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White matter tract injury and cognitive impairment in human immunodeficiency virus–infected individuals

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Approximately half of those infected with the human immunodeficiency virus (HIV) exhibit cognitive impairment, which has been related to cerebral white matter damage. Despite the effectiveness of antiretroviral treatment, cognitive impairment remains common even in individuals with undetectable viral loads. One explanation for this may be subtherapeutic concentrations of some antiretrovirals in the central nervous system (CNS). We utilized diffusion tensor imaging and a comprehensive neuropsychological evaluation to investigate the relationship of white matter integrity to cognitive impairment and antiretroviral treatment variables. Participants included 39 HIV-infected individuals (49% with acquired immunodeficiency syndrome [AIDS]; mean CD4 = 529) and 25 seronegative subjects. Diffusion tensor imaging indices were mapped onto a common whole-brain white matter tract skeleton, allowing between-subject voxelwise comparisons. The total HIV-infected group exhibited abnormal white matter in the internal capsule, inferior longitudinal fasciculus, and optic radiation; whereas those with AIDS exhibited more widespread damage, including in the internal capsule and the corpus callosum. Cognitive impairment in the HIV-infected group was related to white matter injury in the internal capsule, corpus callosum, and superior longitudinal fasciculus. White matter injury was not found to be

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associated with HIV viral load or estimated CNS penetration of antiretrovirals. Diffusion tensor imaging was useful in identifying changes in white matter tracts associated with more advanced HIV infection. Relationships between diffusion alterations in specific white matter tracts and cognitive impairment support the potential utility of diffusion tensor imaging in examining the anatomical underpinnings of HIV-related cognitive impairment. The study also confirms that CNS injury is evident in persons infected with HIV despite effective antiretroviral treatment. *Journal of NeuroVirology* (2009) **15,** 187–195.

Keywords: diffusion tensor imaging; HIV dementia; neuropsychological assessment

Although combination antiretroviral (ARV) treatment has markedly reduced human immunodeficiency virus (HIV)-associated mortality, comparable gains may not have been attained in reducing neurological complications (Tozzi *et al,* 2007; Sevigny *et al*, 2007). Despite a decline in the incidence of HIV-associated dementia, milder forms of HIV-associated neurocognitive disorder (HAND) remain prevalent (Brew, 2004; Antinori *et al*, 2007). The neurobiological underpinnings of HAND remain incompletely understood. Pathological studies have demonstrated preferential damage to cerebral white matter (Bell, 1998). Structural magnetic resonance imaging (MRI) studies have reported diffuse cerebral atrophy and white matter and basal ganglia abnormalities (Stout *et al*, 1998), whereas magnetic resonance (MR) spectroscopy studies have indicated neuronal injury and glial activation in white matter regions (Chang *et al*, 2002).

Diffusion tensor imaging (DTI) permits examination of white matter injury via two common indices, fractional anisotropy (FA), which reflects the orientation specificity of water diffusion, and mean diffusivity (MD), which reflects the degree of water diffusion within an imaging voxel (Parker, 2004). DTI studies in HIV have reported white matter injury (i.e., decreased FA or increased MD) in the subcortical white matter, particularly of the frontal lobes, the genu and splenium of the corpus callosum, and the internal capsule (Filippi *et al*, 1998; Pomara *et al*, 2001; Thurnher *et al*, 2005; Wu *et al*, 2006; Pfefferbaum, *et al* 2007; Chang *et al*, 2008).

We adopted a novel DTI data analysis approach, which focuses on large white matter tracts of the whole brain in a voxelwise manner, which is particularly suited for examining the diffuse white matter injury related to HIV. The unique spatial registration algorithm also facilitates comparison between healthy and atrophied brains secondary to pathological processes such as HIV infection. Specifically, we examined the relationship of DTI indices to presence of HIV infection, ARV experience, and global neurocognitive impairment in those with HIV. We hypothesized that (1) HIV infection would be related to white matter injury, and more white matter injury would be observed in individuals with acquired immunodeficiency syndrome (AIDS); (2) among HIV-infected persons, better ARV response in the central nervous system (CNS), measured by cerebrospinal fluid (CSF) viral load, would be associated with less white matter injury; (3) ARV treatment containing drugs with better CNS penetration would be related to decreased white matter injury; and (4) neurocognitive impairment would be related to more white matter injury.

