Independent Effects of HIV, Aging, and HAART on Brain Volumetric Measures

Beau M. Ances, MD, PhD, Mario Ortega, BS,* Florin Vaida, PhD,† Jodi Heaps, MA,‡ and Robert Paul, PhD‡*

Background: Neurocognitive impairment remains prevalent in HIV-infected (HIV+) individuals despite highly active antiretroviral therapy (HAART). We assessed the impact of HIV, HAART, and aging using structural neuroimaging.

Methods: Seventy-eight participants [HIV− (n = 26), HIV+ on stable HAART (HIV+/HAART+; n = 26), HIV+ naive to HAART (HIV+/HAART−; n = 26)] completed neuroimaging and neuropsychological testing. A subset of HIV+ subjects (n = 12) performed longitudinal assessments before and after initiating HAART. Neuropsychological tests evaluated memory, psychomotor speed, and executive function, and a composite neuropsychological score was calculated based on normalized performances (neuropsychological summary *Z* score, NPZ-4). Volumetrics were evaluated for the amygdala, caudate, thalamus, hippocampus, putamen, corpus callosum, and cerebral gray and white matter. A 3-group 1-way analysis of variance assessed differences in neuroimaging and neuropsychological indices. Correlations were examined between NPZ-4 and volumetrics. Exploratory testing using a broken-stick regression model evaluated self-reported duration of HIV infection on brain structure.

Results: HIV+ individuals had significant reductions in brain volumetrics within select subcortical regions (amygdala, caudate, and corpus callosum) compared with HIV− participants. However, HAART did not affect brain structure as regional volumes were similar for HIV+/HAART− and HIV+/HAART+. No association existed between NPZ-4 and volumetrics. HIV and aging were independently associated with volumetric reductions. Exploratory analyses suggest caudate atrophy due to HIV slowly occurs after self-reported seroconversion.

Conclusions: HIV associated volumetric reductions within the amygdala, caudate, and corpus callosum occurs despite HAART. A gradual decline in caudate volume occurs after self-reported seroconversion. HIV and aging independently increase brain vulnerability. Additional longitudinal structural magnetic resonance imaging studies, especially within older HIV+ participants, are required.

Key Words: HIV, HAART, aging, brain volume

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INTRODUCTION

Before the advent of highly active antiretroviral therapy (HAART), the life expectancy of HIV-infected (HIV+) participants was less than 10 years after initial diagnosis.[1] The introduction of HAART has prolonged the lives of HIV+ participants with many living for more than 20 years after seroconversion. As a result, more HIV+ individuals are reaching advanced ages.[2] If the current trends continue, more than 50% of all HIV+ participants in the United States will be more than 50 years old by 2015.[3] Older HIV+ participants may be at increased risk for accelerated body aging.[4–7] Similar changes could occur in the brain.[8–10] Given the importance of brain function to overall clinical outcomes and HAART compliance, it is critical that sensitive indicators of possible brain disruption are discovered.

Brain volumetric measures may represent a robust method for quantifying brain integrity in the presence of HIV.[11] Brain atrophy has been well described since the early discovery of the virus. Many pre-HAART studies demonstrated HIV associated losses within subcortical regions.[12–15] The degree of atrophy due to HIV has been shown to correlate with poorer neuropsychological performance.[16–19]

Despite the introduction of HAART, changes in brain structure have persisted within impaired and cognitively normal HIV+ individuals.[17,20–23] More recent studies in the HAART era have demonstrated that HIV associated brain atrophy occurs not only within subcortical areas but also cortical regions.[17,21,24,25] However, most of the studies have primarily focused on HIV+ participants on stable therapy (approximately 80% of any cohort)[10,17,20–25] rather than HIV+ participants naive to medications.

Historically, brain atrophy within HIV+ patients has been attributed to direct or indirect effects of the virus; yet, growing concerns exist over possible neurotoxic risks associated with long-term administration of HAART.[26,27] Cell culture studies have demonstrated neuronal loss after the

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From the *Department of Neurology, Washington University School of Medicine, St Louis, MO; †Department of Family and Preventive Medicine, University of California San Diego, San Diego, CA; and ‡Department of Psychology, University of Missouri, St Louis, MO.

