BRIEF REPORT

HIV Infection and Aging Independently Affect Brain Function as Measured by Functional Magnetic Resonance Imaging

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We investigated the interactions between human immunodeficiency virus (HIV) infection and aging and their effects on brain function demands by means of functional magnetic resonance imaging (fMRI). A multiple-regression model was used to study the association and interaction between fMRI measures, HIV serostatus, and age for 26 HIV-infected subjects and 25 seronegative subjects. Although HIV serostatus and age independently affected fMRI measures, no interaction occurred. Functional brain demands in HIV-positive subjects were equivalent to those of HIV-negative subjects who were 15–20 years older. Frailty parallels between HIV infection and aging could result from continued immunological challenges depleting resources and triggering increased metabolic demands. In the future, fMRI could be a noninvasive biomarker to assess HIV infection in the brain.

Biological similarities exist between aging and human immunodeficiency virus (HIV) infection. Both cause a frailty phenotype characterized by generalized decreased physical function caused by enhanced vulnerability to immunological stressors and inflammatory dysregulation [1]. The introduction of highly active antiretroviral therapy (HAART) has increased the population of older HIV-infected individuals (>50 years old) [2]. The physiological impacts of aging and HIV infection have been characterized within multiple organs but not within the brain.

Functional magnetic resonance imaging (fMRI) provides a noninvasive method to study brain metabolic demands. In control participants without HIV infection, aging decreases functional activity within certain brain regions while causing recruitment of additional structures [3]. Statistically significant differences in functional brain activity occur in younger (<40 years old) HIV-infected subjects, compared with HIV-negative subjects [4]. To date, no fMRI studies have investigated the possible interaction between HIV infection and aging. If fMRI is potentially to be used as a preclinical biomarker to assess HIV infection in the brain, then a broader spectrum of ages is required for studying both HIV-positive and HIV-negative subjects.

Methods. HIV-negative ($n = 25$) and HIV-positive ($n = 26$) subjects (age, 20–62 years) provided written consent approved by the University of California, San Diego, institutional review board. A detailed medical history was obtained, and individuals were excluded if they had a previous history of other neurological illness or infections, cerebrovascular disease or strokes, major psychiatric disorders, or substance abuse 3 months prior to imaging. Subjects underwent imaging if reported substance abstinence was confirmed by negative urine toxicology test results. HIV-positive subjects either were not receiving HAART or had been receiving a stable regimen for >3 months. All HIV-positive patients had hematocrit levels, CD4 cell counts, and plasma HIV loads measured. The plasma HIV load was determined by reverse-transcription polymerase chain reaction (Amplicor; Roche Diagnostic Systems) using an ultrasensitive assay (lower quantification limit, <50 copies/mL). CD4 cell count nadir values were obtained by self-report or by direct measurement if the measured CD4 cell count was lower than the self-report measure.

Imaging was performed on a 3 Tesla (3T) whole-body system (3T and Excite; General Electric) using an 8-channel receive head coil. High-resolution structural images for anatomical confirmation were acquired using an inversion recovery prepared 3-dimensional fast spoiled pulse sequence (inversion time [TI], 450 ms; repetition time [TR], 7.9 ms; echo time [TE], 3.1 ms; flip angle, 12°; field of view [FOV], $25 \times 25 \times 16$ cm^3; matrix, $256 \times 256 \times 124$). Images were reviewed to ensure absence of structural lesions. For the activation task, a block de-

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sign was used with functional changes in cerebral blood flow and blood oxygen level–dependent signal determined within the visual cortex for a flickering black and white radial checkerboard (frequency, 8 Hz). Activation periods were 20 s in duration, whereas rest portions were 60 s in duration and consisted of an isoluminant gray screen with a center fixation square. A single-shot proximal inversion sequence that controlled for off-resonance effects by using quantitative imaging of perfusion with a single subtraction (TR, 2.5 s; TI_1, 700 ms; TI_2, 1500 ms; tag width, 20 cm; tag-slice gap, 1 cm), dual-echo gradient echo readout, and spiral acquisition of *k*-space (TE_1, 9.4 ms; TE_2, 30 ms; flip angle, 90°; FOV, 24 cm; matrix, 64×64) allowed for the alternate acquisition of "tag" and "control" images [5]. The difference between images provided cerebral blood flow values, whereas the mean of the images yielded the blood oxygen level–dependent signal. An additional resting cerebral blood flow scan was acquired to quantify baseline values, using methods described elsewhere [6].

