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Independent and Combined Effects of Chronic HIV-Infection and Tobacco Smoking on Brain Microstructure

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Abstract

HIV-infected individuals (HIV+) have 2–3 times higher prevalence of tobacco smoking than the general U.S. population. This study aims to evaluate the independent and combined effects of tobacco-smoking and HIV-infection on brain microstructure and cognition using a 2×2 design. 21 HIV + Smokers, 25 HIV + Nonsmokers, 25 Seronegative (SN)-Smokers and 23 SN-Nonsmokers were evaluated using diffusion tensor imaging. Fractional anisotropy (FA), mean (MD), radial (RD) and axial (AD) diffusivity were assessed in 8 major cerebral fiber tracts and 5 subcortical regions. Cognitive performance in 7 neurocognitive domains was also assessed. Compared to SN, HIV+ had higher AD in genu of corpus callosum (GCC, $p = 0.002$). Smokers also had higher diffusivities in GCC, splenium of corpus callosum (SCC), anterior corona radiata (ACR), sagittal stratum (SS) and superior fronto-occipital fasciculus (SFO), than Nonsmokers (p -values < 0.001 – 0.003). Tobacco-Smoking and HIV-infection showed synergistic effects on AD_SS ($p = 0.002$) and RD_SFO ($p = 0.02$), but opposite effects in FA_putamen ($p = 0.024$). Additive effects from HIV+ and Tobacco-Smoking were observed in 9 other white matter tracts, with highest diffusivities and lowest FA in HIV + Smokers. Higher diffusivities in the GCC, SCC, ACR and SS predicted poorer cognitive performance across all participants ($p = 0.001$). Higher AD_GCC also predicted slower Speed of information processing and poorer Fluency and Attention only in HIV + Smokers ($p = 0.001$ – 0.003). Chronic tobacco smoking and HIV-infection appear to have additive and synergistic adverse effects on brain diffusivities, suggesting greater neuroinflammation, which may contribute to poorer cognition. Therefore, chronic tobacco-smoking may be a risk factor for HIV-associated neurocognitive disorders.

Keywords

HIV; Tobacco use; Microstructure; Diffusion tensor imaging

Introduction

The prevalence of tobacco smoking among HIV seropositive (HIV+) adults was 42% in 2009 and reduced slightly to 36% in 2016 (Centers for Disease Control and Prevention), which is two or three times higher than the 15.5–20.6% of smokers in the general population of the United States (Mdodo et al. 2015; Jamal et al. 2018). Although the progression of HIV disease has slowed significantly since the introduction of combination antiretroviral therapy (cART), smoking has become a significant threat to the long-term survival of people living with HIV (PLWH) or Acquired Immune Deficiency Syndrome (AIDS), as shown in several large cohort studies (Feldman et al. 2006; Hile et al. 2016; Gamarel et al. 2018). The Women's Interagency HIV study showed that cigarette smoking negated some of the benefits from cART, since smoking was associated with poorer HIV prognosis, poorer immune function, less viral suppression, and higher risk of death, compared to non-smokers (Feldman et al. 2006). Similarly, recent or daily tobacco smoking was associated with poorer viral suppression and lower CD4 cell count in a large cohort of PLWH in New York (Hile et al. 2016) and in youth living with HIV across 14 clinics in the United States (Gamarel et al. 2018). In addition, even with effective cART, the milder forms of HIV-associated neurocognitive disorder (HAND) remains prevalent (Saylor et al. 2016). Previously, we demonstrated that tobacco smoking and HIV infection have additive adverse effects on neurocognitive functions, impulsivity, and psychopathological symptoms (Chang et al. 2017), although the underlying mechanisms remain unclear.

Chronic HIV-infection is associated with sustained and widespread neuroinflammation, neuronal loss and axonal damage in the central neuronal system, which may contribute to HAND (McArthur et al. 2010; Saylor et al. 2016). Similarly, tobacco smoke contains a number of compounds that are potentially neurotoxic, either directly by promoting oxidative stress and calcium overload, which cause neuronal and glial cell organelle damage (Durazzo et al. 2010), or indirectly through decreased cerebral blood flow (Domino et al. 2004). An in-vitro study showed that tobacco smoke exacerbated inflammatory events, with release of pro-inflammatory cytokines (TNF- α and IL-6) and nitric oxide (NO), which led to endothelial overexpression of vascular adhesion molecules (VCAM-1, P-selectin and E-selectin), release and activation of matrix metalloproteinases (MMP-2 and MMP-9), and maturation of macrophages that adhere to vascular endothelium (Hossain et al. 2009, 2011). Since matrix metalloproteinases are already elevated in HIV patients with dementia or HAND (Conant et al. 1999; Xing et al. 2017), and may promote microglial activation (Conant et al. 2017) and disrupt the blood brain barrier (Xing et al. 2017), tobacco smoke-mediated release of proinflammatory products may further exacerbate neuroinflammation and increase the risk for HAND in HIV+ patients. Furthermore, exposure to either nicotine-containing cigarette smoke or nicotine alone significantly greater cytokine expression (TNF- α and IL-1) in F344 rodents, but those with HIV1-transgenic (HIV1Tg) showed even higher cytokine expression (TNF- α , IL-6 and IL-1) (Royal et al. 2018), despite nicotine's possible neuroprotective effects in neurodegenerative disorders (Ferrea and Winterer 2009). However, no data are available regarding whether tobacco smoking may exacerbate neuroinflammation in HIV-infected individuals.

Neuroinflammation may be assessed with diffusion tensor imaging (DTI), which is a sensitive noninvasive imaging technique that can evaluate the integrity of brain microstructure by measuring the degree and direction of water diffusion in tissue. In grey matter, water diffusion is mainly limited by the cell membrane and is isotropic; however, in white matter, water diffusion is anisotropic and is affected by axonal density, axonal diameter, and the level of myelination (Feldman et al. 2010). Changes in myelin content, axonal integrity, neuronal or glial damage, or proliferation, and cell swelling, can all lead to changes in water diffusion in the brain. DTI parameters, including fractional anisotropy (FA), mean diffusion (MD), axial diffusion (AD) and radial diffusivity (RD), are highly sensitive to these subtle changes (Alexander et al. 2011), and have been used widely to detect tissue changes induced by diseases (Feldman et al. 2010; Alexander et al. 2011).

Abnormal brain microstructures were reported in both HIV- infected individuals and tobacco smokers. Specifically, DTI studies consistently found reduced FA and greater than normal diffusivities (AD, RD or MD) in various white matter brain regions of HIV-infected adults that are related to cognitive deficits (Cloak et al. 2004; Chang et al. 2008; Pfefferbaum et al. 2009; Masters and Ances 2014; O'Connor et al. 2017; Chang and Shukla 2018). Similarly, chronic smoking has long been associated with cognitive deficits and white matter lesions (Durazzo et al. 2010), as well as reduced cerebral white matter integrity, with lower FA or elevated diffusivities (Paul et al. 2008; Gons et al. 2011; Zhang et al. 2011; Hudkins et al. 2012; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015; Baeza-Loya et al. 2016). However, no study has evaluated the combined effects of HIV-infection and tobacco smoking on brain microstructure, which was the goal of the current study. Based on preclinical studies that demonstrated deleterious effects of tobacco smoke or nicotine on neuroinflammation in in-vitro models (Hossain et al. 2011), the HIV rodent model (Royal et al. 2018), and the poorer cognitive performance and more severe psychiatric symptoms in HIV positive smokers (Chang et al. 2017), we hypothesized that, due to the possible greater neuroinflammation, HIV+ tobacco smokers will show greater diffusivities (AD, RD, MD) and lower FA than either HIV- infection or tobacco smoking alone in the major white matter tracts.