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Correspondence to: Beau M. Ances, MD, PhD, Department of Neurology, Washington University in St Louis, Box 8111, 660 South Euclid, St Louis, MO 63110 (e-mail: bances@wustl.edu).

introduction of HAART.[28,29] In humans, neuropsychological performance improved in a large cohort of HIV+ participants after discontinuing HAART[30] or after participating in a drug holiday.[31] In particular, efavirenz may be neurotoxic as a higher prevalence of HAND has been observed in HIV+ individuals taking this medication.[32] A recent neuroimaging study has also demonstrated a reduction in neuronal function in HIV+ participants after initiating HAART.[33] These findings suggest a need to further investigate the potential side effects of HAART on brain structure.

Given the continued prevalence of HAND despite HAART, isolating additional factors that might influence brain integrity will be crucial for determining if and when neuroprotective interventions should be administered. The aim of this study was to determine the effects of HIV, HAART, and aging on subcortical and cortical brain volumetric measures. We hypothesized that HIV, and to a lesser extent HAART, is associated with structural atrophy. We additionally predicted that HIV and aging would each independently relate to reductions in brain volumetric measures. Results from the study could support the use of structural neuroimaging as a possible tool for differentiating the impact of HIV from other factors associated with loss of brain integrity.

MATERIALS AND METHODS

Participants and Laboratory Testing

Seventy-eight participants provided written consent approved by the institutional review board at Washington University in St Louis. Individuals with a history of confounding neurological disorders including epilepsy, stroke, and head injury resulting in a loss of consciousness of more than 30 minutes; major psychiatric disorders; or active substance abuse were excluded. Serological status of all HIV+ participants was confirmed by documented positive HIV enzyme-linked immunoassay and Western blot or detection of plasma HIV RNA by polymerase chain reaction. All HIV+ participants had laboratory evaluations [plasma CD4 cell count and HIV viral load (VL)]. The cohort was divided into 3 groups: HIV− subjects (n = 26), HIV+ individuals on a stable HAART regimen for at least 3 months before imaging (HIV+/HAART+; n = 26), and HIV+ participants naive to HAART (HIV+/HAART−; n = 26). In addition, a subgroup of the HIV+/HAART− (n = 12) were longitudinally assessed immediately before starting medications and approximately 6 months after being on a stable therapy (see **Figure, Supplemental Digital Content 1,** http://links.lww.com/QAI/A263).

Neuropsychological Evaluation

A standard neuropsychological performance battery [including Trail-Making Tests A and B, the Hopkins Verbal Learning Test-Revised, and the Digit-Symbol Modalities Test (WAIS-IIIR)] was administered to participants. These tests examine memory, psychomotor speed, and executive function and have been previously used to briefly assess HIV− associated neurocognitive disorder.[31,34] Raw scores from each test were standardized using demographic (age, gender, race, education) adjusted normative means.[35] A standardized *z* score was calculated by subtracting the appropriate normative mean from the raw score and then dividing by the normative standard deviation. A composite neuropsychological summary *Z* score (NPZ-4) was calculated by averaging *z* scores from each test. For longitudinal assessments, alternate forms of the Hopkins Verbal Learning Test-Revised were administered. These different versions ensured that subjects were not influenced by previous exposure.

Medications

The specific regimens that HIV+/HAART+ participants (n = 26) received included 14 nucleoside reverse transcriptase inhibitors (NRTIs) + non-NRTIs, 11 NRTIs+ protease inhibitors, or 1 NRTIs only. The longitudinal subgroup of HIV+ participants (n = 12) were followed-up before and after receiving either 7 NRTIs + non-NRTIs or 5 NRTIs+ protease inhibitors. The ability of a particular antiretroviral (ARV) medication to penetrate across the blood–brain barrier has been classified using a central penetration effectiveness (CPE) score.[36] The score for each ARV in a HIV+ participant's regimen was summed and a total CPE was calculated for a particular regimen.

Imaging

Scanning was performed using a 3T Siemens Tim TRIO whole-body magnetic resonance scanner (Siemens AG, Erlangen, Germany) with a product transmit/receive head coil. Structural imaging were acquired using a T1-weighted 3-dimensional magnetization-prepared rapid acquisition gradient echo sequence [time of repetition (TR)/echo time (TE)/inversion time (TI)] = 2400/3.16/1000/milliseconds, flip angle = 8 degrees, 162 slices, and voxel size = $1 \times 1 \times 1$ mm^3). Images were visually inspected at the scanner with an additional scan performed if significant movement was observed.

