

Microstructural Brain Abnormalities in HIV+ Individuals with or without Chronic Marijuana Use

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Research

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Abstract

Objective

Cognitive deficits and microstructural brain abnormalities are well documented in HIV-positive individuals (HIV+). This study evaluated whether chronic marijuana (MJ) use contributes to additional cognitive deficits or brain microstructural abnormalities that may reflect neuroinflammation or neuronal injury in HIV+.

Method:

Using a 2 × 2 design, 44 HIV + participants, [23 minimal/no MJ users (HIV+), 21 chronic active MJ users (HIV + MJ)], were compared to 46 seronegative participants [24 minimal/no MJ users (SN) and 22 chronic MJ users (SN + MJ)] on neuropsychological performance (7 cognitive domains) and diffusion tensor imaging metrics, using an automated atlas to assess fractional anisotropy (FA), axial (AD), radial (RD), and mean (MD) diffusivities, in 18 cortical and 4 subcortical brain regions,

Results

Compared to SN and regardless of MJ use, the HIV + group had lower FA and higher diffusivities in multiple white matter and subcortical structures ($p = 0.001-0.050$), as well as poorer cognition in Fluency ($p = 0.039$), Attention / Working Memory ($p = 0.009$), Learning ($p = 0.015$) and Memory ($p = 0.028$). Regardless of HIV-serostatus, MJ users had lower AD in uncinate fasciculus ($p = 0.016$) but similar cognition as non-users. No additive or interactive effects were found between HIV-serostatus and MJ use on DTI metrics or cognitive function. Furthermore, higher MD in thalamus predicted poorer fluency, learning and memory in HIV + participants, while higher RD in posterior corona radiata predicted poorer learning in MJ users. Lower FA in the anterior internal capsule also predicted worse attention/working memory in all except SN subjects. Lastly, MJ users with or without HIV-infection showed greater than normal age-dependent FA declines in superior longitudinal fasciculus, external capsule and globus pallidus.

Introduction

HIV-infection is associated with chronic neuroinflammation, which contributes to cognitive dysfunction (1) and various brain structural and functional abnormalities in people living with HIV (PLWH) (2). Cannabis, or marijuana (MJ), is the most commonly abused illicit drug worldwide and in the U.S. (3), and is used much more often in PLWH than in the general population (26.4% vs.16%) (4, 5). With the legalization of cannabis for recreational MJ use in many states in the U.S. and in other countries, the prevalence of MJ use amongst PLWH has continued to increase (4).

Despite the highly prevalent MJ use by PLWH, data regarding whether HIV-infection and MJ use may lead to additive or interactive effects on brain function or pathology remain scant (6) and controversial. Several studies found no independent or additive effects on cognitive deficits with chronic MJ users with or without

HIV-infection (7–9). However, MJ use in PLWH was also found to have worse motor learning deficits (10), as well as better verbal fluency and learning (11).

Similar inconsistent findings were reported in MJ users without HIV-infection. Chronic MJ users showed poorer learning, memory, attention, executive planning and lower intelligence quotient (IQ) (12–14). However, deficits in learning and memory normalized within a month after abstinence from MJ use (12, 13). Further, MJ users did not show greater cognitive decline compared with nonusers (15, 16), except for those with adolescent onset of MJ use (12, 14). In addition, chronic MJ use may lead to apathy and lack of motivation (17). Although chronic MJ use may suppress the immune system (18), MJ use in PLWH did not influence viral suppression by cART (19), adherence to cART (20) or mortality (21).

Few neuroimaging studies evaluated the combined effects of HIV-infection and MJ use, but the findings were variable. For instance, chronic MJ use in PLWH did not show additional effects on brain atrophy (8), but had interactive effects on brain glutamate levels on proton MR spectroscopy (7). HIV + MJ smokers also showed greater brain activation in frontal-insular regions effects compared to HIV + individuals or MJ users. (22) Several diffusion tensor imaging (DTI) studies evaluated MJ users, and found disrupted microstructural integrity in corpus callosum (CC), superior longitudinal fasciculus (SLF), thalamic radiation and uncinate fasciculus (UNC), as well as abnormal structural connectivity to the orbitofrontal cortex (OFC) (23–26). Conversely, no group difference between MJ users and nonusers on white matter integrity was also reported (27). Whether chronic MJ use influences brain microstructure in PLWH is unknown.

Therefore, the current study evaluated whether brain microstructure differs between HIV + individuals with and without chronic active MJ use ($\geq 3x/\text{week}$ for past 2 years or longer). We hypothesize that: 1) Consistent with prior reports (7–9), HIV + individuals but not MJ users would have poorer cognitive function compared to seronegative non-MJ users (SN), with no interactive or synergistic effects between HIV + and MJ use on cognitive performance. 2) Based on aforementioned DTI studies, HIV + subjects would show lower FA and higher diffusivities (AD, RD and MD) compared to SN, while MJ users would show minimal or no abnormalities, on DTI in the major fiber tracts and subcortical gray matter. Hence, we expected HIV + MJ users to show minimal or no additional effects on DTI metrics in these brain regions.

Methods

Participants

Using a 2×2 design, 90 participants (ages 18–70 years), including 24 SN, 22 SN with chronic MJ use (SN + MJ), 23 HIV + participants with minimal or no MJ use (HIV+), and 21 HIV + with chronic active MJ use (HIV + MJ), were included in this study. All participants were recruited from the local community, by referrals, on-line advertisements, or flyer postings, and were screened initially by telephone. 330 individuals were screened initially; 182 (55%) potentially eligible participants were invited for further in-person screening. Each signed a written consent form after being verbally informed of the study aims and requirements. The protocol and the consent form were approved by the Cooperative Institutional Review Board of the

University of Hawaii and The Queen's Medical Center, and was Health Insurance Portability and Accountability Act (HIPAA) compliant.

Each participant was additionally screened with detailed medical and drug use histories, medical records reviews, and underwent physical and neuropsychiatric examinations by trained research staff and physicians to ensure they fulfilled the study criteria. 127 (70%) participants fulfilled all study criteria, but only 90 completed the study. They were men or women of any ethnicity, aged 18–70 years, and able to provide informed consent. SN participants were negative on the ClearView® COMPLETE HIV-1/2 test. HIV+ participants fulfilled these inclusion criteria: 1) HIV seropositive (with documentation from medical records); 2) stable on antiretroviral regimen for 6 months or were without antiretroviral treatments for the study duration. MJ participants fulfilled these inclusion criteria: 1) chronic MJ use (> 3 times/week for > 2 years); 2) negative urine toxicology screen for other drugs of abuse (methamphetamine, amphetamine, cocaine, benzodiazepine, barbiturates, and opiates, except for false positive tests from prescribed medications). Exclusion criteria for all participants were similar to those reported previously:(7) 1) history of co-morbid major psychiatric illness; 2) any confounding neurological disorder; 3) significantly abnormal laboratory tests (> 2 standard deviations); 4) moderate to severe substance use disorders (SUD) within the previous 2 years (DSM-5 SUD criteria, other than marijuana and/or tobacco use disorders); 5) positive urine toxicology screen on the day of visit (except for Δ 9-tetrahydrocannabinol or Δ 9-THC in the MJ users); 6) pregnancy; 7) inability to read at the 8th grade level (on Wechsler Test of Adult Reading); 8) contraindications for MRI studies.

Image Acquisition and Processing

All participants were scanned on a 3 T Siemens TIM Trio scanner (Siemens Medical Solutions, Erlangen, Germany). After a localizer, a sagittal 3-D magnetization-prepared rapid gradient-echo scan (TR/TE/TI = 2200/4.47/1000 ms; 1 mm isotropic resolution) and an axial fluid attenuated inversion recovery scan (FLAIR, TR/TE = 9100/84 ms, 3-mm slice thickness, 44 slices) were performed. All structural MR scans were reviewed by an experienced Neurologist (L.C.) to evaluate for any confounding gross structural abnormalities. Five of the 90 total participants had minor structural abnormalities: One had a small area of encephalomalacia in the right posterior parietal region; the second had several small gliotic lesions from old toxoplasma lesions in the anterior frontal lobes, the anterior cingulate cortex and thalamus; the third had a small old lacunar infarct in the ponto-cerebellar junction; the fourth had some small areas of white matter hyperintensities in the U-fibers in the parieto-occipital region; and the fifth had a small area of hyperintense signal at the right frontal and temporal lobe juncture along the Sylvian fissure. These abnormalities did not significantly impact the selected regions of interest (ROIs), based on comparisons of our findings with or without these 5 participants' data. DTI scans were performed with $b = 0$ and 12 directions at 1000s/mm^2 , TR/TE = 3700/88 ms, resolution $1.7 \times 1.7\text{mm}^2$, 4 mm axial slices with 1-mm gap, 4 repetitions.