Methods

Participants

Participants were recruited from the community via the use of flyers, website advertisement, word of mouth, and referrals from local health care providers. In total, 264 participants were screened by phone and 163 were further screened in person; 117 fulfilled the inclusion criteria and 100 completed the parent protocols in addition to the DTI scan (Chang et al. 2017). Participants included 25 seronegative (SN) Nonsmokers, 26 SN Smokers, 25 HIV+ Nonsmokers, and 24 HIV+ Smokers. Due to the relatively small proportions of female (11%) and African American (5%) HIV positive individuals (State of Hawaii Department of Health 2015), we recruited seronegative controls with a similar gender and racial distribution as our HIV positive participants. The subject criteria were detailed in our previous publication regarding their psychopathological symptoms (Chang et al. 2017). Briefly, the inclusion criteria for all participants were (1) age 18–75 years and (2) able to provide

informed consent. SN participants additionally were (1) confirmed to be HIV-1 negative on site with the Clearview® COMPLETE HIV ½ test kits (Alere, Waltham, MA). All HIV+ participants additionally were: (1) HIV-seropositive; and (2) stable on a cART regimen for 6 months or medication naïve. The tobacco smokers were also current daily users, who used at least ½ pack cigarettes per day, for at least the last 2 years. Nonsmokers were defined by no current tobacco cigarette usage, past smoking >2 years ago and < 2 pack-year lifetime smoking. As reported previously (Chang et al. 2017), all subjects were excluded if they had: (1) co-morbid psychiatric illness that may confound the analysis of the study; (2) confounding medical disorders, including active hepatitis C (verified by medical records in the HIV+ participants and by QraQuick® HCV test in the SN controls), other confounding neurological disorder(s), or significant head trauma with coma and prolonged hospitalization; (3) significantly abnormal screening laboratory tests that indicated a chronic confounding medical condition that would affect brain function; (4) medications that could alter functional brain imaging; (5) current or history of moderate or severe substance use disorders (other than smoking) according to the DSM-5 (those with recreational usage or mild substance use disorder were allowed); (6) positive urine toxicology screen for cocaine, methamphetamine, opiates, and benzodiazepines (except for false positives from a prescription medication, e.g., efavirenz); (7) pregnancy in women of child bearing age; (8) inability to read at an 8th grade level; and (9) contraindications for MR studies. All participants also completed detailed clinical, medical, and neuropsychiatric assessments, before their brain MRI studies. All participants signed a written informed consent form which, along with the protocol, was approved by the Committee on Human Studies at the University of Hawaii at Manoa. The study was Health Insurance Portability and Accountability Act (HIPAA) compliant.

Clinical and Medical Assessments

A study physician evaluated each participant, reviewed the medical history, including all HIV disease parameters, such as the nadir and most recent CD4 cell counts and plasma viral loads, and performed the neuropsychiatric examination, including the HIV Dementia Scale and Karnofsky Performance Status Scale. In addition, detailed substance use histories, including tobacco and alcohol usage, depressive symptoms using the Center for Epidemiologic Studies-Depression Scale (CES-D) (Radloff 1991; Lewinsohn et al. 1997), psychopathological symptoms using Symptom Checklist-90, and impulsivity measures using the Barrett Impulsivity scale and the Iowa Gambling Task, were also collected. These measures, along with detailed cognitive assessments from the majority of participants, were reported previously (Chang et al. 2017).

Neurocognitive Tests

Each participant also completed a battery of neuropsychological tests that evaluated the seven cognitive domains, along with assessments of their activities of daily living, which are required to determine the HAND or HAND-equivalent status of the participants (Antinori et al. 2007). The seven domains included the Speed of Information Processing, Learning, Memory, Verbal Fluency, Executive Function, Working Memory/Attention, and Fine Motor function (see Table 2 footnotes for the tests used for each domain). Z-scores were generated for each participant on each of these domains, adjusted for age and education, using our

normative database containing data from 507 SN healthy participants administered the same tests in our laboratory.

Image Acquisition

All participants were scanned on a 3 Tesla TIM Trio scanner (Siemens Medical Solutions, Erlangen, Germany) with a 12- channel head coil. Structural imaging was performed to assess for possible neuroanatomical abnormalities; the protocol included a T1-weight MPRAGE sequence (TR/TE/TI = 2200/4.47/ 1000 ms, flip angle = 12°, FOV = 256 mm, 256*256 matrix, thickness = 1 mm) and a T2-weighted transversal fluid- attenuated inversion recovery (FLAIR) sequence (TR/TE/TI = 9100/84/2500 ms, flip angle = 150°, FOV = 230 mm, 204*256 matrix, thickness = 3 mm). DTI was based on a spin-echo echoplanar series (TR/TE = 3700/88 mm, FOV = 220 mm, 128*128 matrix, thickness = 4 mm, four unweighted scans, 12 directions with b value of 1000s/mm²). DTI scans of 6 participants (2 SN and 4 HIV+ Smokers) were not usable due to excessive head motion. The final DTI analyses included 94 participants.

DTI Processing

The tensor field from the DTI scans for each individual brain was calculated in DtiStudio (www.MriStudio.org) after motion correction (Jiang et al. 2006), and transformed to the JHU-MNI atlas space using dual-channel Large Deformation Diffeomorphic Metric Mapping (LDDMM) (Ceritoglu et al. 2009). The fractional anisotropy (FA), mean diffusivity (MD, mean of the three eigenvalues), axial diffusivity (AD, first eigenvalue), and the radial diffusivity (RD, mean of second and third eigenvalues) of each anatomical region of interest (ROI) defined in the JHU-MNI atlas were obtained as previously described (Oishi et al. 2009; Andres et al. 2016). The ROIs for the current study were selected based on prior DTI studies that found regional brain abnormalities in HIV- infected individuals (Chang et al. 2008; Gongvatana et al. 2009; Pfefferbaum et al. 2009; Masters and Ances 2014; Nir et al. 2014; O'Connor et al. 2017) and in tobacco smokers (Gons et al. 2011; Zhang et al. 2011; Hudkins et al. 2012; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015; Baeza-Loya et al. 2016) that are connected to or near the following 8 major white matter structures (15 substructures): corona radiata (anterior, superior and posterior; or ACR, SCR and PCR), corpus callosum (genu, body and splenium; or GCC, BCC and SCC), sagittal stratum (SS), superiorlongitudinal fasciculus (SLF), superior fronto-occipital fasciculus (SFOF), internal capsule (anterior and posterior limbs and retrolenticular part; or ALIC, PLIC and RLIC), posterior thalamic radiation (PTR) and cingulum (from cingulate gyrus and from hippocampus; or CGC and CGH). Additionally, 5 subcortical regions were selected, which included the caudate, putamen, globus pallidus, thalamus and hippocampus. Since we did not observe differences between the left and right brain regions, they were averaged for each region of interest for the final analyses.