Images were coregistered to correct for subject movement. The visual cortex was defined as the region between the parietal-occipital sulci and was manually delineated on anatomical images. This region was resampled to match functional image scans and corrected for possible white matter involvement and partial volume loss. A general linear model used the stimulus function, acquired cardiac and respiratory data, and constant and linear terms as additional nuisance regressors to identify activated visual cortex voxels with a significance threshold ($P = .05$) that corrected for multiple comparisons [7]. From these activated voxels, functional changes in cerebral blood flow and the blood oxygen level–dependent signal were determined for the task, using methods described elsewhere [7]. The mean baseline cerebral blood flow was calculated by averaging all time points within activated visual cortex voxels.

Wilcoxon rank sum tests were used to assess the differences in fMRI measures between the HIV-positive and HIV-negative subjects. The association and interaction between fMRI measures and HIV status and age (in years) were investigated using a multiple-regression model. Each fMRI variable (baseline cerebral blood flow, functional changes in cerebral blood flow, and blood oxygen level–dependent signal) was $\log_{10}$-transformed to improve normality and homoscedasticity and back-transformed to the natural scale for plotting. An additive regression model was used with *P* values determined from the Wald test. The effects of age and HIV serostatus were determined for each fMRI outcome. The mean change in the fMRI outcome on the log-scale was transformed back to the response scale and expressed as proportional increase (positive) or decrease (negative) in the outcome, with the effect size computed as the regression effect on the logarithmic scale divided by the residual standard deviation. The age-equivalent effect of HIV infection was determined by dividing the HIV effect size by