Results

All group comparisons were matched for age, gender, ethnicity (i.e., proportion of Caucasians), and level of education, $P > .05$. The findings below are summarized in Table 1.

HIV infection

Two primary group comparisons were performed to examine the effect of HIV infection (HIV-seronegative comparison subjects (SC) versus all individuals with HIV infection (HIV+) participants) and the effect of disease stage (medically asymptomatic HIV+ participants versus those with AIDS). Accordingly, α-level for these comparisons was set at .025. FA was found to be significantly higher in SC than HIV+ in the right posterior limb of the internal capsule, the right inferior longitudinal fasciculus, and the right optic radiation, $N = 64$, $P < .025$. No significant differences were found in MD of SC and HIV+, although trends towards higher MD in HIV+ were apparent in the right posterior limb of the internal capsule, and the right optic radiation, $N = 51$, $P = .064$.

No significant differences in FA were found between medically asymptomatic HIV+ participants and those with AIDS, $N = 39$, $P > .05$. However, significantly increased MD in the AIDS group was apparent in the bilateral posterior corona radiata, the bilateral optic radiation, the bilateral superior longitudinal fasciculus, the genu and splenium of the corpus callosum, the left anterior and bilateral posterior limbs of the internal capsule, and the bilateral inferior longitudinal fasciculus, $N = 39$, $P < .025$.

Table 1 Regions within the white matter skeleton with significantly altered FA and MD indicating white matter injury

		SC vs. all HIV+		Asymptomatic HIV+ vs. AIDS		SC vs. AIDS		Cognitively impaired vs. unimpaired HIV+	
		FA	MD	FA	MD	FA	MD	FA	MD
Corona radiata	Voxels				1823		1929		97
	Effect size (*d*)				1.73		1.64		1.13
Optic radiation	Voxels	817			1817	1045	1369		262
	Effect size (*d*)	0.94			1.67	1.50	2.12		1.24
Superior longitudinal fasciculus	Voxels				2115		324		
	Effect size (*d*)				1.70		1.59		
Corpus callosum: genu	Voxels				228		294	884	618
	Effect size (*d*)				1.05		1.24	1.09	1.19
Corpus callosum: body	Voxels						5	259	305
	Effect size (*d*)						.95	1.23	1.16
Corpus callosum: splenium	Voxels				740		147		427
	Effect size (*d*)				1.62		1.24		1.59
Internal capsule: anterior limb	Voxels				357		560		
	Effect size (*d*)				1.10		1.19		
Internal capsule: posterior limb	Voxels	20			57	79	464		85
	Effect size (*d*)	0.38			1.25	0.85	1.72		1.25
Inferior longitudinal fasciculus	Voxels	414			3216	782	1392		
	Effect size (*d*)	0.71			1.84	1.36	1.89		

Note. SC = seronegative control group; HIV+ = HIV-infected group. Results included in the table indicate white matter tract regions with statistically significant group differences (familywise $\alpha = .05$). Significant white matter tract clusters are divided into specific regions as described in the Methods section. Voxels are isometric 1 mm^3. Where regional significant group differences are present, the magnitude of the effect is expressed as Cohen's *d*.

Additional follow-up analyses comparing SC and HIV+ participants with AIDS showed AIDS-associated FA decrease in the right optic radiation, the right posterior limb of the internal capsule, and the right inferior longitudinal fasciculus, $N = 44$, $P < .025$. Significantly increased MD in the AIDS group was apparent in the bilateral corona radiata, the right optic radiation, the bilateral superior longitudinal fasciculus, the genu, body, and splenium of the corpus callosum, the bilateral anterior and posterior limbs of the internal capsule, and the right inferior longitudinal fasciculus, $N = 31$, $P < .05$ (Figure 1).

No significant diffusion differences were found between SC and medically asymptomatic HIV+, $N = 45$ (FA) and 32 (MD), $P > .05$.

ARV treatment effectiveness

Two analyses were performed to evaluate the relationship between ARV treatment effectiveness and white matter injury. First, HIV+ participants with detectable CSF HIV RNA were compared with those with undetectable CSF HIV RNA. No significant group differences were found, $N = 31$, $P > .05$. Additionally, in the nine individuals who had detectable CSF HIV RNA, we correlated this marker variable with FA and MD, after covarying for nadir CD4 level. Similarly, no significant relationships were found between these variables, $N = 9$, $P > .05$, regardless of whether nadir CD4 was used as a covariate.