Statistical Analyses

Statistical analyses were conducted using R (version 3.4.1). Analyses of variance (ANOVA), T-tests, Chi-square, Fisher's exact or Kruskal-Wallis tests, were performed, depending on the type of and distribution of the variable, to assess group differences in the demographic and clinical variables shown in Table 1. Post hoc analysis was performed if a significant

group difference was found. Two-way ANCOVA was used to determine the effects of HIV, tobacco smoking and possible HIV-by-tobacco smoking interaction on DTI measures using only age as a covariate, since IQ, sex, lifetime alcohol and marijuana use did not influence the DTI measures and were removed from the final model. Two-way ANOVA was performed on the cognitive domain z-scores, without additional covariates, since these z-scores were adjusted for age and education. Linear regression models were used to determine if DTI measures that showed HIV or tobacco-smoking effects predicted the cognitive domain z-scores. The Holm method was used to correct for the multiple tests in each DTI metrics separately to determine significance (Holm 1979). Uncorrected p -values <0.05 that did not reach significance after Holm correction were considered trends.

Results

Clinical Characteristics (Table 1)

The four participant groups were similar in mean age, age range, sex proportion, ethnicity, and social economic status. However, Smokers had lower education and IQ than SN-Nonsmokers (for both SN-Smokers and HIV+ Smokers, post hoc p values = 0.005–0.039). HIV+ participants and SN-Smokers had more depressive symptoms (higher CES-D scores) compared to SN- Nonsmokers participants (post hoc p -values between <0.001 –0.015). The two HIV+ groups had similar clinical measures of HIV disease severity, including the HIV-infection duration, recent and nadir CD4 counts, and the percentage of participants taking antiretroviral medications. Four participants had past histories of hepatitis A that was treated, and 12 participants reported asymptomatic hepatitis B ($p = 0.003$, with higher percentage in the HIV Non-Smoker group than other groups). However, liver function blood tests were in normal range for all our participants (based on our screening blood tests or recent medical records). Four participants had stable heart disease that were maintained on medications, 16 had well-controlled hypertension (based on screening physical examinations); no group difference in these co-morbid medical disorders was found. In addition, two participants in the HIV-Smoker group had lung diseases (chronic obstructive pulmonary disease and chronic bronchitis) that were managed with medications. The two Smoker groups had similar tobacco usage. The four groups were similar in the percentage of individuals who smoked marijuana or drank alcohol during the past month, daily amount of marijuana or alcohol use, years of use and years of abstinence from marijuana or alcohol use. However, the two HIV+ groups had more lifetime marijuana users than the SN-Nonsmokers groups (post hoc- $p = 0.041$ and 0.006). Since we excluded individuals with moderate to severe alcohol use disorders, the SN-Smoker group actually had the lowest percentage (57.7%) of lifetime alcohol users across all four groups.

Neuropsychological Performance

Compared to SN controls, and regardless of tobacco use status, HIV+ participants had lower Z scores on cognitive domains for Fluency ($p = 0.049$), Executive function ($p = 0.031$), Speed of Information Processing ($p = 0.043$), Attention/ Working Memory ($p = 0.036$), Motor function ($p = 0.036$) and Global functions ($p = 0.016$). Compared to NonSmokers, and regardless of HIV-serostatus, Smokers had lower Z scores on Executive domain ($p = 0.019$), learning ($p = 0.019$), memory ($p = 0.038$) and Global function ($p = 0.016$) function.

No HIV infection-by-tobacco smoking interactions on cognitive domain Z scores were observed (Table 2).

Group Differences on Brain Diffusivities

Regardless of smoking status, HIV+ participants showed relatively normal FA in all brain regions (Table 3), but five white matter subregions (GCC, SCC, CGC, ALIC and SFO) showed HIV serostatus effects on brain diffusivities (Table 4 and Fig. 1). However, after Holm correction, the HIV effect remained significant only in the GCC_AD (Fig. 1).

Compared to non-smokers, regardless of HIV-serostatus, Tobacco Smokers not only had lower FA in the GCC, BCC, SCC, ACR, and CGC (Table 3, Fig. 2a, b), but also higher diffusivities (RD, AD or MD) in 12 of the 15 subregions of the major white matter tracts (Table 4, Fig. 2c–e). After Holm correction, chronic tobacco smoking effects remained significant in GCC (MD- $p = 0.003$), SCC (MD & RD, both p -values = 0.001) and the ACR (MD & AD, both p -values = 0.002) (Fig. 2c, e, f). In addition, the significant Tobacco-Smoking effect in the SS (AD- $p = 0.001$) (Fig. 2c) and the SFO (RD- $p = 0.001$) (Fig. 2d) led to the trend for the Smoking effect in MD in the SS ($P = 0.005$) and SFO ($P = 0.004$) (Fig. 2e).

In addition, a significant HIV-by-tobacco smoking interaction was found in the Putamen (FA: interaction- $p = 0.024$); while HIV+ Nonsmokers had lower FA than SN Nonsmokers, HIV+ Smokers had higher FA than SN Smokers (Fig. 2f). Furthermore, HIV-by-tobacco smoking interactions were found in the AD_SS (interaction- $p = 0.002$) (Fig. 2c), RD_SFO (interaction- $p = 0.020$) (Fig. 2d), which led to trends for interactions in the MD_SS ($p = 0.037$) and MD_SFO ($p = 0.039$) (Fig. 2e). For both of these brain regions, while HIV + Smokers had significantly higher AD or MD compared to SN Smokers, HIV+ Nonsmokers had similar AD or MD compared to SN-Nonsmokers. In addition, HIV+ status and tobacco smoking had additive effects on diffusivities in the other nine cerebral white matter tracts (GCC, BCC, SCC, ACR, SCR, PCR, CGC, ALIC and PTR); therefore, the HIV + Smoker group presented the highest diffusivities or lowest FA values across study groups. Lastly, no age-by- HIV interaction or age-by-Smoking interaction was found on the diffusivity measures in any of these white matter or grey matter regions. Co-varying for group difference in the percentage of lifetime marijuana users, or the percentage of lifetime alcohol user, did not change the significance of the results.

Abnormally Higher Brain Diffusivity Predicted Poorer Cognitive Performance

A linear regression model was used to examine whether abnormal DTI measurements predicted cognitive domain scores (with age as a covariate). The regressions that remain significant after the Holm correction included four brain regions (GCC, SCC, ACR and SS) and four cognitive domains (fluency, motor, learning and speed). Across the four participant groups, higher GCC_AD and SS_AD predicted slower motor performance (Fig. 3a, b), while higher ACR_AD or SCC_RD predicted poorer learning (Fig. 3 c, d). In addition, higher MD in GCC (which is due to the higher AD) predicted slower Speed of information processing and Motor performance, as well as poorer executive function and Learning (P -values between <0.001 – 0.001 , Table 5). Overall, across all participants, higher diffusivities

in most regions (GCC_AD and MD, SCC_MD, ACR_AD and MD, SS_AD) predicted poorer global function (all p values < 0.001 , Table 5). However, higher GCC_AD predicted poorer Fluency and Attention, as well as slower Speed of information processing, only in the HIV + Smoker group (group-interaction- $p = 0.002$, 0.003 and 0.001 respectively, Fig. 3e–g, Table 5).