A general automated processing stream was performed using a previously described software package (Freesurfer Version 4.5 (2008) developed at the Martinos Center, Harvard University, Boston, MA; http://surfer.nmr.mgh.harvard.edu).[37,38] Briefly, magnetization-prepared rapid acquisition gradient echo scans were transformed into a format compatible for Freesurfer. Brain segmentation was accomplished with voxel intensity normalization applied to remove magnetic resonance bias. Skull stripping was performed to remove the surrounding tissue. The brain was subsequently registered to a template volume in Talairach space. Based on prior probabilities of voxel identity assigned by the Freesurfer atlas,[39] subcortical and cortical volumes were subsequently delineated.[38] The quality of segmentation was independently confirmed by 2 reviewers extensively trained in Freesurfer processing (M.O. and J.H.). Segmented volumes were manually edited only when outliers were identified. Once corrected, these scans were reanalyzed.[40] Using a least squares residual regression model, normalized values were obtained from 6 bilaterally selected regions of interest[41] (ROIs). These ROIs included

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the amygdala, caudate, thalamus, hippocampus, and putamen (Fig. 1). The total corpus callosum value was obtained by summing the posterior, posterior-middle, middle, anterior-middle, and anterior portions. In addition, total cerebral cortex, gray matter, and white matter volumes were determined. For the subgroup that was assessed before and after starting HAART, the longitudinal option of the Freesurfer 4.5 pipeline was used.

Statistical Analysis

Cross-sectional Analysis

Brain volumetric values were transformed to the natural logarithmic scale to improve the normality of distribution and to facilitate the interpretation of the results. The differences among groups on the logarithmic scale were then suitably transformed and reported in terms of proportional differences of mean volume among groups. The logarithm transformation allowed for comparisons of relative differences across brain regions. A 1-way analysis of variance was first conducted for each of the brain regions, comparing the 3 groups: HIV−, HIV+/HAART−, and HIV+/HAART+. The P values for the 9 analysis of variances were adjusted for multiple comparisons using the Hochberg algorithm.[42] Volumes that showed a significant difference at the 0.10 level were considered for further assessment. The exploratory nature of the study justified using this less strict level of selection. In particular, 3 ROIs were subsequently studied.

Within these 3 ROIs, a linear model analysis was performed to investigate the difference in the log volumes between groups. These analyses were adjusted for age and/or sex and if these variables were significant at $P < 0.20$. Among the HIV+ subjects (HIV+/HAART− and HIV+/HAART+), the association between laboratory values (current CD4 cell count, nadir CD4 cell count, $\log_{10}$ plasma HIV VL) and brain volumetric measures were assessed via simple linear regression models. However, for duration of infection, we also controlled for age to detect any effects of HIV infection in addition to natural aging.

Longitudinal Analysis

In a subgroup of HIV+/HAART− participants, structural imaging was performed immediately before starting medications and at approximately 6 months after the subject