Following motion correction (28), the tensor field for each individual brain was calculated using DTIStudio (www.MriStudio.org) and automatically fit to JHU-MNI atlas space using Large Deformation Diffeomorphic Metric Mapping (28, 29). Fractional anisotropy (FA), and axial (AD, first eigenvalue) and radial diffusivity (RD, mean of second and third eigenvalues), were measured in anatomical regions defined in the JHU-MNI

atlas (30). Based on prior DTI studies that demonstrated regional white matter abnormalities in MJ users. (23–26) and in HIV-infected individuals,(2) FA, AD, and RD were assessed in the following 11 major white matter structures (18 including the 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), SLF, superior fronto-occipital fasciculus (SFO), inferior fronto-occipital fasciculus (IFO), internal capsule (anterior and posterior limbs and retrolenticular part; or ALIC, PLIC and RLIC), external capsule (EC), posterior thalamic radiation (PTR), UNC and cingulum (connecting to the cingulate gyrus and to the hippocampus; or CGC and CGH). Due to excessive high proportion of crossing fibers, only FA and mean diffusivity (MD, average of the three eigenvalues) were assessed in the four subcortical regions (caudate, putamen, globus pallidus and thalamus).

Neuropsychological Testing

Cognitive function was assessed in 7 domains:1) Learning was assessed with the Rey Auditory Verbal Learning Test (RAVLT, immediate recall) and the Rey-Osterreith Complex Figure Test (RCFT, immediate recall). 2) Memory was assessed with the RAVLT (delayed recall) and RCFT (delayed recall). 3) Executive function was assessed with the Delis-Kaplan Executive Function System (D-KEFS) Stroop Color-Word Interference Test and Trail-making (Number-Letter Switching). 4) Attention/Working memory was assessed with the Wechsler Adult Intelligence Scale-Fourth Edition and Wechsler Memory Scale-Fourth Edition. 5) Speed of Information Processing was assessed with the D-KEFS Trail-making (Number Sequencing) Test, D-KEFS Stroop Color Naming Test, the mean simple reaction time from the California Computerized Assessment Package. 6) Design and Verbal Fluency were assessed with D-KEFS Design Fluency Test and D-KEFS Controlled Oral Word Association Test. 7) Fine Motor Skill was assessed with the Grooved Pegboard Test. All z-scores, adjusted for age and education, were generated with a normative database from 547 SN healthy participants who were administered the same tests in a standardized manner in the same laboratory.

Statistics

All analyses were performed using R (version 3.5.2 <https://www.R-project.org/>). One-way analysis of variance (ANOVA), Chi-square, Mann-Whitney Test, Kruskal-Wallis Test and Fisher's Exact Test were used to compare the demographic measures and clinical variables depending on the variable types and distributions. Two-way analyses of co-variance (ANCOVAs) were performed to evaluate the independent and interactive effects of HIV-serostatus and chronic active MJ use across the four groups, on the cognitive domain Z-scores and on DTI metrics in the 22 ROIs. Two-way ANCOVAs for cognitive domain Z-scores did not include covariates since the Z-scores were adjusted for age and education levels. However, 2-way ANCOVA models for DTI metrics included age and Index of Social Position [calculated from the Hollingshead Four-factor index of social status (31)], as covariates. A p-value < 0.05 was considered significant for cognitive domain z-scores. However, ROI-based analyses on DTI were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Since adding the lifetime marijuana use or lifetime regular alcohol use as covariates did not change the DTI findings, they were removed from the final models.

Exploratory correlations were performed between DTI metrics or cognitive domain z-scores that showed group differences, using the following general linear models: cognitive domain z-scores as dependent variables; DTI metrics, HIV-status, MJ use-status and their 2-way and 3-way interactions as independent variables; and age as a covariate. Similar methods were used to explore the correlations between DTI metrics and age, HIV-related clinical variables and MJ use patterns.

Results

Participant Characteristics (Table 1)

Table 1
Participant Demographics and Clinical Characteristics (mean ± S.E.)

	SN Nonusers (N = 24)	SN-MJ user (N = 22)	HIV Nonusers (N = 23)	HIV + MJ user (N = 21)	p-value
Age (years)	44.6 ± 2.8	45.3 ± 2.1	46.8 ± 2.4	46.3 ± 1.9	0.904 ^a
Age range	(18.5–68.9)	(25.9–65.8)	(28.3–70.3)	(26–60.7)	
# Men (%)	21 (87.5%)	19 (86.4%)	22 (95.7%)	20 (95.2%)	0.567 ^b
Education (years)	14.8 ± 0.5	13.7 ± 0.5	15.2 ± 0.4	14.2 ± 0.5	0.167 ^a
WTAR Predicted Verbal IQ	109.8 ± 1.6	106.4 ± 2.1	107.9 ± 1.6	103.7 ± 1.9	0.103 ^a
Race (W/As/B/NH/NA/Mixed)	12/4/1/1/1/5	13/0/1/1/0/3	11/7/1/0/0/5	13/3/1/1/0/4	0.629 ^c
Index of Social Position (8–66)	36.3 ± 3.4	40.2 ± 3.8	35.8 ± 3.3	44.8 ± 3.6	0.233 ^a
CES-Depression score (0–60)	5.9 ± 0.9	13.0 ± 3.3	14.3 ± 2.0	13.0 ± 1.9	0.015^a
HIV disease-related					
Duration (months)	-	-	327.4 ± 29.9	339.4 ± 31.5	0.837 ^e
# With Detectable HIV RNA (> 40 copies/mL, %)	-	-	3 (13.0%)	4 (19.1%)	0.488 ^b
Log plasma HIV RNA	-	-	1.8 ± 0.2	2.0 ± 0.3	0.420 ^d
Plasma HIV RNA (copies/mL)*	-	-	4,064 ± 3,970	2,933 ± 1,774	0.814 ^d
# (%) on combined antiretrovirals	-	-	23 (100%)	21 (100%)	
CD4 count (#/mm ³)	-	-	511.9 ± 42.4	563.4 ± 65.4	0.514 ^e
Nadir CD4 count (#/mm ³)	-	-	250.6 ± 46.4	218.1 ± 46.0	0.622 ^e
HIV dementia Scale (0–16)	-	-	14.3 ± 0.5	14.2 ± 0.6	0.886 ^e
Karnofsky score (0-100)	-	-	94.5 ± 1.4	92.9 ± 1.4	0.405 ^e
# (%) with HAND or equivalent**	5 (20.8%)	6 (27.3%)	8 (34.8%)	6 (28.6%)	0.705 ^b
Marijuana usage, Median (range)					