Exploratory analyses between HIV disease severity and brain diffusivity showed that CD4 cell counts, but not nadir CD4 counts, tended to correlate with GCC_AD ($p = 0.061$). Furthermore, higher viral loads (Log transformed) tended to correlate with lower GCC_AD and higher Putamen_FA in HIV + Smokers, but not in HIV+ Smokers, but not in HIV+ Nonsmokers (GCC_AD_interaction- $P = 0.08$ and Putamen_FA_interaction- $P = 0.09$).

Exploratory analyses between DTI metrics and smoking variables showed that only longer smoking duration correlated with higher diffusivities in the ACR, only in SN-Smokers but not in HIV + Smokers (MD- interaction- $P = 0.034$; RD-interaction- $P = 0.024$). Similarly, longer duration of smoking correlated with lower FA in the putamen only in SN-Smokers but not in HIV + Smokers (interaction- $P = 0.018$).

Discussion

This study found that HIV-infection and chronic tobacco-smoking has independent and combined (either additive, synergistic or opposite) effects on white matter diffusivity (AD, RD and MD) in multiple regions of the major white matter tracts (GCC, BCC, SCC, ACR, SCR, ALIC, SS, SFO and PTR). Higher white matter diffusivity in GCC, SCC, ACR and SS predicted slower motor performance and worse Learning in all participants, while higher white matter diffusivity in GCC also predicted poorer Attention and Fluency and slower Speed of information processing in only HIV + Smokers.

HIV Effects on DTI

Regardless of smoking status, HIV+ individuals had higher diffusivities in several major white matter regions, especially the genu of corpus callosum, compared to SN participants. High RD or AD mainly reflects greater water diffusivity, which can result from axonal demyelination (Alexander et al. 2011), axonal swelling (Singh et al. 2016), or axonal loss (Hikishima et al. 2015). Disrupted fiber microstructure integrity might also result from persistent neuroinflammation that is common in HIV+ patients (Chang et al. 2003; McArthur et al. 2010; Chang et al. 2014; Saylor et al. 2016). The findings from our current study are consistent with numerous studies that documented elevated diffusivity in widespread white matter regions (Chang et al. 2008; Gongvatana et al. 2009; Pfefferbaum et al. 2009; Masters and Ances 2014; Nir et al. 2014; O'Connor et al. 2017), and most frequently in the corpus callosum, of HIV+ patients (Masters and Ances 2014; O'Connor et al. 2017). Our findings of elevated mean diffusivity, primarily due to elevated AD, with normal FA, in our HIV+ participants are consistent with prior reports (Pfefferbaum et al. 2009; Li et al. 2018). However, lower FA and higher RD in HIV+ compared to SN participants were also reported by others (Leite et al. 2013; Underwood et al. 2017). Some argued that axonal-related AD changes might occur earlier than demyelination-related RD elevation after HIV-infection (Li et al. 2018). For example, myelin basic protein, the marker

of demyelination was elevated in the cerebrospinal fluid (CSF) of all HIV+ participants with severe dementia but not in HIV+ participants without neurological disorders (Liuzzi et al. 1992). Furthermore, individuals with HAND showed elevated RD in more widespread regions than non-HAND individuals (Oh et al. 2018).

Smoking Effects on DTI

Regardless of HIV-infection status, tobacco smokers showed higher diffusivities (primarily MD and RD) in GCC, SCC, ACR, SS, and SFO compared with nonsmokers after correction for multiple comparisons. Our findings are consistent with prior DTI studies of tobacco smokers that typically found abnormal white matter integrity, with lower FA (Gons et al. 2011; Zhang et al. 2011; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015; Baeza-Loya et al. 2016) and higher diffusivity (MD and RD) (Gons et al. 2011; Lin et al. 2013; Savjani et al. 2014) in the corpus callosum (Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015) or frontal white matter regions (Zhang et al. 2011). However, both elevated (Yu et al. 2016) and reduced (Lin et al. 2013) AD were reported in elderly tobacco smokers, while elevated FA were found in younger smokers (Paul et al. 2008; Hudkins et al. 2012; Huang et al. 2013; Yu et al. 2016). Typically, these studies found that greater tobacco usage, and higher dependence scores (Zhang et al. 2011; Hudkins et al. 2012; Huang et al. 2013; Baeza-Loya et al. 2016), were associated with higher brain diffusivity, such as higher RD and MD in the corpus callosum and cingulum (Lin et al. 2013; Savjani et al. 2014), lower FA in the corpus higher dependence scores (Zhang et al. 2011; Hudkins et al. 2012; Huang et al. 2013; Baeza-Loya et al. 2016), were associated with higher brain RD and MD and higher FA in normal appearing white matter globally (Gons et al. 2011). In contrast, two studies in younger smokers (19 years of age) found positive (Yu et al. 2016) or no (Li et al. 2016) correlation between smoking severity and the DTI metrics (FA or RD). Taken together, DTI studies suggest an adverse effect of chronic heavy smoking on white matter microstructure, with FA, MD and RD more affected than AD. The abnormal diffusivity, involving RD more than AD in many brain regions of chronic smokers suggest that tobacco smoking may lead to dysmyelination and eventually neuronal or axonal damage, which is consistent with tobacco smoke exposure mediated white matter degeneration, resulting from interruption of white matter myelin lipid composition in mice (Nunez et al. 2016). Future longitudinal studies, including a larger sample size of never smokers, are needed to determine whether some of these adverse effects are reversible in smokers with prolonged abstinence.

Combined HIV and Smoking Effects on DTI

HIV+ status and tobacco smoking appear to have synergic adverse effects on RD in the SFO and AD in the SS, and additive adverse effects on diffusivities in the other 9 subregions of the white matter tracts (GCC, BCC, SCC, ACR, SCR, PCR, CGC, ALIC and PTR). Earlier DTI studies of HIV+ participants typically did not report or specifically evaluate the participants' smoking status (Chang et al. 2008; Gongvatana et al. 2009; Tate et al. 2011; Hoare et al. 2012; Towgood et al. 2012; Nir et al. 2014; Correa et al. 2015; Wright et al. 2015). Prior studies of asymptomatic (no history of AIDS or cognitive impairments) HIV+ participants typically showed similar FA and/or MD (Gongvatana et al. 2009; Tate et al. 2011; Hoare et al. 2012; Towgood et al. 2012; Correa et al. 2015) or even higher FA (Hoare et al. 2012) compared to SN participants. However, a DTI study of asymptomatic HIV+