CNS penetration of ARV regimens

We correlated CNS penetration scores of HIV+ participants with FA and MD, after covarying for nadir CD4 level. No significant relationships were found between these variables, $N = 32$, $P > .05$, regardless of whether nadir CD4 was used as a covariate.

Neurocognitive impairment

To examine the relationship between white matter injury and neurocognitive impairment in HIV-infected individuals, FA and MD were compared between HIV+ participants with and without neurocognitive impairment. The two groups are comparable regarding proportions with AIDS, $\chi^2(1, N = 38) = .04$, $P > .05$. Neurocognitively impaired individuals exhibited significantly decreased FA in the genu and body of the corpus callosum, $N = 38$, $P < .05$, and significantly increased MD in the right corona radiata, the right optic radiation, the right posterior internal capsule, and the genu, body, and splenium of the corpus callosum, $N = 38$, $P < .05$ (Figure 2). Follow-up analyses were performed to examine the relationship between the degree of neurocognitive impairment reflected by the Global Deficit Score (GDS), and DTI indices averaged across regions where significant differences were found between the two groups. Average MD in these regions was found to be significantly related to GDS, $r(36) = .37$, $P < .05$, whereas average FA showed a trend toward significant relationship, $r(36) = .29$, $P = .077$.

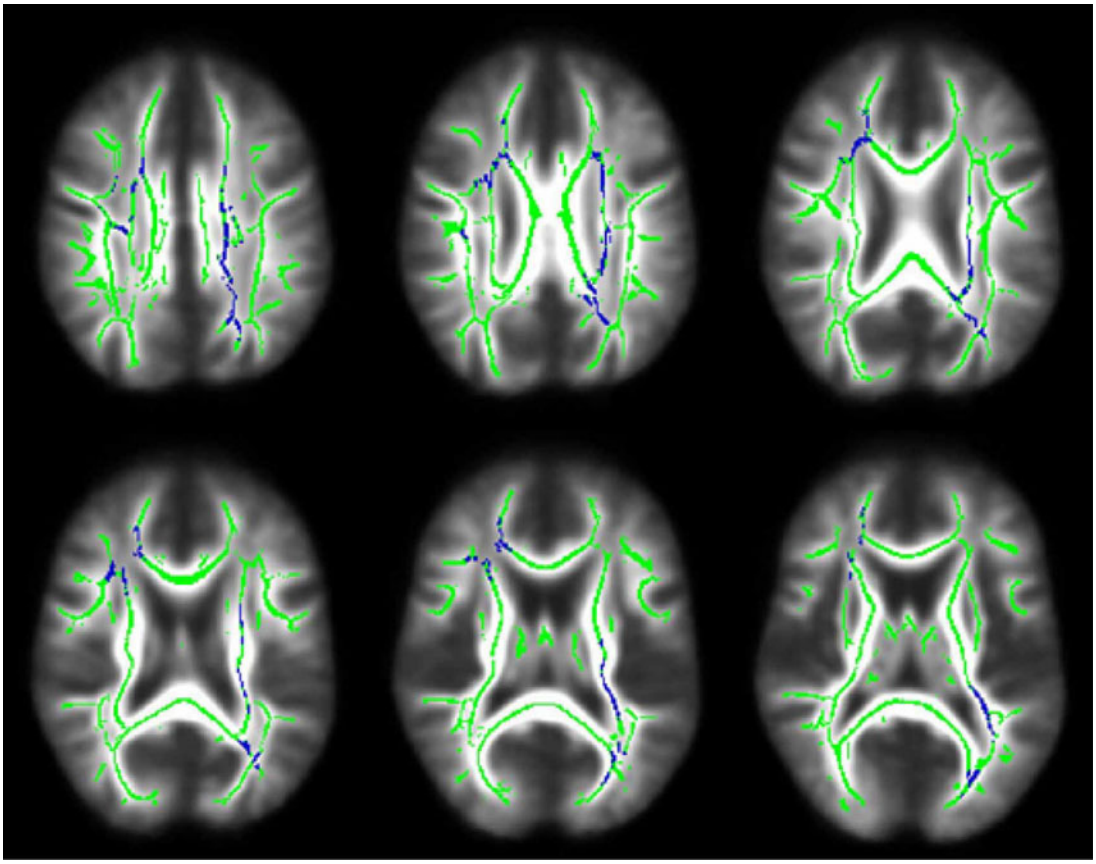

Figure 1 Sample consecutive 5-mm slices showing voxels with significantly increased MD (*blue*) in HIV+ participants with AIDS relative to seronegative participants, overlaid on white matter tract skeleton (*green*), and averaged FA image (*grayscale*).

Discussion

DTI as implemented here appears to be sensitive to HIV-related microstructural white matter abnormalities in the overall HIV+ group, consisting of individuals both with and without AIDS. However, the most robust differences were noted in those with AIDS. These include MD and FA changes in the internal capsule, inferior longitudinal fasciculus, and optic radiation. Differences in MD (but not FA) suggested that there may also be injury to the corpus callosum, superior longitudinal fasciculus, and corona radiata.