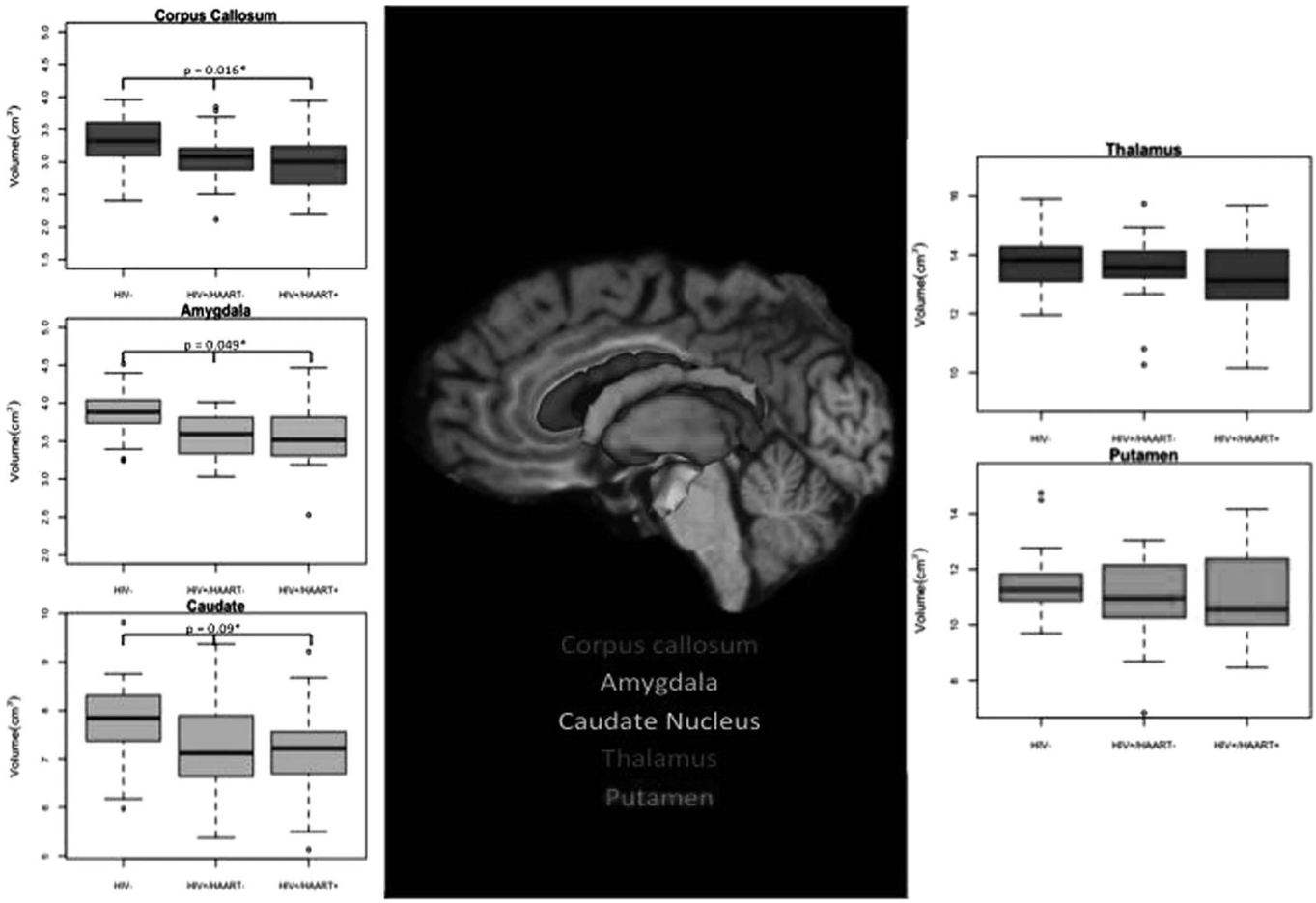

FIGURE 1. Using Freesurfer, the corpus callosum, amygdala, caudate, thalamus, and putamen were determined for each subject. *P value for brain regions that were significantly different after a 1-way analysis of variance (correcting for multiple comparisons). All error bars are presented as quartiles.

was successfully on a stable ARV regimen. Brain volumetric measures for each of the above ROIs, laboratory values, and NPZ-4 scores were compared for the 2 time points using simple paired *t* tests. In addition to main effects of HIV and age, an interaction between HIV and age on brain volume was included if significant at the 0.05 level.

