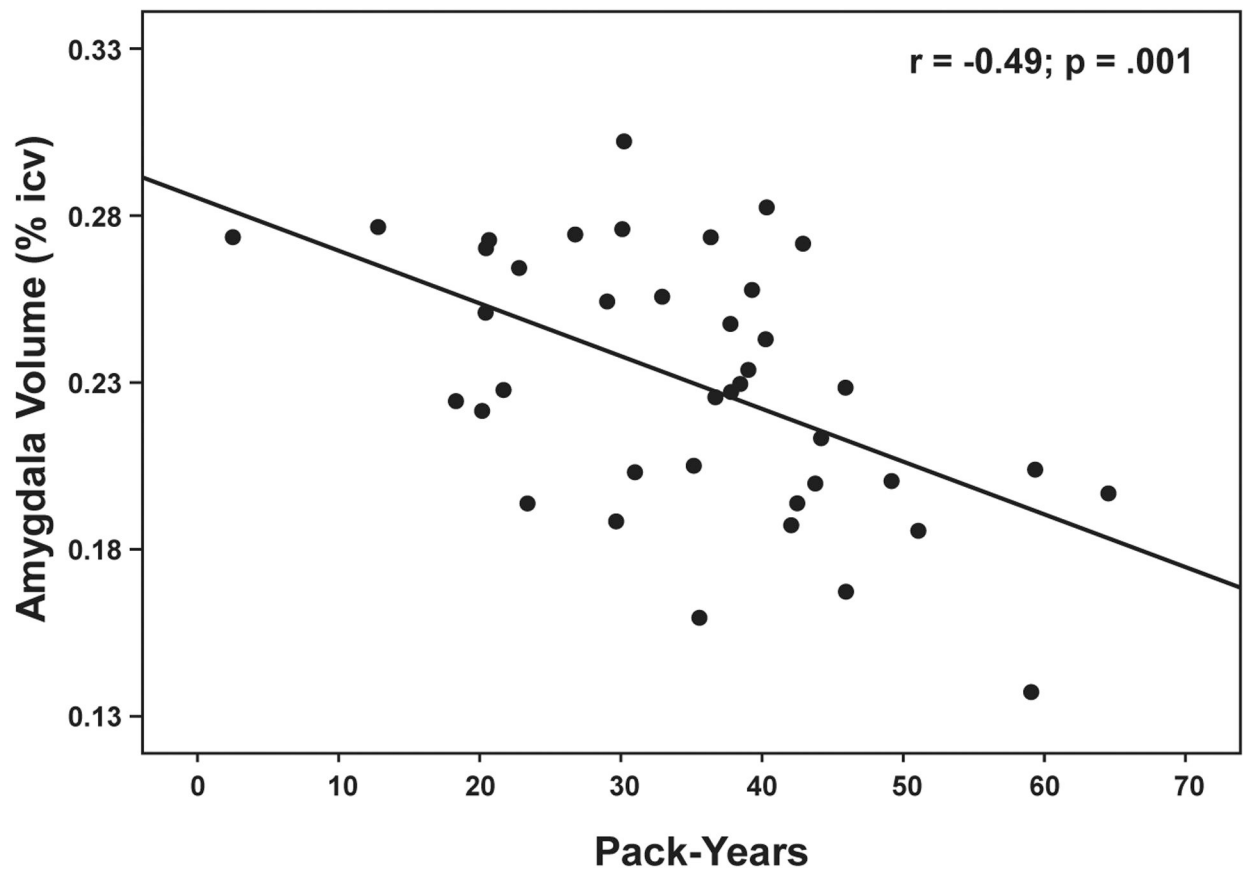


Fig. 7.
Association of amygdala volume with pack-years.

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).

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)				YoungS vs. OlderNS	YoungS vs. OlderS	OlderNS vs. OlderS
					YoungNS vs. YoungS	YoungNS vs. OlderNS	YoungNS 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).

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.