Diffuse cerebral white matter damage is a hallmark pathological feature of HIV encephalitis. The changes to the corpus callosum apparent here are consistent with neuropathologic reports of heavy histological labeling of HIV DNA and RNA in the corpus callosum in HIV encephalitis (Gosztonyi *et al*, 1994), as well as callosal thinning on anatomical MRI (Thompson *et al*, 2006). Histopathological data also indicate increased presence of HIV in the internal capsule (Gosztonyi *et al*, 1994). More specifically, the demonstrated preferential injury to the posterior limb of the internal capsule has been previously reported in another DTI study (Pomara *et al*, 2001). Using the current voxelwise data analysis approach, we also found significant results in the superior/inferior longitudinal fasciculi, the optic radiation, and the corona radiata, which have not been previously reported in the absence of secondary conditions. Such widespread findings are consistent with a previous DTI study of HIV utilizing a voxelwise approach (Stebbins *et al*, 2007). Apparent white matter injury to these regions may therefore reflect the diffuse white matter pathology associated with progression to AIDS.

The lack of association between diffusion alterations and the ARV treatment variables—i.e., virus suppression in the CSF, and degree of CNS penetration of ARV regimens—was contrary to expectation. This is notable in light of previously reported associations between elevated CSF viral load and subsequent development of HIV-associated dementia (HAD) (Ellis *et al*, 2002), and between ARV regimens with good CNS penetration and improved cognitive performance (Letendre *et al*, 2004). The current results should be interpreted in the context of notable limitations of our study. First, only nine HIV-infected participants (23%) had detectable CSF viral load, suggesting possible statistical power limitation in the analyses involving the variable. Second, the implication of these results may be limited by the cross-sectional and uncontrolled