participants found higher RD in the corpus callosum and AD in the internal capsule and superior cingulate bundle than SN participants, but the HIV+ group also had more tobacco smokers than the SN group (Pfefferbaum et al. 2009), which is consistent with our current findings. Taken together, chronic tobacco smoking appears to exacerbate HIV-associated brain injury in these white matter fibers. Although the mechanism for this deleterious effect remains unclear, a possible etiology may be due to tobacco-mediated accelerated HIV replication (Andeet et al. 2015; Rao et al. 2016), since higher plasma HIV viral load was related to higher AD in the occipital forceps (Pfefferbaum et al. 2009) and with both higher MD and lower FA in corpus callosum (Wright et al. 2015). However, our HIV+ tobacco smokers with higher brain diffusivity did not have higher plasma viral load compared to our HIV-Nonsmokers. A more likely mechanism is that tobacco smoking may exacerbate neuroinflammation in HIV+ participants. Rodent models indeed demonstrated that tobacco smoke exposure led to greater astroglial and microglial activation in an Alzheimer rodent model (Moreno-Gonzalez et al. 2013) and greater pro-inflammation cytokine gene expression in HIV-1 transgenic rats compared to the F344 control rats (Royal et al. 2018). Tobacco smoke also stimulated the release of proinflammatory cytokines, matrix metalloproteinases (MMPs), and nitric oxide, as well as maturation of monocytes into macrophages that adhere to the vascular endothelium (Hossain et al. 2011). These products and events can lead to blood-brain barrier damage and promote microglial activation (Conant et al. 2017), which may contribute to the pathogenesis of HAND (Xing et al. 2017; Singh et al. 2018). Furthermore, plasma MMPs were also associated with white matter diffusivity and viral load in HIV+ participants (Li et al. 2013). In contrast, in the putamen, our HIV+ Tobacco Smokers appeared to show normalization of the FA, suggesting a possible neuroprotective effect of chronic smoking in this brain region with high density of dopaminergic synapses. In support of this possibility, nicotine may be neuroprotective (Quik et al. 2012), and chronic tobacco smoking was associated lower risk, for Parkinson's disease (Ascherio and Schwarzschild 2016). Furthermore, although rodents exposed to cigarette smoke or nicotine showed greater inflammation (cytokine expression) in the frontal cortex (Royal et al. 2018), nicotine inhibited the microglial activation in the striatum (Serriere et al. 2015). Nevertheless, despite the possible neuroprotective effect of nicotine and smoking in the putamen, our HIV+ Smokers did show better motor performance than the nonsmokers.

Higher Diffusivities Predicted Poorer Cognitive Performance

We showed that the DTI diffusivities (MD, AD and RD) that were higher in specific brain regions (GCC, SCC, ACR and SS) predicted poorer performance on Speed of Information Processing, Fine Motor performance, Learning and Global cognition across all participants. The association between DTI diffusivities and cognition suggest that neuroinflammation might have contributed to axonal or neuronal injury (Masliah et al. 1997; Weed et al. 2003). Our findings are consistent with higher diffusivity in the corpus callosum (Wu et al. 2006) and corona radiata (Salami et al. 2012) in relation to deficits in Speed and Motor function in HIV- infected individuals (Wu et al. 2006; Nir et al. 2014) and in an aging population (Salami et al. 2012). Similarly, we also found poorer global cognition were predicted by the higher MD, AD and RD in the corpus callosum (Chang et al. 2008; Nir et al. 2014) and corona radiata (Nir et al. 2014) in HIV+ participants and chronic smokers (Gons et al. 2011).

However, the current study is the first to show the combined effects of HIV and tobacco smoking on the elevated diffusion in relation to cognitive performance, especially in the SS.

Lack of Age by HIV Effect on White Matter Microstructures

Whether HIV infection accelerates the aging effect on white matter microstructural degeneration remains controversial. The findings from the current study is consistent with some (Towgood et al. 2012; Nir et al. 2014) but not other previous studies (Chang et al. 2008; Seider et al. 2016), showing relatively normal aging effect on white matter microstructures. Prior studies were primarily cross-sectional studies (Towgood et al. 2012; Nir et al. 2014; Seider et al. 2016), and are inadequate to detect the age-related changes over time. For example, one study that evaluated HIV+ participants over time showed a greater increase in MD in the genu of the CC than that in SN controls over a year period, but the age-by-HIV effect was not significant when the baseline DTI values were analyzed cross-sectionally (Chang et al. 2008). Therefore, due to the large intersubject variability across participants, longitudinal follow-up studies using DTI to monitor age-related changes in brain microstructure in HIV+ participants compared to those in SN controls matched for various clinical and substance use variables (including tobacco smoking) are needed.

Limitations

A main limitation of this study is the cross-sectional design which limited the interpretation of a causal relationship between HIV-infection and / or chronic tobacco smoking on white matter microstructure. Although it would be extremely difficult to evaluate individuals before they are infected with HIV or before they start smoking, and follow them afterwards, longitudinal studies of HIV+ individuals with or without tobacco smoking may demonstrate whether chronic tobacco smoking or HIV-infection would accelerate white matter alterations and cognitive decline over time. Another limitation of this study is the highly prevalent co-morbid substance use, similar to the reported prevalence of alcohol (95% lifetime, 62% last month) and marijuana use (75% lifetime, 25% last month) in HIV positive individuals (Shiau et al. 2017), which may confound the DTI measurements. We tried to mitigate the potential confounding effects of heavy marijuana or alcohol use on the DTI metrics by excluding individuals with moderate or severe alcohol or marijuana use disorders, and ensuring that their usage patterns were similar across groups. Despite the higher prevalence of lifetime use marijuana or alcohol use in the HIV+ participants, covarying for these variables did not change our findings. Other potential confounding factors on the DTI measurements, such as other cardiovascular risk factors (e.g., fast blood glucose level) (Nakamoto et al. 2012) and genetic vulnerability (Hoare et al. 2013; Chang et al. 2014; Singh et al. 2017) were not considered in this study. We also did not exclude individuals with second hand smoke exposure; however, if we had excluded the non-smokers exposed to secondhand smoke, we might have found even stronger tobacco effects in the smoker groups. Lastly, the HIV-infected population in Hawaii is predominantly male and White/Asian; therefore, our findings may not be generalizable to the HIV-infected population in the United States or other countries. The very few women and small proportion of African Americans also did not allow us to assess possible additional group differences due to gender or race/ethnicity on these DTI measurements.

Conclusions

Chronic tobacco smoking and HIV+ infection appear to have additive and synergistic adverse effects on white matter microstructure and cognitive function. The etiology of this additive deleterious effect of greater diffusivity is unclear; however, tobacco smoking's possible proinflammatory effects, the dysmyelination effect and its indirect effects on the cerebrovasculature all may contribute to the additional white matter microstructural abnormalities in our HIV+ Smokers. The poorer cognitive performance in the HIV+ Smokers with abnormally higher brain diffusivity also suggests that chronic tobacco-smoking may be a risk factor of HAND. Therefore, effective smoking cessation programs are important to prevent further neuroinflammation and additional risk for HAND in HIV+ individuals.

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Fig. 1.

a The white matter regions of interest with HIV main effects (left to right) are shown: SCC = Splenium of the Corpus Callosum, CGC = Cingulum Cingulate Gyrus, SFO = Superior Fronto-Occipital Fasciculus, GCC = Genu of Corpus Callosum, ALIC = Anterior Limb of Internal Capsule. **b** HIV+ participants had higher axial diffusivities than SN participants in these white matter regions (GCC, SCC, CGC, ALIC, and SFO). **c** Radial diffusivity in the CGC was higher in HIV+ than SN control participants, with or without tobacco smoking. **d** Mean diffusivity in four regions (GCC, SCC, CGC, ALIC) was higher in HIV+ than controls. Only AD in GCC remained significant after Holm correction. SN=Seronegative Nonsmokers, SN_S = Seronegative_Smokers, HIV = HIV + Nonsmokers, HIV + S=HIV + Smokers

Fig. 2.

a White matter brain regions that show Tobacco-Smoking effects or Smoking-by-HIV Interaction Effects are shown (left to right): SS=Sagittal Striatum, SCC=Splenium of the Corpus Callosum, CGC=Cingulum Cingulate Gyrus, SFO=Superior Fronto-Occipital Fasiculus, BCC=Body of Corpus Callosum, GCC = Genu of Corpus Callosum, ACR = Anterior Corona Radiata, Put = Putamen. **b & c** Bargraphs showing Smokers typically had lower FA and higher axial diffusivity (AD) than nonsmokers in these white matter regions. **d & e** Smokers also had higher radial (RD) and mean diffusivities (MD) across all these brain regions; however, in the SS and SFO, only HIV+ Smokers had higher diffusivities than SN_Smokers, while HIV had similar diffusivities as the SN (interaction effects). **f** While HIV+ Smokers had higher FA than HIV + Nonsmokers, SN smokers had lower FA than SN-Nonsmokers in the putamen. Asterisks indicate p-values that remained significant after Holm correction for multiple comparisons. SN=Seronegative Nonsmokers, SN_S=Seronegative_Smokers, HIV = HIV+ Nonsmokers, HIV + S=HIV+ Smokers

Fig. 3.