	SN Nonusers (N = 24)	SN-MJ user (N = 22)	HIV Nonusers (N = 23)	HIV + MJ user (N = 21)	p-value
Age at first use (years)	-	15.5 (8–39)	-	16 (9–40)	0.906 ^e
Daily average use (gram)	-	0.5 (0.02–3.5)	-	0.4 (0.04–3.6)	0.699 ^f
Total lifetime use (kg)	-	3.5 (0.06–54.5)	-	3.2 (0.08–39.6)	0.941 ^d
Duration of MJ use (years)	-	27.3 (1.9–45.3)	-	31.8 (3.7–41.7)	0.784 ^d
Tobacco Smoking, Median (range)					
# Lifetime Tobacco Smokers (%)	12 (50.0%)	18 (81.8%)	11 (47.8%)	17 (81.0%)	0.016^b
# Smokers in the past month (%)	9 (37.5%)	13 (59.1%)	5 (21.7%)	8 (40.0%)	0.085 ^b
Daily average use (g, range)	24.4 (0.5–76.3)	19.9 (0.002–61)	29.9 (0.01–61)	27.5 (2.0–39.3)	0.774 ^f
Total lifetime use (pack-year) ^a (range)	10.8 (0.01–39.9)	16.1 (0.0001–59.3)	14.0 (0.0002–50.4)	16.4 (0.2–37.0)	0.733 ^f
Duration of use (year, range)	14.8 (0.6–32.5)	23.9 (1–48.1)	17.9 (0.6–35.9)	25.8 (2.9–41.7)	0.241 ^f
Duration of abstinence (months) ^a	0 (0–390)	0 (0–483)	3 (0–332)	0 (0–370)	0.367 ^f
Alcohol usage, Median (range)					
# Lifetime Alcohol users (%)	22 (91.7%)	19 (86.4%)	21 (95.5%)	20 (95.2%)	0.651 ^b
Regular Use (> 1/week) in the past month (%)	6 (25.0%)	14 (63.6%)	6 (27.3%)	7 (33.3%)	0.004^b
Daily average use (mL) ^a	9.4 (0.06–48.9)	12.3 (0.4–41.8)	6.1 (0.2–63.0)	7.7 (0.4–186.9)	0.632 ^f
Total lifetime use (Liter, range)	71.5 (0.1–655)	110.3 (0.9–613)	86.8 (0.4–968)	56.6 (0.1–2491)	0.767 ^f
Duration of use (years) ^a	22.9 (2.3–46.5)	25.0 (5.7–45.5)	23.2 (9.0–46.6)	29.1 (0.9–44.8)	0.792 ^f
Duration of abstinence (months) ^a	0 (0–63)	0 (0–59)	1 (0–89)	0 (0–91)	0.197 ^f
a: ANOVA; b: χ^2 ; c: Fisher's Exact test; d: Mann-Whitney U; e: Student t-test; f: Kruskal-Wallis Test.					

	SN Nonusers (N = 24)	SN-MJ user (N = 22)	HIV Nonusers (N = 23)	HIV + MJ user (N = 21)	p-value
P-values < 0.05 are bolded					
WTAR = Wechsler Test of Adult Reading					
CES-D = Center for Epidemiological Studies – Depression Scale					
Index of Social Position: assessed using the Hollingshead Four Factor Index of Social Position					
Race = White/ Asian/ Black/Native Hawaiian/Native American/More than one race					
# Tobacco use status was missing in 5 participants; alcohol use status was missing in 3 participants.					
* Plasma HIV RNA was calculated from 7 (HIV+) and 5 (HIV + Smoker) participants with detectable viruses.					
** HIV-associated Neurocognitive Disorder (HAND) diagnoses: HIV-Nonuser (3 ANI, 3 MND, 2 HAD), HIV + MJ users (2 ANI, 2 MND, 2 HAD); or HAND-equivalent in the SN groups: SN-Nonuser (4 ANI and 1MND), SN-MJ (4 ANI and 2 MND)					
¶ These variables were calculated only among the users.					

All four groups had similar age, sex and racial distributions, socioeconomic status, years of education and predicted verbal intelligence quotient (IQ). SN had the fewest depressive symptoms on the CES-D across all four groups ($p = 0.015$). The two HIV + groups had similar duration of HIV infection, current plasma RNA levels, nadir and recent CD4 cell counts, percentage of participants on stable combination antiretroviral therapy (cART) regimens, and HIV dementia scores and Karnofsky scales. The two MJ user groups also had similar age of first MJ use, duration of MJ use, daily MJ use, and total lifetime MJ usage. Although more MJ users reported tobacco use in their lifetime ($p = 0.016$) and regular alcohol use (> once/week, $p = 0.004$) than non-MJ users in the past month, the four groups had similar total lifetime amount and duration of alcohol or tobacco use.

HIV and chronic MJ use on neuropsychological test performance (Fig. 1)

Regardless of MJ use, HIV + individuals had lower Z-scores than SN controls in the domains of Fluency (0.039), Attention/Working Memory ($p = 0.009$), Learning ($p = 0.015$), Memory ($p = 0.028$) and Global function ($p = 0.012$). Trends for similar HIV effects were found in the Executive function ($p = 0.055$) and Speed of Information Processing ($p = 0.064$) (Fig. 1). With or without HIV infection, those who chronically used MJ did not perform worse in any of these domains. Although no significant HIV-by-MJ interaction was observed in any cognitive domain, HIV + MJ users performed slightly and non-significantly better than HIV + subjects in many of the domains.

HIV-infection and chronic marijuana use on DTI metrics:

Independent of MJ use, HIV + participants had lower FA than SN controls in the internal capsule (ALIC and PLIC), cingulum (CGC and CGH), posterior thalamic radiata, superior fronto-occipital fasciculus, sagittal stratum (SS) and caudate (p-values between 0.001–0.041); only ALIC_L and PLIC_L remained significant after correction for multiple comparisons (Table 2; Figs. 2A and 2B). HIV + group also had higher diffusivities (AD and/or RD) in multiple white matter structures (Table 3). For example, compared to SN controls and regardless of MJ use, HIV + had higher AD in the right BCC, left SCC and left SLF, as well as higher RD in the left ALIC, right ALIC, left PCR, left SCR and right SFO (p-values between 0.003–0.019; Figs. 3A-C). In subcortical regions, HIV + had lower FA in the caudate (Fig. 2B) and higher MD in the caudate, globus pallidus, and thalamus (p-values between 0.007–0.025) (Table 3; Fig. 3D). Regardless of HIV-serostatus, MJ users had lower AD in the uncinate fasciculus than nonusers (p = 0.016, Fig. 3B). In addition, a trend for HIV-by-MJ interaction (p = 0.054) was observed in the MD in the right GP; while SN + MJ had lower MD than SN nonusers, HIV + MJ had higher MD than HIV+ (Table 3, Fig. 3D). We also co-varied for the lifetime tobacco and alcohol use, since these variables showed group difference, but all the findings remained the same. In our exploratory analyses, these abnormal DTI metrics did not correlate with HIV-related clinical variables and MJ use patterns.

Table 2
Fractional Anisotropy in Regions of Interest (ROIs) that Showed HIV Effects

Region	SN	SN + MJ	HIV	HIV + MJ	HIV Effect	MJ Effect
ALIC_L	0.4676 ± 0.005	0.4694 ± 0.005	0.4518 ± 0.0054	0.4536 ± 0.005	0.001*	0.712
ALIC_R	0.4656 ± 0.0057	0.4689 ± 0.0057	0.4514 ± 0.0062	0.4547 ± 0.0057	0.009	0.533
PLIC_L	0.5547 ± 0.0047	0.5568 ± 0.0047	0.5419 ± 0.0051	0.544 ± 0.0047	0.005*	0.631
CGC_L	0.3842 ± 0.0035	0.3846 ± 0.0036	0.3766 ± 0.0039	0.3771 ± 0.0036	0.026	0.885
CGH_L	0.3695 ± 0.0035	0.3672 ± 0.0036	0.3617 ± 0.0038	0.3594 ± 0.0036	0.022	0.496
PTR_R	0.4561 ± 0.0056	0.4581 ± 0.0056	0.445 ± 0.006	0.447 ± 0.0056	0.036	0.702
SFO_L	0.4059 ± 0.0056	0.4007 ± 0.0057	0.3948 ± 0.0061	0.3897 ± 0.0057	0.041	0.338
SFO_R	0.4211 ± 0.0051	0.4187 ± 0.0052	0.4093 ± 0.0056	0.4069 ± 0.0052	0.017	0.631
SS_R	0.4125 ± 0.0039	0.4135 ± 0.0039	0.4024 ± 0.0042	0.4034 ± 0.0039	0.007	0.787
Caudate_L	0.3553 ± 0.0039	0.3613 ± 0.0039	0.3472 ± 0.0042	0.3532 ± 0.0039	0.029	0.108
All uncorrected-p-values < 0.05 from two-way ANCOVA are shown; the other 11 ROIs not listed showed no HIV or MJ effects. No HIV-by-MJ interaction was found for FA measurement in any of the ROIs. *p-values remained significant after Holm correction for multiple comparisons. Bold: uncorrected p-value < 0.05.						
Abbreviations: ALIC = anterior limb of internal capsule, CGC = cingulum, cingulate gyrus part, CGH = cingulum (hippocampal part), PTR = posterior thalamic radiation, PLIC = posterior limb of the internal capsule, SS = sagittal stratum, SFO = superior fronto-occipital fasciculus, R = right, L = left, HIV = HIV seropositive, SN = HIV seronegative, MJ = marijuana.						