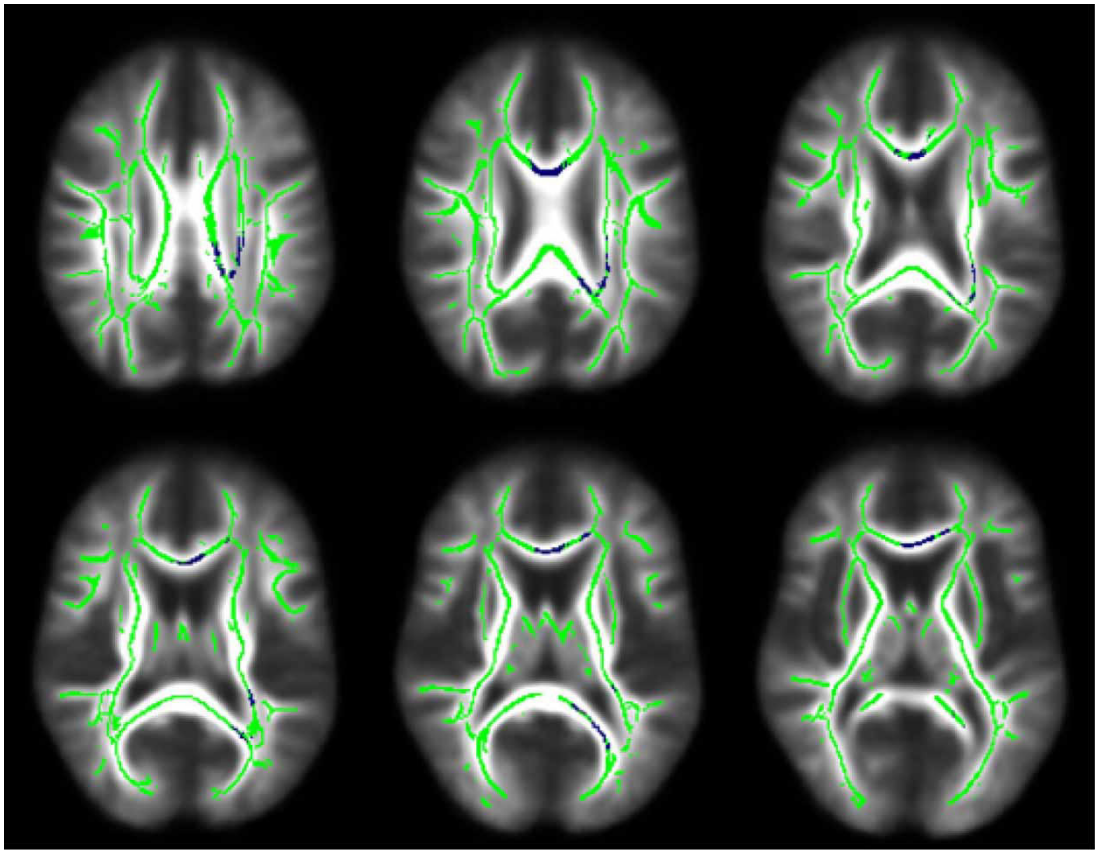

Figure 2 Sample consecutive 5-mm slices showing voxels with significantly increased MD (*blue*) in neurocognitively impaired relative to unimpaired HIV+ participants, overlaid on white matter tract skeleton (*green*), and averaged FA image (*grayscale*).

design of our study. Important variables such as durations of HIV disease and ARV treatment, and history of ARV regimens and treatment adherence, may be confounded with CD4 and viral load variables. This may be addressed longitudinally in future studies, i.e., by examining changes over time of DTI indices in the context of CSF viral load and estimated ARV CNS penetration. It is also possible, however, that the current DTI implementation is not adequately sensitive to detect the hypothesized relationships. We are not aware of other published studies examining diffusion alterations in the context of these treatment variables, and this remains an issue that requires further investigation.

Finally, we also found significant association between diffusion alterations and the presence of neurocognitive impairment in HIV-infected individuals. In impaired individuals, white matter injury indicated by increased MD was apparent in the genu, body, and splenium of the corpus callosum, the posterior limb of the internal capsule, the corona radiata, and the optic radiation, whereas voxels with decreased FA were limited to the genu and body of the corpus callosum. As noted above, callosal damage has been observed in HIV infection. Although frank disconnection symptoms have not been associated with HIV infection, general cognitive impairment with motor dysfunction has been reported in individuals who exhibited callosal thinning on MRI examinations (Thompson *et al*, 2006). Abnormal FA and MD in the corpus callosum have also been associated with increased dementia severity and decreased motor speed (Wu *et al*, 2006), and increased global cognitive deficit (Chang *et al*, 2008). In addition, in a simian model of AIDS, motor impairment was associated with axonal injury in the corpus callosum, indicated by accumulation of β-amyloid precursor protein (Weed *et al*, 2003).

Damage to the posterior limb of the internal capsule would be consistent with the motor deficits commonly observed in HIV-related cognitive disorders, though the current study provides no specific evidence for this association. We further conducted a preliminary analysis of the relationship between diffusion alteration and fine motor performance. Although this failed to yield significant results, we believe that future studies specifically designed to address this issue are warranted. The additional novel findings in the corona radiata and the optic radiation, again, have not been previously reported, and may reflect the diffuse nature of HIV-related white matter pathology.