Brain regions with abnormal white matter diffusivity predicted performance on the motor function, learning, fluency, attention and speed. **a-b** Slower Fine Motor Performance is predicted by higher diffusivity in the Genu of the Corpus Callosum (GCC_AD) and Sagittal Striatum (SS_AD). **c-d** Poorer Learning is predicted by higher diffusivity in the Anterior Corona Radiata (ACR_AD) and Splenium of the Corpus Callosum (SCC_RD). **e-g** Higher axial diffusivity in the Genu of the corpus callosum (GCC_AD) predicted Slower Speed of Information Processing, Fluency and Attention only in the HIV + Smoker group but not in the other groups (group-interaction- p values = 0.001–0.003). SN=Seronegative Nonsmokers, SN_S=Seronegative Smokers, HIV = HIV+ Nonsmokers, HIV + S=HIV+ Smokers

Table 1

Participant demographics and clinical characteristics (mean $\pm$ S.E)

	SN Non smokers <i>N</i> = 25	SN-Tobacco smokers <i>N</i> = 26	HIV Non smokers <i>N</i> = 25	HIV + Tobacco smokers <i>N</i> = 24	p-value
Age (years)	43.8 $\pm$ 2.3	44.2 $\pm$ 1.8	47.4 $\pm$ 2.4	45.1 $\pm$ 2.4	0.656 ^a
Age range	(23.8–60.2)	(25.9–59.1)	(24.9–74.5)	(22.2–64.8)	
# Men (%)	22 (88.0%)	24 (92.3%)	25 (100.0)	22 (91.7%)	0.517 ^b
Education (years)	15.6 $\pm$ 0.7	13.5 $\pm$ 0.4	15.6 $\pm$ 0.4	14.0 $\pm$ 0.5	0.008^a
WTAR Predicted Verbal IQ	108.7 $\pm$ 1.7	101.4 $\pm$ 1.7	106.9 $\pm$ 1.7	101.6 $\pm$ 2.2	0.008^a
Race (W/As/B/NH/Mixed)	12/8/0/2/3	10/6/1/3/6	13/6/2/3	13/4/1/1/5	0.950 ^c
Index of Social Position (8–66)	36.0 $\pm$ 3.3	41.6 $\pm$ 2.1	36.7 $\pm$ 3.1	44.3 $\pm$ 3.1	0.138 ^a
CES-D scale score (0–60)	5.1 $\pm$ 0.7	11.4 $\pm$ 1.9	11.9 $\pm$ 2.0	18.0 $\pm$ 2.1	<0.001^a
# On antidepressants (%)	2 (8.0%)	4 (15.4%)	9 (36.0%)	10 (41.7%)	0.016^b
HIV disease-related					
Duration (months)	-	-	195.2 $\pm$ 51.8	129.1 $\pm$ 26.7	0.257 ^d
# with Detectable HIV RNA (>40 copies/mL, %)	-	-	8 (33.3%)	3 (12.5%)	0.170 ^b
Log plasma HIV RNA Plasma HIV RNA (copies/mL) *	-	-	2.1 $\pm$ 0.2 9641 $\pm$ 8225	1.6 $\pm$ 0.1 142 $\pm$ 97	0.063 ^e
# (%) on antiretrovirals	-	-	23 (92.0%)	23 (95.8%)	0.051 ^d
CD4 count (#/mm ³)	-	-	483 $\pm$ 46.9	545.4 $\pm$ 45.4	1.0 ^b
Nadir CD4 count (#/mm ³)	-	-	195.4 $\pm$ 26.3	258.3 $\pm$ 50.3	0.344 ^e
HIV dementia Scale (0–16)	-	-	14.6 $\pm$ 0.4	12.7 $\pm$ 0.8	0.268 ^e
Karnofsky score (0–100)	-	-	93.0 $\pm$ 1.4	93.0 $\pm$ 1.8	0.052 ^e
# (%) with HAND or equivalent **	6 (24.0%)	9 (34.6%)	7 (28.0%)	13 (54.2%)	0.985 ^e
Tobacco usage, Median (range)					0.125 ^b
Daily average use (# cigarettes)	-	17.9, (5–40)	-	16.4, (3.0–40.0)	0.716 ^e
Total lifetime use (pack years)	-	20.1 (1.3–59.3)	-	22.8 (3.6–68.0)	0.725 ^e

	SN Non smokers <i>N</i> = 25	SN-Tobacco smokers <i>N</i> = 26	HIV Non smokers <i>N</i> = 25	HIV + Tobacco smokers <i>N</i> = 24	p-value
Duration of tobacco use (years)	-	24.9 (3.2–41.1)	-	26.4 (3.8–45.6)	0.799 ^d
Marijuana usage, Median (range)					
# Lifetime marijuana users (%)	13 (52.0%)	15 (57.7%)	20 (80.0%)	22 (91.7%)	0.006^b
# Users in the past month (%)	3 (12.0%)	6 (23.1%)	7 (28.0%)	7 (29.2%)	0.462 ^b
Daily average use (g) ^f (range)	0.02 (0.001–7.1)	0.16 (0.01–0.8)	0.04 (0.0002–1.5)	0.12 (0–3.5)	0.460 ^f
Total lifetime use (kg) ^f (range)	0.01 (0.0003–51.8)	0.7 (0.0008–4.7)	0.04 (0.0003–14.7)	0.7 (0–39.3)	0.214 ^f
Duration of use (year) ^f (range)	3.0 (0.01–29.3)	19.8 (0.003–34.7)	11.0 (0.003–44.0)	11.4 (0.01–41.1)	0.381 ^a
Duration of abstinence (years) ^f	12.2 (0–39.8)	1.2 (0–29.9)	2.2 (0–30.4)	1.1 (0–39.7)	0.194 ^a
Alcohol usage, Median (range)					
# Lifetime Alcohol users (%)	21 (84.0%)	15 (57.7%)	19 (76.0%)	23 (95.8%)	0.010^b
# Users in the past month (%)	14 (56.0%)	8 (30.8%)	9 (36.0%)	9 (37.5%)	0.282 ^b
Daily average use (mL) ^f	8.5 (0.1–59.8)	12.0 (0.2–152.6)	9.2 (1.2–127.5)	9.3 (0.04–222.0)	0.553 ^f
Total lifetime use (Liter) ^f (range)	65.8 (0.004–596.4)	68.4 (2.2–1533.0)	86.0 (9.5–640.9)	53.1 (0.33–2491.4)	0.798 ^f
Duration of use (years) ^f	24.1 (0.1–44.4)	28.5 (4.1–40.8)	21.3 (1.0–40.7)	22.5 (5.1–46.6)	0.348 ^a
Since last Alcohol use (Days) ^f	0 (0–12,926)	20 (0–2002)	30 (0–9235)	90 (0–3666)	0.270 ^f