Effects of Age

For the caudate where both age and HIV effects were observed, an exploratory analysis was performed investigating the hypothesis that the HIV effect develops gradually, from no effect at the time of infection, to a steady-state effect, after a certain period of time. In this scenario, the effect of HIV infection on the brain region acts in 2 stages: stage 1 is a transition stage, in which brain loss is gradual, from the uninfected levels to the long-term infected volumes. Stage 2 is the steady-state situation, in which the effect of HIV translates into a constant reduction in brain volume. At this stage, the impact of HIV is in addition to the natural aging process. To this end, we used cross-sectional data using self-reported duration of infection (di). The HIV− group was included with di = 0, that is, as a surrogate for subjects just before infection. This is a natural exploratory hypothesis and model because it is hard to conceive that the difference in brain volumes between the HIV+ and HIV− subjects would occur immediately. For additional clarity, we included a smooth estimation of brain volumes. Accordingly, first, a nonparametric lowess smoother[43] was used to explore the effect of time since infection on brain volumes for HIV+ subjects only. Second, the HIV effect was estimated using a "broken-stick" (or piecewise linear) regression model,[44] in which the transition stage between time 0 and T has a linear effect on the log-brain volume, and the steady state, from T onwards, where the effect of HIV infection is constant. T was the time required for the brain volume at seroconversion to transition to the level of a chronic HIV infection. For this analysis, the effect of self-reported duration of infection (D) on the log volume was equal to 0 if $D = 0$ (ie, for HIV− subjects), βD if $0 < D < T$, and βT if $D \geq T$. As a consequence, βT was the age-adjusted difference in mean response between the HIV− and the chronically infected HIV+ group. The coefficient β was determined from the regression model. The breakpoint T, representing self-reported duration of transition time associated with atrophy due to HIV, was determined through a grid search that used a maximum likelihood. A 95% confidence interval for T was calculated via parametric bootstrap. To determine the line of best fit, a more gradual change in brain volume was compared with abrupt brain atrophy by the Akaike information criterion.[45] All analyses were performed using *R* statistical software (version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

HIV+ Participants Performed Worse on Neuropsychological Performance Testing than HIV−

Cross-sectional demographic variables for the 3 groups (HIV−, HIV+/HAART−, and HIV+/HAART+) are shown in Table 1. No significant differences were noted among the groups with regard to age, sex, or education. In general, the HIV+ subjects performed poorly on neuropsychological performance testing compared with HIV− subjects ($P = 0.02$). This difference was primarily driven by the HIV+/HAART− group who had worse NPZ-4 scores than HIV− ($P = 0.03$) but who were not different from the HIV+/HAART+ ($P = 0.09$). Differences between HIV+/HAART+ and HIV+/HAART− included self-reported duration of disease with HIV+/HAART+ participants infected for a significantly longer time period. The 2 HIV+ groups also differed on HIV markers (CD4 nadir, CD4 current, and $\log_{10}$ plasma HIV VL). The HIV+/HAART+ subjects had a significantly lower nadir and a higher current CD4 cell count. Log_{10} plasma HIV VL was

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TABLE 1. Demographic, Medical, Neuropsychological, and Laboratory Values for all Subjects

	HIV− (n = 26)	HIV+/HAART− (n = 26)	HIV+/HAART+ (n = 26)	*P*
Demographics				
Mean age, yr	34 ± 11	37 ± 13	40 ± 12	0.27
Education, yr	14 ± 3	13 ± 2	13 ± 2	0.14
Sex, % male	77	92	85	0.13
Medical and neuropsychological				
Duration of HIV infection, months	NA	36 ± 69	126 ± 89	<0.001
Duration on HAART, months	NA	NA	66 ± 78	NA
Central nervous system penetration effectiveness score	NA	NA	7.0 ± 0.4	NA
NPZ-4 score	−0.03 ± 0.19	−0.76 ± 0.20	−0.16 ± 0.19	0.02
Laboratory				
Median CD4, cells/μL (quartiles)	NA	363 (198–429)	462 (353–771)	0.004
Median CD4 nadir, cells/μL (quartiles)	NA	287 (195–403)	193 (52–377)	0.003
Median Log plasma viral load, copies (quartiles)	NA	3.51 (3.51–5.06)	1.69 (1.69–1.81)	<0.001
% Virologically suppressed, <50 copies/mL	NA	NA	77	NA

All errors are reported as SD of the mean, and quartiles for laboratory values.
NA, not available.

significantly lower in HIV+/HAART+ group with more than 3 quarters of these participants having an undetectable value (<50 copies/mL). These results likely reflect successful reconstitution with ARVs. The average CPE score for the HIV+/HAART+ participants was 7 with regimens chosen by health practitioner in conjunction with patient preferences.