HIV-related neuronal injury is generally believed to be secondary to neurotoxic cascades associated with the virus itself or infection/activation of other CNS cell types. Such injury typically occurs without substantial neuronal loss and may help explain the relative sensitivity of MD to HIV-related white matter injury. Increased extracellular space due to atrophied neurons would be consistent with a general increase in water diffusivity (i.e., MD). Diffusion anisotropy (i.e., FA), however, may be more associated with frank axonal or neuronal loss, which may be less evident in HIV infection, except in late stage disease. If this is correct, it is possible that the significant AIDS-related FA alterations found here in the optic radiation, the posterior internal capsule, and the inferior longitudinal fasciculus may reflect a distinctive nature of white matter injury in these regions. The same may apply to the relative sensitivity of FA to white matter injury in the anterior corpus callosum in individuals with cognitive impairment.

This study provides evidence that injury to white matter tracts can be demonstrated in HIV infected individuals receiving ARV treatment. The relationship between DTI indices and neurocognitive impairment suggests that such white matter changes may have clinical significance. Longitudinal investigations are needed to determine whether long-term successful viral suppression relates to improvement in these indices of white matter injury.

Methods

Participants

Participants included 39 individuals with HIV+ and 25 SC subjects. HIV infection was verified by enzyme-linked immunosorbent assay (ELISA) and Western blot confirmatory test. Half of the HIV+ group (49%, $N = 19$) met the AIDS diagnosis according to the Centers for Disease Control and Prevention (CDC) 1993 criteria (CDC, 1992). HIV+ participants were recruited as part of the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) and other cohort studies at the University of California, San Diego, HIV Neurobehavioral Research Center.

Plasma was available for 38 (97%) and CSF was available for 31 participants (79%). Most HIV+ participants (82%, $N = 32$) reported current ARV use. Twenty-two participants had undetectable CSF HIV RNA levels (< 50 copies/ml). Nine participants had detectable values ranging in $\log_{10}$ from 2.42 to 3.79 ($M = 3.06$, $SD = 0.46$).

HIV-seronegative control (SC) participants were from healthy control cohorts from a study of HIV infection ($N = 12$), and a study of alcoholism ($N = 13$). SC and HIV+ participants were comparable demographically (Table 2).

Participants were excluded for history of (1) head injury with loss of consciousness > 10 minutes; (2) neurological or psychiatric illness that would impact cognitive functioning; (3) substance use disorder (except marijuana) within the past 6 months.

CNS penetration of ARV drugs

ARV treatment information was obtained through written questionnaire and clinician interview. The degree of CNS penetration of ARV regimens was quantified by assigning a value of 0, 0.5, or 1 to each drug based on published data on CSF concentrations and/or chemical properties. The ranks for all drugs in a regimen were summed to estimate penetration and effectiveness in the CNS (Letendre *et al*, 2008). In this cohort, penetration scores for the 32 ARV-treated individuals ranged from 0.5 to 3.5 ($M = 1.72$, $SD = 0.91$).

Neurocognitive evaluation

Thirty-eight HIV+ participants received comprehensive neurocognitive evaluation examining seven cognitive domains most implicated in HIV (Rippeth *et al*, 2004): speed of information processing, learning, delayed recall, executive functions, verbal fluency, working memory, and motor skills.

Table 2 Demographic information and relevant lab results for participant subgroups

		HIV+				
	SC	All	Non-AIDS	AIDS	Cognitively unimpaired	Cognitively impaired
N	25	39	20	19	28	10
Age (years)	38.7 (13.0)	42.4 (8.3)	42.7 (9.4)	42.0 (7.3)	42.5 (8.5)	41.1 (8.1)
Education (years)	13.8 (2.5)	13.6 (2.5)	14.3 (2.3)	12.8 (2.5)	13.8 (2.3)	13.1 (3.2)
% male	84	92	95	89	93	90
% Caucasians	88	72	85	58	68	80
% AIDS	—	49	0	100	46	50
Current CD4 level	—	529 (243)	635 (212)	411 (225)	530 (267)	523 (171)
Nadir CD4 level	—	219 (159)	333 (121)	100 (90)	233 (170)	190 (131)
% with detectable plasma HIV RNA	—	29 (11/38)	25 (5/20)	33 (6/18)	25 (7/28)	22 (2/9)
% with detectable CSF HIV RNA	—	29 (9/31)	25 (4/16)	33 (5/15)	27 (6/22)	25 (2/8)

Note. SC = seronegative control group; HIV+ = HIV-infected group.