Index of Social Position: assessed using the Hollingshead Four Factor Index of Social Position. *p*-values < 0.05 are bolded. *WTAR*, Wechsler Test of Adult Reading; *CES-D*, Center for Epidemiological Studies - Depression Scale; *Race*, White/ Asian/ Black/Native Hawaiian/ More than one race

^aANOVA

^b χ^2

^cFisher's Exact test

^dMann-Whitney U

^eT test

^fKruskal-Wallis test

* Plasma HIV RNA was calculated from 8 (HIV+) and 3 (HIV+ Smoker) participants with detectable viruses

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HAND = HIV-associated Neurocognitive Disorder - Subgroup diagnosis: HIV + Non-Smoker (5 ANI, 1 MND, 1 HAD), HIV + Smoker (3 ANI, 5 MND, 5 HAD); or HAND-equivalent: SN (6 ANI), SN smoker (8 ANI and 1 MND)

##

These variables were calculated only among the users

Table 2

Cognitive domain Z-scores of the study participants (Mean $\pm$ S.E.) and *p* values from two-way ANOVA

	SN Non-smokers (N = 25)	SN Tobacco smokers (N = 26)	HIV+ Non smokers (N = 25)	HIV+ Tobacco smokers (N = 24)	ANOVA p-values	
					HIV effect	Tobacco smoking effect
Fluency	0.36 $\pm$ 0.18	0.03 $\pm$ 0.15	0.01 $\pm$ 0.16	-0.26 $\pm$ 0.20	0.049	0.070
Executive Function	0.18 $\pm$ 0.18	-0.25 $\pm$ 0.17	-0.22 $\pm$ 0.20	-0.64 $\pm$ 0.22	0.031	0.019
Speed	-0.07 $\pm$ 0.14	-0.23 $\pm$ 0.13	-0.28 $\pm$ 0.13	-0.63 $\pm$ 0.19	0.043	0.091
Attention / Working Memory	-0.02 $\pm$ 0.19	-0.39 $\pm$ 0.14	-0.50 $\pm$ 0.19	-0.63 $\pm$ 0.20	0.036	0.128
Learning	0.11 $\pm$ 0.18	-0.30 $\pm$ 0.16	-0.13 $\pm$ 0.20	-0.70 $\pm$ 0.24	0.133	0.019
Memory	0.13 $\pm$ 0.18	-0.13 $\pm$ 0.14	-0.09 $\pm$ 0.19	-0.65 $\pm$ 0.21	0.063	0.038
Motor	-0.05 $\pm$ 0.19	-0.16 $\pm$ 0.14	-0.28 $\pm$ 0.19	-0.79 $\pm$ 0.25	0.036	0.140
Global	0.08 $\pm$ 0.13	-0.20 $\pm$ 0.10	-0.18 $\pm$ 0.14	-0.61 $\pm$ 0.19	0.016	0.016

Z-Scores for each domain for each participant are derived from a normative database of 507 seronegative healthy controls, studied with the same cognitive tests below (adjusted for age and education)

Fluency, DKEFS or Ruff Figural Design Fluency and Verbal Fluency (with letters FAS); *Executive Functions*, DKEFS CWI or Stroop Interference and Trail Making Test B; *Speed of Information Processing*, Symbol Digit, DKEFS Trail-making Number Sequencing or Trail Making Test A, DKEFS Color naming or Stroop Color Naming, and California Computerized Assessment Package (CalCAP) Simple Reaction Time; *Attention/Working Memory*, Arithmetic from Wechsler Adult Intelligence Scale-VI, Digit Span Backward, Letter-Number Sequencing, Arithmetic and Paced Auditory Serial Addition Test 1; *Learning*, Rey Auditory Verbal Learning Test Trial 5 and Rey-Osterreith Complex Figure Test-Immediate Recall; *Memory*, Rey Auditory Verbal Learning Test Delayed Recall (Trial 7) and Rey Complex Figure-Delayed Recall; *Motor Skills*, Grooved Pegboard Dominant and Non-dominant hands. *p*-values <0.05 are bolded

Table 3

HIV-infection and tobacco-smoking effects on fractional anisotropy (Mean $\pm$ SE)

	SN Non-smoker (N = 23)	SN Tobacco smoker (N = 25)	HIV + Nonsmoker (N = 25)	HIV tobacco smoker N = 21	2-way ANCOVA <i>p</i> -values		
					HIV effect	Tobacco smoking effect	HIV x tobacco interaction
GCC	0.54 $\pm$ 0.006	0.53 $\pm$ 0.007	0.54 $\pm$ 0.006	0.51 $\pm$ 0.013	ns	0.007	ns
BCC	0.47 $\pm$ 0.006	0.47 $\pm$ 0.006	0.47 $\pm$ 0.007	0.45 $\pm$ 0.008	ns	0.034	ns
SCC	0.56 $\pm$ 0.004	0.56 $\pm$ 0.004	0.57 $\pm$ 0.004	0.55 $\pm$ 0.006	ns	0.012	ns
ACR	0.39 $\pm$ 0.004	0.39 $\pm$ 0.003	0.4 $\pm$ 0.003	0.39 $\pm$ 0.004	ns	0.047	ns
CGC	0.38 $\pm$ 0.003	0.38 $\pm$ 0.003	0.38 $\pm$ 0.003	0.37 $\pm$ 0.004	ns	0.03	ns
Put	0.35 $\pm$ 0.001	0.34 $\pm$ 0.001	0.34 $\pm$ 0.002	0.35 $\pm$ 0.002	ns	ns	0.024*

All uncorrected *p*-values <0.05 from two-way ANCOVA are shown

* *p* value remained significant after Holm correction for multiple tests; *ns*, not significant (*p* > 0.05)

GCC, Genu of corpus callosum; BCC, Body of corpus callosum; SCC, Splenium of corpus callosum; ACR, Anterior corona radiate; CGC, Cingulum Cingulate Gyrus; Put, Putamen

HIV-infection and tobacco-smoking effects on brain diffusivities (Mean $\pm$ SE, unit $\times 1000 \text{ m}^2/\text{s}$)