Brain Volumes From Select Regions Were Reduced in HIV+ Individuals

Brain volumetric measures were obtained from the ROIs (Fig. 1). Only 3 ROIs were significantly different (amygdala, corpus callosum, and caudate) among the groups (adjusted P values = 0.016, 0.049, and 0.090, respectively). For the 3 ROIs, HIV+/HAART− and HIV+/HAART+ participants had significantly smaller volumetric measures compared with who are HIV−. HAART did not affect brain structure as volumetric measures for each of the regions were similar for HIV+/HAART− and HIV+/HAART+. The total cerebral cortex volume as well as gray and white matter volumes was similar among the groups (see **Table, Supplemental Digital Content 2,** http://links.lww.com/QAI/A264).

Changes in Brain Volumetrics Did Not Correlate With Typical Laboratory Markers of HIV Disease

For the HIV+ groups, the relationship between known markers of HIV disease and volumetric measures were also assessed. No significant correlations existed between volumetric measures within the 3 ROIs and either current CD4 cell count or CD4 nadir cell count. HIV VL was correlated with caudate volume for HIV+/HAART+ participants (P = 0.001). However, these results were primarily weighted by 6 HIV+/HAART+ participants who had escaped viral suppression (<50 copies/mL) despite being on HAART. If these subjects were removed from the analysis, then no significant differences were seen. In addition, no significant correlations existed between $\log_{10}$ plasma HIV VL and brain volumetric measures for HIV+/HAART− subjects. With regard to neuropsychological performance, no significant correlations existed between NPZ-4 scores and volumetric measurements for each of the 3 regions (all $P > 0.30$).

Brain Volumetric Measures Were Inversely Correlated With Age in HIV+ Participants

A decrease in caudate volume was seen with increasing age for the 3 groups. Because HIV+/HAART− and HIV+/HAART+ participants had similar results (data not shown), these 2 groups were combined into a single group (HIV+) and compared with HIV− subjects (Fig. 2). Both increasing age and HIV infection were each associated with significant decreases in caudate volume, but no significant interaction was present. For every 10 years, HIV led to a 6% decrease in caudate volume, whereas aging resulted in a 4% decrease (Table 2). Overall, for a given age, caudate volumetric values in a HIV+ subject were equivalent to a HIV− participant who was approximately 17 years older. For the amygdala and the corpus callosum, significant effects of HIV but not aging were observed. No significant age by HIV interactions were seen within these 2 regions (Table 2).

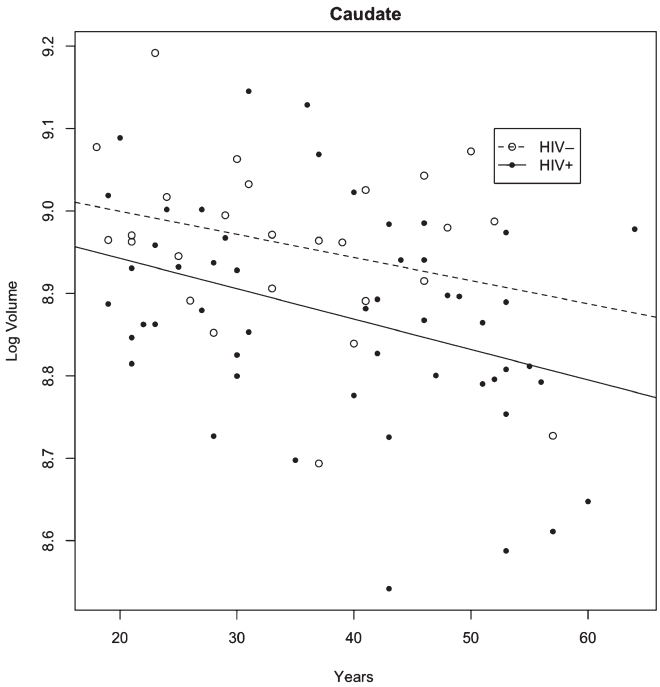

FIGURE 2. Effects of HIV and aging within the caudate. For both HIV+ and HIV− subjects, a significant reduction in caudate volume was seen with aging. Overall, HIV+ participants had a greater atrophy than HIV− participants. For this analysis, HIV+/HAART+ and HIV+/HAART− were combined into a single group as similar results were seen for the 2 subgroups.