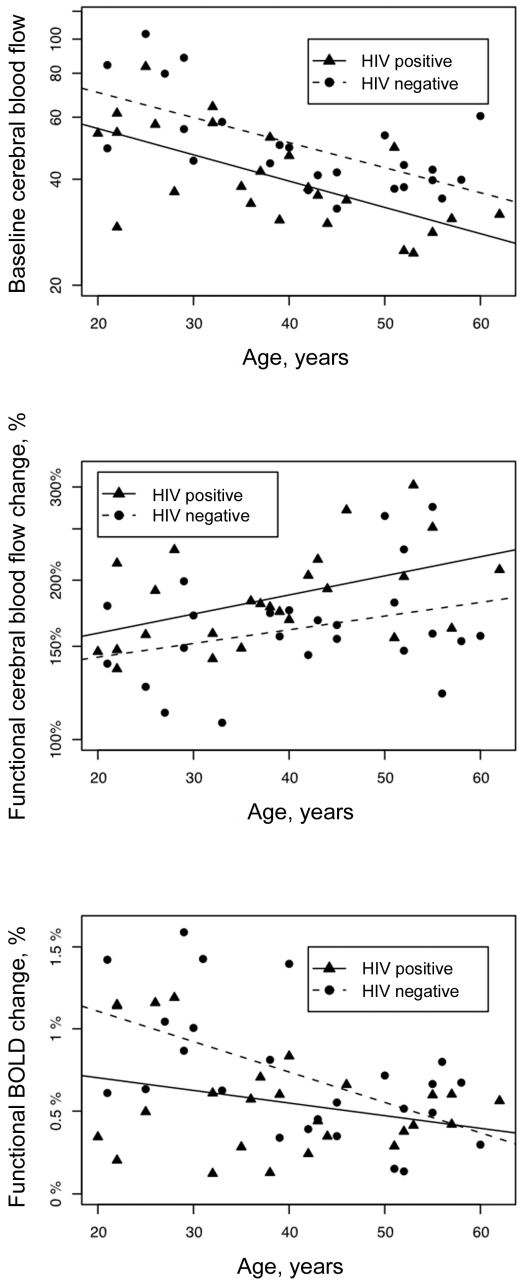

Figure 1. Effects of human immunodeficiency virus (HIV) infection and aging on baseline cerebral blood flow (*top*), functional changes in cerebral blood flow (*middle*), and functional changes in the blood oxygen level–dependent (BOLD) signal (*bottom*) in the visual cortex. Both HIV infection and aging led to statistically significant decreases in baseline cerebral blood flow and functional changes in the BOLD signal, but no interaction was observed. HIV infection and aging also led to statistically significant increases in functional changes in cerebral blood flow, but no interaction was observed. The plots include regression lines for the baseline levels in each of the functional magnetic resonance imaging (fMRI) measures for the HIV-positive subjects and the HIV-negative control participants. Each of the fMRI measures was $\log_{10}$-transformed to improve normality and homoscedasticity and then back-transformed to the natural scale.

Table 1. Effect of Age and HIV Infection on Functional Magnetic Resonance Imaging Variables

Variable, characteristic	Change in outcome, % (95% CI)	Effect size[a] (95% CI)	*P*
Baseline CBF (R^2 = 47%)			
15-year age increase	−22.2 (−32.4 to −10.7)	−1.02 (−1.59 to −0.46)	<.001
HIV infection	−22.2 (−28.7 to −15.3)	−1.02 (−1.38 to −0.67)	<.001
Age and HIV interaction	…	…	.89
Functional CBF changes, % (R^2 = 22.7%)			
15-year age increase	11.3 (3.5–19.7)	0.74 (0.17–1.32)	.005
HIV infection	16.3 (3.4–30.8)	0.74 (0.16–1.32)	.013
Age and HIV interaction	…	…	.64
Functional BOLD signal changes, % (R^2 = 20.0%)			
15-year age increase	−0.19 (−0.30 to −0.08)	…	.001
HIV infection	−0.19 (−0.37 to −0.03)	…	.048
Age and HIV interaction	…	…	.16

NOTE. The change in outcome was calculated on the basis of the analysis on a logarithmic scale, which was then back-transformed. A positive change represents an increase, and a negative change represents a decrease. BOLD, blood oxygen level–dependent; CBF, cerebral blood flow; CI, confidence interval; HIV, human immunodeficiency virus.

[a] The effect size was computed as the regression effect on the logarithmic scale divided by the residual standard deviation.

the yearly effect size of age. The age effect was expressed per 15 years of aging for comparison.

Results. Both groups were comparable in regard to age (mean, 41 years for HIV-negative subjects and 39 years for HIV-positive subjects), sex (male sex, 14 [56%] of 25 HIV-negative subjects and 20 [77%] of 26 HIV-positive subjects), and education level (mean, 15 years for HIV-negative subjects and 16 years for HIV-positive subjects). HIV-positive subjects had a median CD4 cell count of 486 cells/μL and a median CD4 nadir of 278 cells/μL. Approximately 60% (15 of 26) of the HIV-positive subjects were receiving stable HAART regimens.

HIV-positive subjects had a lower baseline cerebral blood flow, compared with HIV-negative subjects (Figure 1, *top*). Increasing age and HIV infection caused statistically significant decreases in baseline cerebral blood flow, but no interaction occurred (Table 1). For a given age, baseline cerebral blood flow values for HIV-positive subjects were equivalent to those for HIV-negative subjects who were 15 years older. Functional cerebral blood flow changes were also greater for HIV-positive subjects than for HIV-negative subjects (Figure 1, *middle*). Both aging and HIV infection caused statistically significant increases in functional changes in cerebral blood flow, but no interaction was present. HIV infection was equivalent to a 21-year increase in brain age, compared with HIV-negative control subjects (Table 1). Functional changes in the blood oxygen level–dependent signal were reduced for HIV-positive subjects, compared with HIV-negative subjects (Figure 1, *bottom*). Aging and HIV infection independently caused statistically significant decreases in functional blood oxygen level–dependent signal. Although the age-related regression lines intersected for the 2 groups, no statistically significant interaction was observed ($P = .16$). For a given age, functional changes in the blood oxygen level–dependent signal of HIV-positive subjects were equivalent to those of HIV-negative control subjects who were 15 years older (Table 1).