A statistically derived summary score, the GDS was used to represent the severity of neurocognitive impairment (Heaton *et al*, 1995). GDS values range from 0 (normal) to 5 (severely impaired). Prior research supports the construct validity of the GDS in HIV (Carey *et al*, 2004), for which a cutpoint of 0.5 demonstrates optimal classification accuracy for identifying global cognitive impairment. Using the recommended GDS cutpoint, we observed a 26% ($N = 10$) prevalence of neurocognitive impairment in this cohort, in which GDS values ranged from 0 to 3.47 ($M = 0.39$, $SD = .63$).

DTI data acquisition and processing protocols
All neuroimaging was performed on one GE Echospeed LX 1.5-T imager at the VA San Diego Healthcare System. DTI for HIV+ and 12 SC participants were performed with a single-shot spiral spin-echo acquisition with TE = 120 ms, TR = 6000 ms, FOV = 250 mm, slice thickness = 3.9 mm, image matrix = 64 × 64, and b-value = 2416 s/mm^2. Diffusion-weighted images were acquired in 42 diffusion directions, in addition to a T2-weighted image with no diffusion encoding (Frank, 2001). Images were derived by averaging four identical acquisitions. For 13 SC participants, data were collected with the same parameters except for TE = 100 ms, TR = 6000 ms, FOV = 240 mm, slice thickness = 3.8 mm, and b-value = 1745 s/mm^2.

We utilized a voxelwise data-processing method, which focuses on whole-brain large white matter tracts. FA and MD were calculated using nonlinear estimation of the diffusion tensor model with the *3dDWItoDT* program of the AFNI package (Cox, 1996) based on standard formulas (Parker, 2004). White matter tract maps were generated with the Tract-Based Spatial Statistics (TBSS) tool from the FMRIB Software Library (FSL; Smith *et al*, 2004). The process involved nonlinear registration of each participant's FA images to a study-specific template, before affine transformation to MNI152 space. All images were then averaged to create a representative FA image from which a white matter tract skeleton was generated. The skeleton was mapped onto individual participants' FA images via a spatial searching algorithm. This processing approach thus minimizes the necessity of nonlinear registration with higher degrees of freedom, which typically introduces significant distortion to images and limits comparability between subjects with healthy and significantly atrophied brains such as typically found in advanced HIV disease. The previous processing steps yielded identical skeletons for all participants, with voxel values representing white matter tract centers, allowing between-subject voxel-by-voxel comparisons without additional spatial registration. The spatial mapping results were also used to map MD images to the skeleton (Smith *et al*, 2006).

White matter tracts were identified using human brain atlases (Woolsey *et al*, 2002; Mori *et al*, 2005). Subregions of the corpus callosum were defined by dividing the anterior-to-posterior length at midline into four equal sections. The anterior quarter was designated as the genu, the middle quarters as the callosal body, and the posterior quarter as the splenium.

Statistical analysis
Voxelwise statistical analyses inherently involve a large number of comparisons. Parametric statistical techniques are commonly used to control for type I error in such analyses, especially in studies utilizing voxel-based morphometry. Valid application of such approach, however, requires gaussian distribution of image data. Such a requirement is not met in the case of the current study due to processing steps involving nonlinear image registration and the lack of spatial smoothing. We therefore utilized a nonparametric permutation-based approach with suprathreshold cluster test. This involved creating a sampling distribution of the *t*-statistic based on a large number of random permutations of the data. The *t*-statistic image was then thresholded at a predetermined level, and the cluster sizes of suprathreshold voxels were nonparametrically tested. The critical cluster size was the $\alpha N+1$ highest ranked member of the distribution (α = type I error level, N = number of permutations) (Nichols and Holmes, 2002). All permutation-based analyses were performed using the *randomise* program of the FSL package, with 5000 permutations to construct the sampling distribution, and a threshold *t*-value of 2.

Thirteen SC participants received a slightly different DTI protocol than the others. Because FA values are normalized to a common metric, all SC were treated as equivalent (both protocols had identical resolution, and comparable echo times). A permutation-based test confirmed that the two SC groups were comparable in FA, $N = 25$, $P > .1$. MD values, however, are not normalized. Thus, significant differences in MD between the SC groups were found in the majority of skeleton voxels, $N = 25$, $P < .01$. These 13 participants were therefore excluded from statistical analyses involving MD.

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