Table 3
Diffusivities in Regions of Interest (ROIs) that Showed HIV or MJ Effects

Regions	SN	SN + MJ	HIV	HIV + MJ	HIV Effect	MJ Effect	HIV x MJ Effect
Axial Diffusivity							
BCC_R	1.518 ± 0.014	1.503 ± 0.014	1.549 ± 0.015	1.534 ± 0.015	0.019	0.248	ns
SCC_L	1.486 ± 0.014	1.495 ± 0.015	1.528 ± 0.016	1.537 ± 0.015	0.003*	0.526	ns
SLF_L	1.067 ± 0.009	1.078 ± 0.009	1.087 ± 0.009	1.098 ± 0.009	0.017	0.170	ns
UNC_R	1.104 ± 0.011	1.078 ± 0.011	1.113 ± 0.012	1.088 ± 0.012	0.361	0.016	ns
CGC_R	1.182 ± 0.013	1.167 ± 0.013	1.209 ± 0.014	1.194 ± 0.014	0.031	0.219	ns
EC_L	1.108 ± 0.011	1.105 ± 0.011	1.129 ± 0.012	1.127 ± 0.012	0.039	0.799	ns
SCR_L	1.034 ± 0.015	1.051 ± 0.015	1.063 ± 0.017	1.080 ± 0.016	0.050	0.256	ns
SFO_R	1.101 ± 0.024	1.106 ± 0.024	1.149 ± 0.026	1.154 ± 0.026	0.037	0.839	ns
Radial Diffusivity							
ALIC_L	0.534 ± 0.008	0.537 ± 0.008	0.557 ± 0.009	0.559 ± 0.009	0.005*	0.750	ns
ALIC_R	0.548 ± 0.008	0.545 ± 0.009	0.573 ± 0.009	0.570 ± 0.009	0.003*	0.719	ns
PCR_L	0.574 ± 0.008	0.580 ± 0.008	0.596 ± 0.009	0.602 ± 0.008	0.005*	0.439	ns
PCR_R	0.604 ± 0.012	0.606 ± 0.012	0.626 ± 0.013	0.628 ± 0.012	0.042	0.862	ns
SCR_L	0.536 ± 0.009	0.542 ± 0.009	0.559 ± 0.009	0.565 ± 0.009	0.006*	0.515	ns

All uncorrected-p-values < 0.05 from two-way ANCOVA are shown. ns = not significant ($p \geq 0.05$). *p values remained significant after Holm correction for multiple comparisons. P-values < 0.05 are shown in bold.

Abbreviations: ALIC = Anterior limb of internal capsule, BCC = Body of corpus callosum, CGC = Cingulum Cingulate Gyrus, EC = external capsule, GP = globus pallidus, IFO = inferior fronto-occipital fasciculus, PCR = Posterior corona radiata, PLIC = posterior limb of the internal capsule, RLIC = retrolenticular part of the internal capsule, SCC = Splenium of corpus callosum, SCR = Superior corona radiata, SLF = superior longitudinal fasciculus, SFO = Superior fronto-occipital fasciculus, Thal = Thalamus, UNC = uncinatofasciculus.

Regions	SN	SN + MJ	HIV	HIV + MJ	HIV Effect	MJ Effect	HIV x MJ Effect
SCR_R	0.527 ± 0.009	0.534 ± 0.009	0.546 ± 0.010	0.552 ± 0.010	0.037	0.475	ns
SFO_L	0.546 ± 0.014	0.563 ± 0.014	0.576 ± 0.015	0.593 ± 0.015	0.026	0.196	ns
SFO_R	0.545 ± 0.015	0.551 ± 0.015	0.583 ± 0.016	0.589 ± 0.016	0.007*	0.649	ns
PLIC_L	0.454 ± 0.006	0.457 ± 0.006	0.468 ± 0.007	0.470 ± 0.007	0.022	0.661	ns
RLIC_L	0.571 ± 0.006	0.570 ± 0.006	0.585 ± 0.006	0.585 ± 0.006	0.009	0.938	ns
EC_L	0.599 ± 0.008	0.596 ± 0.008	0.616 ± 0.008	0.613 ± 0.008	0.019	0.672	ns
IFO_L	0.593 ± 0.007	0.585 ± 0.007	0.607 ± 0.008	0.599 ± 0.008	0.045	0.231	ns
SLF_L	0.564 ± 0.007	0.564 ± 0.007	0.578 ± 0.007	0.579 ± 0.007	0.028	0.935	ns
SLF_R	0.580 ± 0.009	0.577 ± 0.009	0.600 ± 0.010	0.597 ± 0.010	0.027	0.706	ns
Mean Diffusivity							
Tha_L	0.750 ± 0.006	0.749 ± 0.006	0.762 ± 0.006	0.762 ± 0.006	0.025	0.922	ns
Caudate_R	0.807 ± 0.011	0.801 ± 0.011	0.835 ± 0.012	0.829 ± 0.012	0.007*	0.532	ns
GP_L	0.758 ± 0.015	0.764 ± 0.015	0.791 ± 0.016	0.797 ± 0.016	0.020	0.697	ns
GP_R	0.786 ± 0.013	0.785 ± 0.013	0.769 ± 0.013	0.810 ± 0.014	0.929	0.288	0.054
All uncorrected-p-values < 0.05 from two-way ANCOVA are shown. ns = not significant (p ≥ 0.05). *p values remained significant after Holm correction for multiple comparisons. P-values < 0.05 are shown in bold.							
Abbreviations: ALIC = Anterior limb of internal capsule, BCC = Body of corpus callosum, CGC = Cingulum Cingulate Gyrus, EC = external capsule, GP = globus pallidus, IFO = inferior fronto-occipital fasciculus, PCR = Posterior corona radiata, PLIC = posterior limb of the internal capsule, RLIC = retrolenticular part of the internal capsule, SCC = Splenium of corpus callosum, SCR = Superior corona radiata, SLF = superior longitudinal fasciculus, SFO = Superior fronto-occipital fasciculus, Thal = Thalamus, UNC = uncinatofasciculus.							

Correlations between Abnormal DTI Metrics and Abnormal Cognitive Domain Z-scores

In the two HIV + groups, but not SN subjects, higher MD in the left thalamus predicted poorer Fluency (interaction- $p = 0.004$), Learning (interaction- $p = 0.026$) and Memory Z-scores (interaction- $p = 0.048$); Fig. 4A-C. However, higher RD in the PCR_L predicted poorer learning (interaction- $p = 0.050$) across all MJ users regardless of HIV-serostatus (Fig. 4D). In SN + MJ and HIV groups, higher RD in the SCR_L (3-way-interaction- $p = 0.014$) and lower FA in the ALIC_L (3-way-interaction- $p = 0.038$) predicted poorer Attention/Working Memory, but no such correlations were found in the SN and HIV + MJ groups (Fig. 4E-F). No correlations were found between the DTI metrics and HIV-related clinical features (e.g. nadir CD4 and current CD4 counts and duration of HIV infection) and MJ usage patterns (age of onset, daily average use, duration and lifetime amount of MJ use).

Age-Related Changes in DTI

Although HIV + showed lower FA and higher diffusivities than SN in multiple white matter and subcortical regions, regardless of MJ use, HIV + and SN showed similar age-dependent decreases in FA in 5 brain regions (left and right ACR, left, and right GCC, and right SS), and age-dependent increases in diffusivities in 16/44 regions (including left and right hemispheres, p -values between 0.001–0.049, data not shown, except for BCC-AD and ALIC-FA, Figs. 5A & B). In addition, independent of MJ use, HIV + showed greater age-related decline in the R_BCC-FA than SN (HIV x Age-interaction $p = 0.037$; Fig. 5C). Furthermore, regardless of HIV-serostatus, MJ users showed greater age-related decline than non-users in left EC-FA (MJ x Age-interaction $p = 0.007$) and in left SLF-FA (MJ x Age-interaction- $p = 0.044$; Figs. 5D & E). Lastly, age-related decline in the right GP-FA was found only in MJ users (HIV x MJ x Age-interaction- $p = 0.02$, Fig. 5F), since the other three groups showed relatively lower right GP_FA at younger age.