Discussion. As the number of older HIV-positive individuals continues to increase, a growing need exists to understand the potential interactions between HIV and aging within the brain. Our results suggest that HIV infection and aging independently affect brain functional demands that are measurable by fMRI.

The lack of a synergistic interaction between age and HIV infection, although surprising, is comparable to the findings of a larger neuropsychological study that showed that age and HIV serostatus were independent risk factors for the development of HIV-associated neurocognitive disorders [8]. Although our sample size was statistically significantly smaller than that in the previous descriptive study, our cohort included women, which may better reflect the diversity of the disease among the sexes.

Conflicting results concerning the relationship between HIV and aging have been noted in studies using structural neuroimaging [9, 10]. An interaction occurred in HAART-naive HIV-positive subjects observed using magnetic resonance spectroscopy, with a 5-fold increase present in frontal white matter inflammatory and glial metabolites [10]. In contrast, diffusion tensor imaging of tissue water molecules did not show age-dependent changes in HIV-positive subjects receiving stable HAART regimens [9]. Discrepancies in these results could reflect differences in neuroimaging techniques or in the effects of HAART.

Although most neuroimaging studies of HIV infection have investigated the role of subcortical or frontal areas in HIV-associated neurocognitive disorders, primary cortical areas have been shown to be affected [11]. Our analysis was not focused

on studying the effects of HIV-associated neurocognitive disorders on fMRI measures; instead, we investigated the impact of HIV infection itself on a cortical structure—the visual cortex. The effects of "normal" aging have been previously characterized in this region [12]. Observed decreases with increasing aging in baseline cerebral blood flow in HIV-negative control subjects are similar to the results found in previous neuroimaging studies [12, 13]. In general, a decrease in baseline cerebral blood flow occurs with HIV infection [14]. Derangements within primary cortical areas may cause disconnections among higher processing networks despite the absence of abnormalities on traditional structural images. The etiology of observed decreases in baseline cerebral blood flow remains unknown, but it could result from deleterious effects of HIV infection on platelet function, endothelial function, or dendritic arborization [14].

In this cross-sectional cohort, observed parallel trends for HIV and aging in fMRI measures suggest pathophysiological similarities not only throughout the body but in the brain. Body frailty caused by aging and HIV infection results from a complex interplay among viral factors, host responses, and immune dysfunction, causing (1) a shift in T cell phenotypes from naive T cells to memory T cells, (2) an increase in replicative senescence by reductions in T cell proliferation, and (3) an increase in the production and release of cytokines [1]. Within the brain, continued immunological challenges from "normal" aging could deplete available resources, triggering increased functional metabolic demands. Although we did not directly correlate neuroimaging measures with markers of brain immune dysfunction, it is reasonable to hypothesize that persistent low HIV loads and increased inflammation levels may deplete brain reserves, augmenting functional metabolic requirements. Observed increases in metabolic demands could result from excessive neurotransmission from oxidative stress within signaling pathways [15]. Multiple neurodegenerative and neuroimmune mechanisms may converge in older HIV-positive individuals. Future longitudinal analyses are needed, because the effects of HIV infection and aging could proceed in parallel, making it difficult to distinguish between them.

A number of limitations exist with this study. Comorbid substance abuse, such as alcoholism or stimulant dependence, could influence fMRI outcomes. Many of our subjects had a previous history of drug abuse or illegal recreational drug use. However, approximately equal numbers of the HIV-negative control subjects (11 of 25) and HIV-positive subjects (10 of 26) met these criteria. Future studies investigating the interaction between substance abuse, HIV infection, and age are needed. Second, although our cross-sectional cohort was modest in size, a larger sample may have demonstrated even greater differences. Despite somewhat large variability among HIV-positive patients, observed effect sizes for fMRI measures were quite robust (Table 1). Longitudinal studies of older HIV-positive patients are required to confirm these initial findings. Third, the impact of HAART on aging could not be analyzed, because most of the older HIV-positive subjects were receiving medications. Prospective studies among older HIV-positive subjects are needed to address the appropriateness of current HAART regimens, the susceptibility to the adverse effects of HAART, and the potential for increased vulnerability to adverse drug reactions within this population.