Table 4

Brain region	SN Non-smoker (N = 23)	SN tobacco smoker (N = 25)	HIV + Nonsmoker (N = 25)	HIV + Tobacco smoker (N = 21)	2-way ANCOVA <i>p</i> -values		
					HIV effect	Tobacco smoking effect	HIV x tobacco interaction
GCC	MD	0.89 $\pm$ 0.007	0.9 $\pm$ 0.007	0.95 $\pm$ 0.02	0.013	0.003 *	ns
	RD	0.58 $\pm$ 0.008	0.59 $\pm$ 0.008	0.63 $\pm$ 0.02	ns	0.006	ns
	AD	1.5 $\pm$ 0.008	1.51 $\pm$ 0.009	1.55 $\pm$ 0.014	0.002 *	0.024	ns
BCC	RD	0.68 $\pm$ 0.009	0.69 $\pm$ 0.011	0.72 $\pm$ 0.012	ns	0.029	ns
	MD	0.87 $\pm$ 0.006	0.88 $\pm$ 0.007	0.88 $\pm$ 0.006	0.038	0.001 *	ns
SCC	RD	0.55 $\pm$ 0.005	0.56 $\pm$ 0.007	0.55 $\pm$ 0.006	ns	0.001 *	ns
	AD	1.51 $\pm$ 0.011	1.53 $\pm$ 0.009	1.53 $\pm$ 0.01	0.016	0.005	ns
	MD	0.78 $\pm$ 0.006	0.8 $\pm$ 0.008	0.79 $\pm$ 0.007	ns	0.002 *	ns
ACR	RD	0.6 $\pm$ 0.006	0.61 $\pm$ 0.007	0.6 $\pm$ 0.007	ns	0.003	ns
	AD	1.14 $\pm$ 0.006	1.16 $\pm$ 0.01	1.16 $\pm$ 0.009	ns	0.002 *	ns
	MD	0.71 $\pm$ 0.006	0.72 $\pm$ 0.006	0.71 $\pm$ 0.005	ns	0.011	ns
SCR	RD	0.54 $\pm$ 0.006	0.55 $\pm$ 0.006	0.55 $\pm$ 0.005	ns	0.034	ns
	AD	1.05 $\pm$ 0.007	1.05 $\pm$ 0.007	1.05 $\pm$ 0.007	ns	0.01	ns
	MD	0.79 $\pm$ 0.007	0.8 $\pm$ 0.008	0.79 $\pm$ 0.006	ns	0.03	ns
PCR	AD	1.15 $\pm$ 0.01	1.16 $\pm$ 0.01	1.15 $\pm$ 0.009	ns	0.02	ns
	MD	0.83 $\pm$ 0.006	0.83 $\pm$ 0.007	0.84 $\pm$ 0.007	0.014	0.037	ns
	RD	0.65 $\pm$ 0.006	0.66 $\pm$ 0.007	0.67 $\pm$ 0.007	0.015	0.028	ns
CGC	AD	1.18 $\pm$ 0.008	1.18 $\pm$ 0.009	1.19 $\pm$ 0.009	0.037	ns	ns
	MD	0.75 $\pm$ 0.006	0.76 $\pm$ 0.007	0.76 $\pm$ 0.006	0.049	0.012	ns
	RD	0.54 $\pm$ 0.006	0.55 $\pm$ 0.007	0.55 $\pm$ 0.006	ns	0.021	ns
ALIC	AD	1.17 $\pm$ 0.007	1.18 $\pm$ 0.009	1.19 $\pm$ 0.007	0.019	0.019	ns
	MD	0.84 $\pm$ 0.006	0.84 $\pm$ 0.006	0.83 $\pm$ 0.006	ns	0.005	0.037
	RD	0.63 $\pm$ 0.006	0.64 $\pm$ 0.006	0.64 $\pm$ 0.005	ns	0.024	ns
SS	AD	1.24 $\pm$ 0.007	1.24 $\pm$ 0.008	1.22 $\pm$ 0.009	ns	0.001 *	0.002 *
	MD	0.74 $\pm$ 0.008	0.74 $\pm$ 0.008	0.74 $\pm$ 0.006	ns	0.004	0.038

Brain region	SN Non-smoker (N = 23)	SN tobacco smoker (N = 25)	HIV + Nonsmoker (N = 25)	HIV + Tobacco smoker (N = 21)	2-way ANCOVA <i>p</i> -values		
					HIV effect	Tobacco smoking effect	HIV x tobacco interaction
PTR	RD	0.56 ± 0.007	0.57 ± 0.007	0.56 ± 0.006	ns	0.001 *	0.02 *
	AD	1.1 ± 0.011	1.1 ± 0.010	1.11 ± 0.009	0.015	0.025	ns
	MD	0.85 ± 0.006	0.86 ± 0.007	0.85 ± 0.008	ns	0.011	ns
	RD	0.62 ± 0.007	0.63 ± 0.007	0.62 ± 0.008	ns	0.007	ns
Tha	MD	0.75 ± 0.004	0.75 ± 0.004	0.75 ± 0.004	ns	0.036	ns
	RD	0.58 ± 0.004	0.58 ± 0.003	0.58 ± 0.003	ns	0.033	ns
	AD	1.08 ± 0.005	1.09 ± 0.007	1.08 ± 0.007	ns	0.037	ns

All uncorrected *p*-values < 0.05 from two-way ANCOVA are shown. ns = not significant (*p* > 0.05)

GCC, Genu of corpus callosum; *BCC*, Body of corpus callosum; *SCC*, Splenium of corpus callosum; *ACR*, Anterior corona radiata; *SCR*, Superior corona radiata; *PCR*, Posterior corona radiata; *COC*, Cingulum Cingulate Gyrus; *ALIC*, Anterior limb of internal capsule; *SS*, Sagittal stratum; *SFO*, Superior fronto-occipital fasciculus; *PTR*, Posterior thalamic radiation (include optic radiation); *Tha*, Thalamus

* *p* values remained significant after Holm correction for multiple comparisons

Table 5

Regional DTI metrics (with significant HIV or Smoking effects) predicted cognitive performance (p values)

Regional DTI		Fluency	Executive function	Speed of information processing	Attention	Learning	Memory	Motor	Global Z-score
Domain Z-score									
p-values from linear regression across all groups									
GCC	AD	ns	0.026	0.006	ns	0.005	0.003	<0.001*	<0.001*
	MD	0.019	0.001*	<0.001*	0.013	<0.001*	0.002	<0.001*	<0.001*
SCC	RD	ns	0.042	0.050	ns	<0.001*	0.004	0.014	0.002
	MD	0.01	0.005	0.015	0.005	<0.001*	0.001*	<0.001*	<0.001*
ACR	AD	ns	ns	0.013	0.016	0.001*	0.002	0.009	<0.001*
	MD	ns	0.022	0.003	0.014	<0.001*	<0.001*	0.011	<0.001*
SS	AD	ns	0.041	0.044	0.012	0.002	0.004	<0.001*	<0.001*
	RD	ns	0.018	0.002	0.017	0.011	ns	0.015	0.001
p-values from one-way ANCOVA showing interactions									
GCC	AD	0.002*	ns	0.001*	0.003*	ns	ns	ns	0.002*
	MD	ns	ns	ns	0.02	ns	ns	ns	ns
SCC	RD	ns	ns	ns	ns	ns	0.022	ns	ns
	MD	ns	ns	ns	ns	ns	ns	ns	ns
ACR	AD	ns	ns	0.050	0.03	ns	ns	ns	ns
	MD	ns	ns	ns	0.047	ns	ns	ns	ns
SS	AD	ns	0.044	ns	0.037	0.029	ns	ns	0.022
	RD	ns	ns	0.007	0.014	0.038	0.011	ns	0.019

* p-values that remained significant after Holm correction for multiple tests, ns = not significant

GCC, Genu of corpus callosum; SCC, Splenium of corpus callosum; ACR, Anterior corona radiata; SS, Sagittal stratum; SFO, Superior fronto-occipital fasciculus