Discussion

The main findings of this study are: 1) As hypothesized, compared to SN and regardless of MJ use status, HIV + group had poorer cognitive performance. 2) In contrast, regardless of HIV-serostatus, MJ users and non-users had similar cognitive performance; hence, no interactions or additive deleterious effects were found in HIV + MJ on cognitive test scores. 3) Similarly, DTI measures in HIV + group, regardless of MJ use status, had lower FA and higher diffusivities than SN controls in multiple white matter and subcortical brain regions, indicating greater neurodegeneration and neuroinflammation. 4) Regardless of HIV-serostatus, MJ users had lower AD in the right UNC than Nonusers. Furthermore, we observed a trend for HIV-by-MJ interaction in the right GP_MD, indicating possible differential MJ effects on neuroinflammation in this brain region of HIV patients compared to SN controls.

Cognitive Performance in Chronic MJ Users with and without HIV-infection

The poorer performance in HIV + compared to SN controls, regardless of MJ Use, in the Fluency, Attention/Working Memory, Learning, Memory, and Global Function, domains are consistent with prior studies in HIV + individuals (1). These persistent cognitive abnormalities were attributed primarily to ongoing neuroinflammation (23–26). Also similar to prior reports (7–10), regardless of HIV status, MJ users

had similar performance across all cognitive domains as Nonusers. The lack of cognition deficits in our adult MJ users suggest little or no neurotoxic effects associated with chronic MJ use, which is supported by the lack of decline in IQ in adult onset MJ users (14). In contrast, the developing brain of adolescents may be more vulnerable to the neurotoxic effects of MJ, since earlier onset or regular (weekly) MJ use was associated with lower cognition (15, 16), decline in IQ and cognitive function (between ages 13–38 years) (14).

In the current study, although no significant HIV-by-MJ interaction was found in any of the cognitive domains, SN + MJ tended to have poorer performance than SN Nonusers in Learning, Memory and Motor domains, while HIV + MJ tended to perform better than HIV + Nonusers in Fluency, executive function and speed of information processing. These trends are consistent with a recent large study that found MJ use was associated with lower odds of neurocognitive impairment, and higher verbal fluency and learning performance, in PLWH, but not in the SN participants (11). This paradoxical effect of MJ use in SN and HIV + individuals might be related to the anti-inflammatory effects from some of the MJ constituents on the neuroinflammation in PLWH (32, 33). For example, $\Delta 9$ -THC suppresses cytokine-induced T-cell activation (32, 33) and lowers the monocyte-derived pro-inflammatory factor IP-10 *in vitro* (33). Furthermore, MJ-using HIV + participants showed faster decline of cellular HIV DNA levels during the first 4 months of cART, compared with those who did not use MJ or used other substances (34). In addition, HIV + light MJ users had better verbal fluency than SN light users (9), but this advantage was not found in HIV + heavy MJ users (8). How the dosage and the potency of $\Delta 9$ -THC in MJ, which has quadrupled in the past two decades (35), may impact cognition in PLWH will need to be evaluated in future studies.

DTI Metrics in Chronic MJ Users with and Without HIV-infection

Consistent with prior DTI studies (2, 36–38), our HIV + participants, regardless of MJ use, had lower FA and/or higher diffusivities in the corpus callosum, coronal radiata, internal capsule, the cingulum, SLF, SFO and SS. Lower FA and higher diffusivities in HIV + individuals most likely reflect disrupted white matter microstructure, perhaps due to neurodegeneration and chronic neuroinflammation induced by ongoing HIV + infection (39). Our HIV + participants also showed lower FA in the caudates and higher MD in subcortical gray matter (caudate, globus pallidus and thalamus), suggesting lesser microstructural integrity and possible demyelination in these regions. The elevated MD in the caudates of our HIV + individuals is consistent with the findings in early HIV infection (40), while the elevated MD in the GP is consistent with higher 18 kDa translocator protein (TSPO) binding, reflecting microglial activation, in this region in PLWH (39). Furthermore, relatively lower FA in the globus pallidus, along with poorer motor skills, were found in HIV + women, but not HIV + men (41).

In contrast, the right uncinate fasciculus (UNC) was the only brain region that showed abnormally lower AD in MJ users, regardless of HIV status. The lower AD in the UNC indicates reduced water movement along the axonal fibers, suggesting lesser axonal fiber density, accumulated cellular debris from damaged axonal, or extracellular space tortuosity (42). In preclinical studies, reduced AD was consistently found at early stages of brain injury from models of multiple sclerosis, and correlated with the axonal damage or loss (42, 43).

Furthermore, the UNC in MJ users were found to have reduced FA and elevated MD (25), as well as shorter than normal fiber bundle (44). Lower FA in the UNC was also associated with higher scores for apathy in MJ users (25). The UNC fasciculi are long-range projecting fibers that connect the orbitofrontal cortex with entorhinal and fusiform cortices, which have densely localized CB1 receptors that are target receptors for $\Delta 9$ -THC (44). Lower AD in the UNC might lead to lesser connectivity among these regions, which were found to have abnormally thinner cortices and associated poorer verbal memory in cannabis users (44).

The right globus pallidus showed a trend for HIV-by-MJ interaction on diffusivity, with relatively lower MD in SN + MJ users but relatively higher MD in the HIV + MJ users. This trend parallels the interactive effects in an earlier study that showed relatively lower levels of myoinositol, a glial marker, in the basal ganglia of SN + MJ users but relatively higher myoinositol levels in HIV + MJ users (7). In HIV + patients, higher diffusivity was associated with higher myoinositol level, indicating greater neuroinflammation, which in turn correlated with poorer cognitive performance (45). Therefore, this interactive trend suggests while chronic MJ use might suppress glial activation in the GP of SN, chronic MJ use might exacerbate the glial activation in HIV + users. However, future studies with other glial markers to assess these possible differential effects of MJ use on neuroinflammation in the GP between PLWH and SN are needed.

DTI Metrics Predicted Cognitive Performance in HIV + individuals and MJ users

Microstructural abnormalities predicted poorer cognitive function in our HIV + and SN + MJ groups. Specifically, in our HIV + group, the higher MD in thalamus, suggesting lesser microstructural integrity or greater neuroinflammation, predicted poorer fluency, learning and verbal memory. Consistent with our study, a $[(11)C]PBR28$ PET study that measured the 18 kDa TSP0 found greater binding in several subcortical regions, including the thalamus, suggesting greater microglial activation; the higher tracer binding also correlated with higher MD on DTI, as well as poorer memory, verbal learning and global function (39). Also consistent with our findings, lower FA in the thalamic radiation predicted greater intra-individual variability on neuropsychological performance in HIV patients (46). Lastly, higher RD in posterior corona radiata (PCR) in our MJ users predicted poorer learning, similar to the correlations between higher MD in corona radiata and slower processing speed in the aging population (47), as well as between higher ACR_AD and poorer learning in HIV + participants (37).

Age-Related and MJ-Related Changes in DTI Metrics in HIV + individuals and MJ users

Our HIV + participants had greater than normal age-dependent declines in FA in the ACR, CC and SS, regardless of MJ usage; this is consistent with findings in prior DTI studies (36, 48). In addition, we observed greater than normal age-related FA decline in the EC and SLF of MJ users regardless of HIV-serostatus, which is consistent with the greater than normal age-related FA decline in multiple white matter regions in MJ users (24). Lastly, age-dependent decline was observed in the right globus pallidus FA only in SN-MJ users, but not in the other three groups (HIV, HIV + MJ or MJ), since they already had lower GP_FA, indicating lesser microstructural integrity, at younger ages. However, due to the limited sample size in each of the subgroups, these exploratory observations will need to be confirmed in future studies.