Despite these limitations, our results highlight the potential role for fMRI in the evaluation of possible impacts of HIV infection and aging on brain function. We found that both HIV infection and advancing age challenge brain perfusion and functional metabolic pathways within the visual cortex. This may represent diminished capacity and functional frailty within this brain region in HIV-positive and older adults. We do not advocate the use of fMRI for routine diagnostic evaluation of HIV-positive individuals because of its expense and relatively limited availability. However, noninvasive fMRI in research settings could provide valuable information concerning basic pathophysiology of HIV infection within certain brain areas and the potential neurotoxic actions of HAART, and it could assist in the evaluation of neuroprotective therapeutic strategies for older HIV-positive patients.

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References

1. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci **2001**; 56:M146–M156.
2. Valcour V, Shikuma C, Shiramizu B, et al. Higher frequency of dementia in older HIV-1 individuals: the Hawaii Aging with HIV-1 Cohort. Neurology **2004**; 63:822–827.
3. Buxton RB, Uludag K, Dubowitz DJ, Liu TT. Modeling the hemodynamic response to brain activation. Neuroimage **2004**; 23(suppl 1): S220–S233.
4. Chang L, Tomasi D, Yakupov R, et al. Adaptation of the attention network in human immunodeficiency virus brain injury. Ann Neurol **2004**; 56:259–272.
5. Wong EC, Buxton RB, Frank LR. Quantitative imaging of perfusion using a single subtraction (QUIPSS and QUIPSS II). Magn Reson Med **1998**; 39:702–708.
6. Wang J, Qiu M, Constable RT. In vivo method for correcting transmit/receive nonuniformities with phased array coils. Magn Reson Med **2005**; 53:666–674.
7. Ances BM, Liang CL, Leontiev O, et al. Effects of aging on cerebral blood flow, oxygen metabolism, and blood oxygenation level dependent responses to visual stimulation. Hum Brain Mapp **2009**; 30:1120–1132.
8. van Gorp WG, Miller EN, Marcotte TD, et al. The relationship between age and cognitive impairment in HIV-1 infection: findings from the

Multicenter AIDS Cohort Study and a clinical cohort. Neurology **1994**; 44:929–935.
9. Chang L, Wong V, Nakama H, et al. Greater than age-related changes in brain diffusion of HIV patients after 1 year. J Neuroimmune Pharmacol **2008**; 3:265–274.
10. Ernst T, Chang L. Effect of aging on brain metabolism in antiretroviral-naive HIV patients. AIDS **2004**; 18(suppl 1):S61–S67.
11. Thompson PM, Dutton RA, Hayashi KM, et al. Thinning of the cerebral cortex visualized in HIV/AIDS reflects $CD4^+$ T lymphocyte decline. Proc Natl Acad Sci U S A **2005**; 102:15647–15652.
12. Ross MH, Yurgelun-Todd DA, Renshaw PF, et al. Age-related reduction in functional MRI response to photic stimulation. Neurology **1997**; 48:173–176.
13. Buckner RL, Snyder AZ, Sanders AL, Raichle ME, Morris JC. Functional brain imaging of young, nondemented, and demented older adults. J Cogn Neurosci **2000**; 12(suppl 2):24–34.
14. Ances BM, Roc AC, Wang J, et al. Caudate blood flow and volume are reduced in HIV+ neurocognitively impaired patients. Neurology **2006**; 66:862–866.
15. Haughey NJ, Steiner J, Nath A, et al. Converging roles for sphingolipids and cell stress in the progression of neuro-AIDS. Front Biosci **2008**; 13:5120–5130.