Limitations

Our study has several limitations. 1) Our cohort included primarily men; therefore, we were not able to assess sex-specific differences on brain microstructure in relation to the possible additive or interactive effects of HIV-infection and chronic MJ use. 2) Since this is a cross-sectional study, we could not determine the causality of chronic MJ use on altered DTI metrics or cognitive deficits in HIV + individuals. Future longitudinal studies are necessary to further delineate the independent and combined effects of chronic MJ use and HIV-infection on brain microstructure. 3) Self-report of MJ use or other substances used may be inaccurate or under-reported, and might have confounded our results.

Conclusions

Adding to previous studies that did not find additional adverse effects of chronic MJ use on cognition (7–9), brain morphometry (8), and clinical outcomes, such as viral load, CD4 cell count and total mortality (19, 21) in HIV + participants, our findings suggest that chronic MJ use has no additional negative influence on brain microstructure or neurocognitive deficits in PLWH. However, the lower AD in the UNC of MJ users suggests axonal loss in this white matter tract that connects to CB1 receptor rich brain regions that are involved in verbal memory (44) and emotionality. Furthermore, the greater than normal age-dependent FA declines in several white matter tracts and the GP in SN-MJ users suggest that older MJ users may eventually have lesser neuronal integrity in these brain regions.

Abbreviations

ACR anterior corona radiata

AD axial diffusivity

ALIC anterior limb of internal capsule

AN(C)OVA analysis of (co-) variance

BCC body of corpus callosum

cART combination antiretroviral therapy

CC corpus callosum

CES-D Center for Epidemiological Studies – Depression Scale

CGC cingulum, cingulate gyrus part

CGH cingulum (hippocampal part)

D-KEFS Delis-Kaplan Executive Function System

DTI diffusion tensor imaging

EC external capsule

FA fractional anisotropy

GCC genu of corpus callosum

GP globus pallidus

HIV + HIV-seropositive

HIV + MJ HIV-seropositive marijuana user group

IFO inferior fronto-occipital fasciculus

IQ intelligence quotient

ISP Index of Social Position

L Left

MD mean diffusivity

MJ marijuana

PCR posterior corona radiata

PLIC posterior limb of the internal capsule

PLWH people living with HIV

PTR posterior thalamic radiation

R Right

RAVLT Rey Auditory Verbal Learning Test

ROCFT Rey-Osterreith Complex Figure Test

RD radial diffusivity

RLIC retrolenticular part of the internal capsule

SCC splenium of corpus callosum

SCR superior corona radiata

SFO superior fronto-occipital fasciculus

SLF superior longitudinal fasciculus

SN Seronegative (for HIV)

SN + MJ Seronegative (for HIV) and marijuana user group

SS sagittal stratum

SUD substance use disorder

Thal Thalamus

UNC uncinata fasciculus

WTAR Wechsler Test of Adult Reading

Declarations

Ethics approval and consent to participate: The protocol and the consent form were approved by the Cooperative Institutional Review Board of the University of Hawaii and The Queen's Medical Center, and was Health Insurance Portability and Accountability Act (HIPAA) compliant. All participants signed a written consent form.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interest

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Authors' contributions: HW analyzed the data and co-drafted and revised the manuscript; HL analyzed the data, interpreted the data; co-drafted and revised the manuscript; TE supervised the data acquisition and interpretation, critically reviewed the statistics, reviewed and revised the manuscript. KO supervised and processed the DTI scans to generate the data, reviewed and revised the manuscript; LC designed and conceptualized the study, supervised the data collection, co-drafted, critically reviewed, revised and approved all aspects of the data analyses. All authors have approved the manuscript.

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Figures

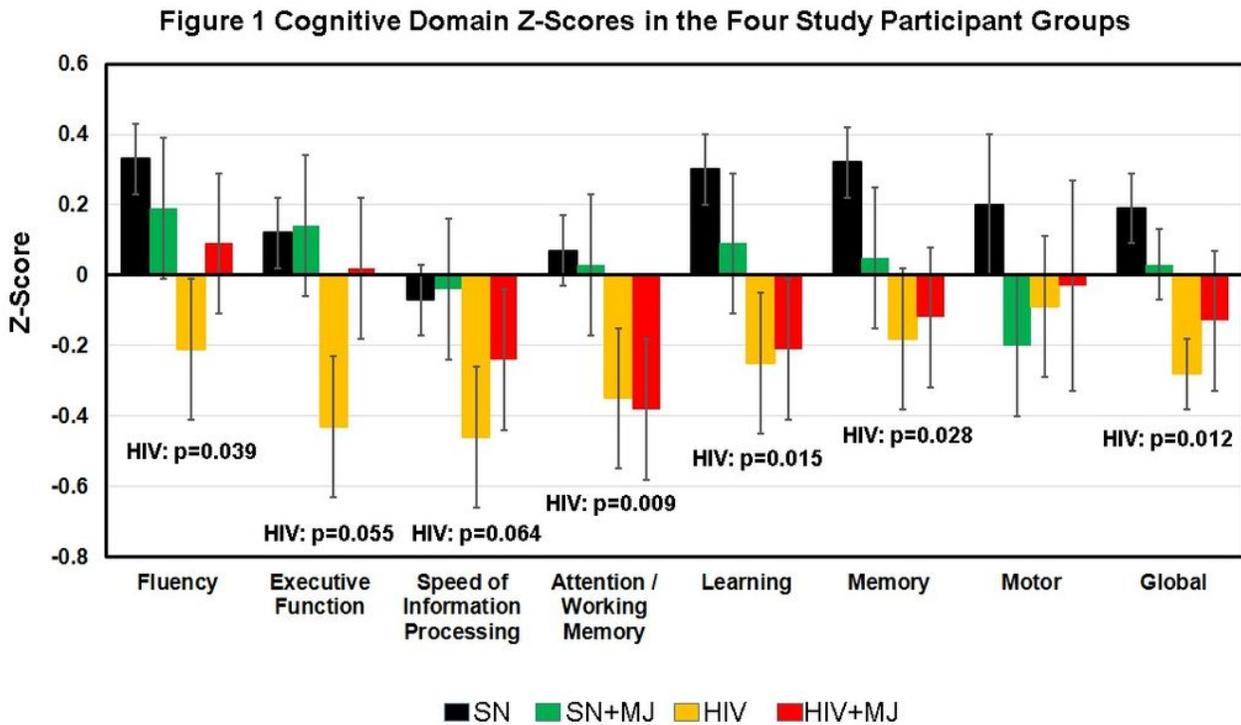


Figure 1

Cognitive Domain Z-Scores in the Four Study Participant Groups. Regardless of marijuana Use, HIV+ participants had significantly poorer performance in the Fluency ($p=0.039$), Attention / Working Memory ($p=0.009$), Learning ($p=0.015$) and Memory ($p=0.028$) domains, as well as the Global z-score ($p=0.012$), compared to HIV-seronegative participants. Error bars=Standard Errors.

Figure 2. HIV+ Participants Showed Lower Regional Fractional Anisotropy (FA) than SN Subjects on DTI

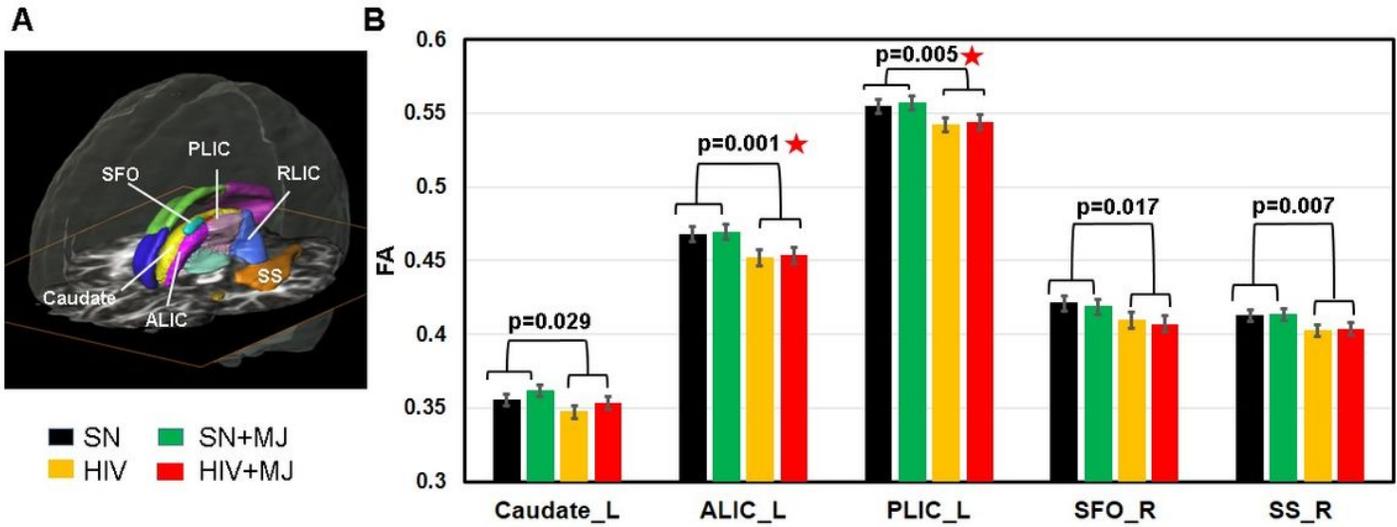


Figure 2

HIV+ Participants Showed Lower Regional Fractional Anisotropy (FA) than SN Subjects on DTI. A) Regions of interest automatically segmented for DTI-FA measures showing significant HIV effects. B) Regardless of MJ use status, HIV+ had lower FA in the caudate_L ($p=0.029$), ALIC_L ($p=0.001$), PLIC_L ($p=0.005$), SFO_R ($p=0.017$) and SS_R ($p=0.007$) than SN participants. Abbreviations: ALIC=anterior limb of internal capsule, PLIC=posterior limb of internal capsule, SFO=superior fronto-occipital fasciculus, SS=sagittal stratum, R=right, L=left, SN=HIV-seronegative nonuser group, SN+MJ= HIV-seronegative marijuana user group, HIV=HIV seropositive nonuser group, HIV+MJ=HIV-seropositive marijuana user group. Red star: p-values that remained significant after Holm-Bonferroni correction for multiple comparisons.

Figure 3. Group Differences in DTI Diffusivities

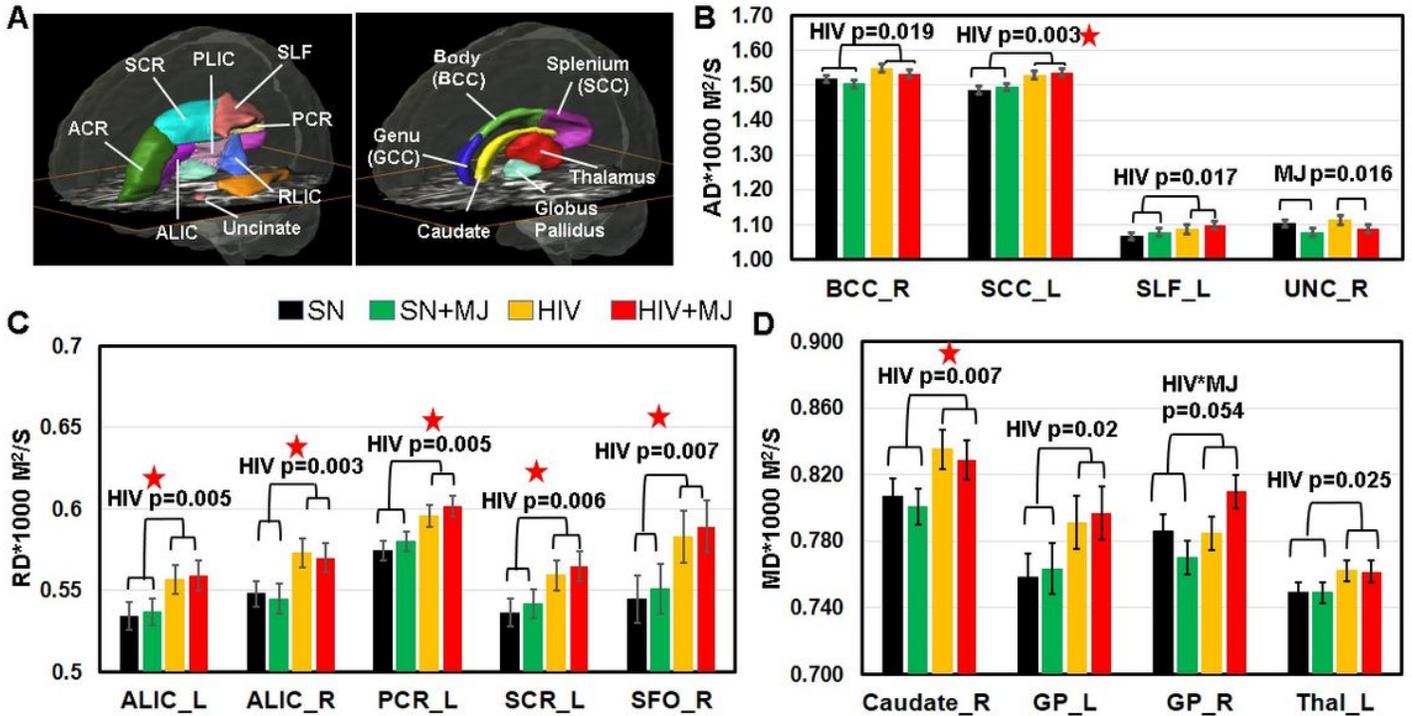


Figure 3

Group Differences in DTI Diffusivities. A) Regions of interest automatically segmented for DTI measures that showed significant HIV or MJ main effects. B) Regardless of MJ use status, HIV+ group showed higher axial diffusivity (AD) in the BCC_R ($p=0.019$), SCC_L ($p=0.003$) and in SLF_L ($p=0.017$) than SN participants. However, regardless of HIV serostatus, MJ users had lower AD in the UNC_R ($p=0.016$) than Nonusers. C) Compared to SN controls and regardless of MJ use status, HIV+ had higher radial diffusivity (RD) than SN participants in the ALIC_L ($p=0.005$), ALIC_R ($p=0.003$), PCR_L ($p=0.005$), SCR_L ($p=0.006$) and SFO_R ($p=0.007$). D) Regardless of MJ use status, HIV+ group had higher mean diffusivity (MD) in the right caudate ($p=0.007$), left globus pallidus ($p=0.02$) and left thalamus ($p=0.025$) than SN groups. In additional, there was a trend of HIV-by-Marijuana interaction in the right GP ($p=0.054$); while HIV+MJ users had higher MD than HIV non-users, MJ users had lower MD than SN nonusers. Abbreviations (in order of appearance in graphs): BCC=body of corpus, SCC=splenium of corpus callosum, SLF=superior longitudinal fasciculus, UNC=uncinate fasciculus, ALIC=anterior limb of internal capsule, PCR posterior corona radiate, SCR=superior corona radiate, SFO=superior fronto-occipital fasciculus (see Figure 2A), GP=globus pallidus, Thal=thalamus; R=right, L=left, SN = HIV-seronegative nonuser group, SN+MJ = HIV-seronegative marijuana user group, HIV = HIV- seropositive nonuser group, HIV+MJ = HIV-seropositive marijuana user group. Red star: p-value that remained significant after Holm-Bonferroni correction for multiple comparisons.

Figure 4. Correlations between DTI Metrics And Cognitive Domain Z-scores performance

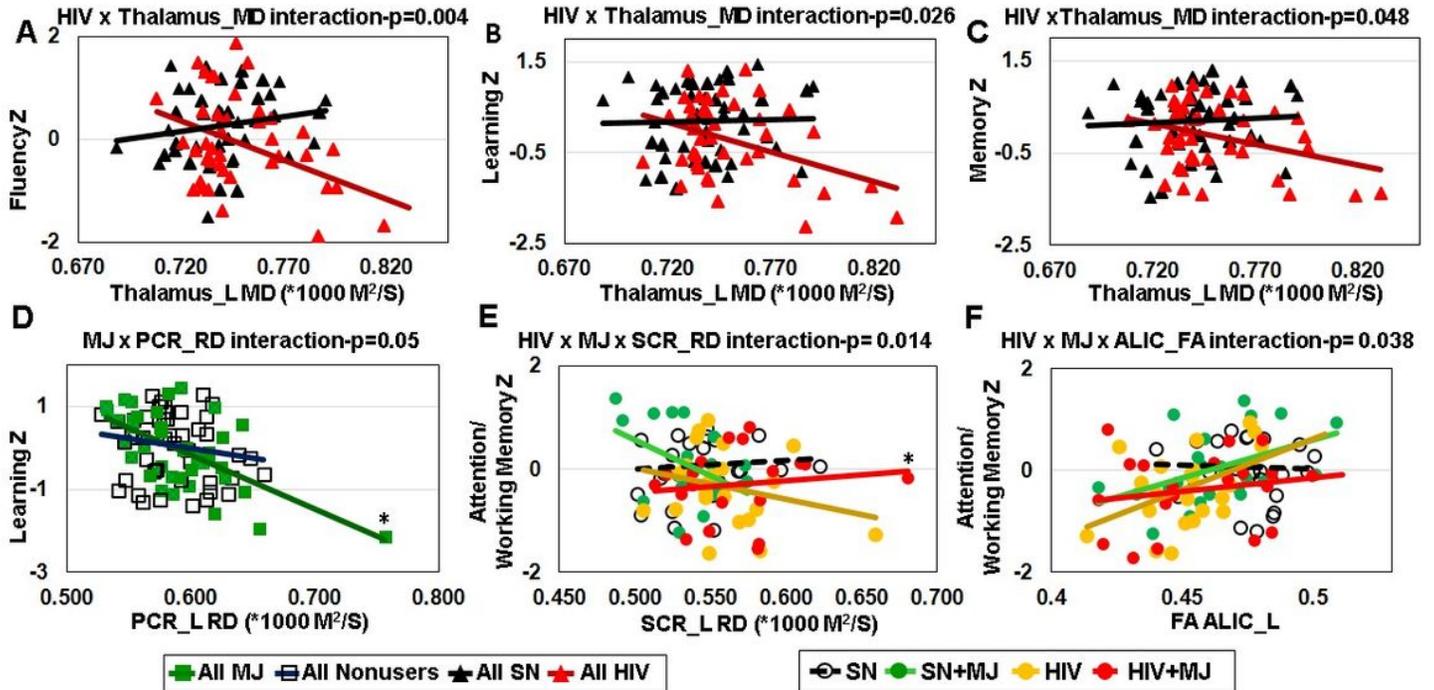


Figure 4

Correlations between DTI Metrics And Cognitive Domain Z-scores. A-C Higher mean diffusivity (MD) in the left thalamus correlated with poorer Fluency, Learning and Memory Z-scores only in HIV+ individuals (interaction-p=0.004, 0.026 and 0.048 respectively) D) Higher radial diffusivity (RD) in the left PCR correlated with poorer Learning z-scores (interaction-p=0.050) in MJ users regardless of HIV serostatus. *The interaction-p value became more significant (p=0.037) if this highest RD values is removed. E-F) Poorer Attention/Working Memory was predicted by higher RD in the left SCR (p=0.014) and lower fractional anisotropy (FA) in the left ALIC (p=0.038) in SN+MJ and HIV groups, but not in the SN and HIV+MJ groups. *The 3-way interaction p-value for the SCR_L remained the same (p=0.014) after removal the highest RD value. Abbreviations: PCR=posterior corona radiata, SCR=superior corona radiata, ALIC=anterior limb of internal capsule, SN = HIV-seronegative nonuser group, SN+MJ = HIV-seronegative marijuana user group, HIV = HIV- seropositive nonuser group, HIV+MJ = HIV-seropositive marijuana user group.

Figure 5. Correlations Between DTI Metrics and Age

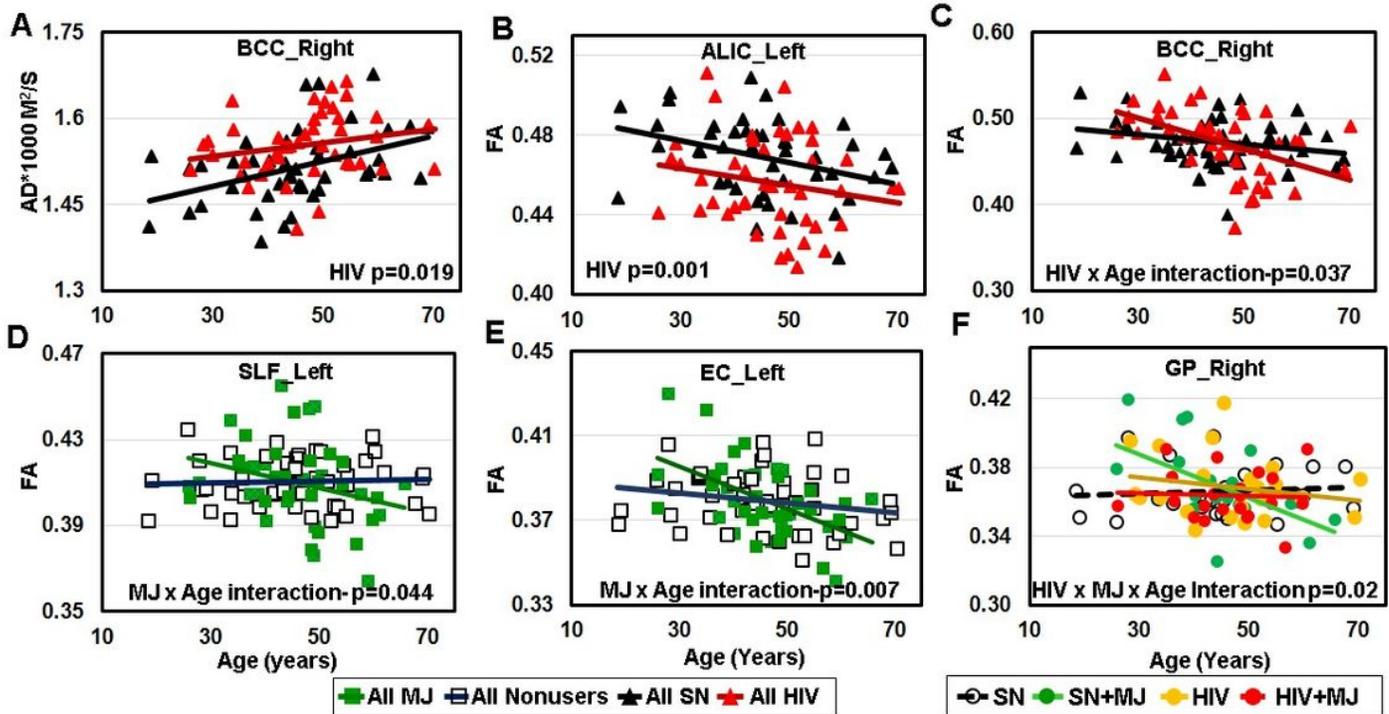


Figure 5

Correlations Between DTI Metrics and Age. A) Despite the higher AD in HIV than SN (regardless of MJ use), the two groups showed similar slopes of age-dependent increases in AD. The BCC_AD is illustrated as an example. B) Although HIV+ had lower FA than SN, both groups (regardless of MJ use) showed similar age-dependent declines; see in left ALIC-FA for example. C) Greater age-related declines in the BCC-FA was observed in HIV+ individuals, with or without MJ use, than SN subjects (HIV x Age-interaction- $p=0.037$). D) and E) Compared to all Nonusers (blue line), MJ users with or without HIV (dark green line), showed greater age-related declines in FA in the left EC (MJ x Age-interaction- $p=0.007$) and left SLE (MJ x Age-interaction- $p=0.044$). F) Age-related decline in the right GP_FA was observed in SN-MJ users only (3-way interaction- $p=0.02$); the other three groups already showed lower levels of FA at younger ages. Abbreviations (in order of appearance): BCC=body of corpus callosum, ALIC=anterior limb of internal capsule, SLF=superior longitudinal fasciculus, EC= external capsule, GP=globus pallidus, SN = HIV-seronegative nonuser group, SN+MJ = HIV-seronegative marijuana user group, HIV = HIV- seropositive nonuser group, HIV+MJ = HIV-seropositive marijuana user group.