HAART Does Not Change Brain Volumetrics

Within the subset of the HIV+/HAART− that started medications, neuroimaging was performed both before and after starting HAART. The introduction of HAART led to a significant reduction in $\log_{10}$ plasma HIV VL ($P <$ 0.001) and an increase in CD4 cell count (P = 0.03; data not shown). However, there was no change in volumetric measures within select ROIs (ie, caudate) after starting HAART (see **Figure, Supplemental Digital Content 3,** http://links.lww.com/QAI/A265). Some improvements on neuropsychological performance testing were seen at the second assessment, but this difference was not statistically significant (P = 0.65). For each of the ROIs, there was no significant effect of self-reported duration of HAART on brain volume (data not shown).

Caudate Volume Atrophies After Approximately 13 Years of HIV Infection

The difference in brain volume between the HIV+ and HIV− subjects suggests that following the time of self-reported infection, certain regions may be at increased risk for atrophy. Based on an exploratory model that uses the

TABLE 2. Effects of HIV and Aging on Select Regions of Interest

	Difference (95% CI)	Cohen *d* (95% CI)	*P*
Caudate			
HIV effect	−6.1% (−11.2% to −0.7%)	0.55 (0.06 to 1.05)	**0.028**
Age effect (per 10 yr)	−3.6% (−5.6% to −1.5%)	0.32 (0.13 to 0.51)	**0.001**
HIV and age interaction			0.62
Amygdala			
HIV effect	−6.8% (−10.9% to −2.6%)	0.78 (0.29 to 1.27)	**0.002**
Age effect (per 10 yr)	−1.2% (−2.9% to 0.5%)	0.14 (0 to 0.33)	0.15
HIV and age interaction			0.21
Corpus callosum			
HIV effect	−8.2% (−13.9% to −2.2%)	0.66 (0.17 to 1.15)	**0.009**
Age effect (per 10 yr)	−0.7% (−3.1% to 1.8%)	0.05 (0 to 0.24)	0.57
HIV and age interaction			0.091

An effect of HIV was seen within each of these regions. Only within the caudate was there an effect of HIV and aging. No interaction was seen between HIV and aging in the 3 regions. AU9

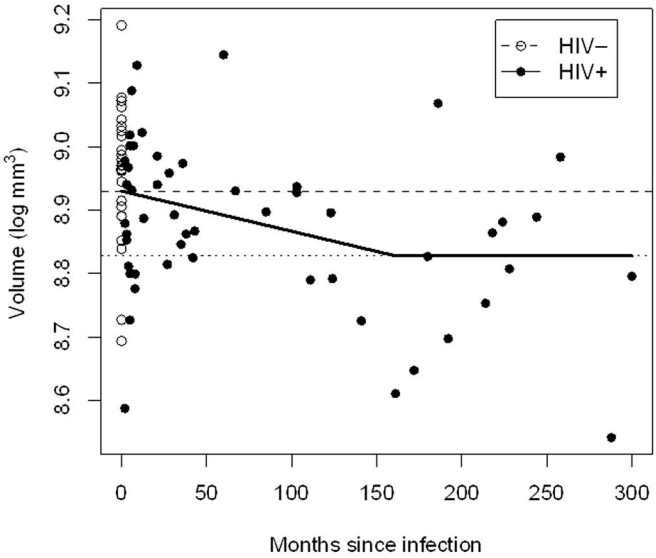

FIGURE 3. Effects of self-reported duration of HIV infection on caudate volume. HIV− are represented at $T = 0$. This group can be seen as subjects just before HIV infection. The upper dashed line is the mean log volume for HIV−. The lower dotted line is the mean log volume for chronically infected HIV. The dashed smooth line (lowess) and the solid broken-stick line (piecewise linear model) show the progression of caudate atrophy from the HIV− status to the chronically infected HIV+ status. Approximately 160 months (approximately 13 years) is required for this transition. A large amount of variability exists. All lines were age adjusted.

broken-stick method, the estimated time (T) needed for the brain volume to transition from self-reported date of HIV+ infection to HIV−, the level for chronically HIV+ infection was 161 months (13.4 years), 95% confidence interval (3–300 months) (Fig. 3). Although a significant degree of variability was observed, a more gradual decline in brain volume fit the data better than a one implying abrupt atrophy (improvement in Akaike information criterion = 0.57). F3

DISCUSSION

HIV is associated with a significant reduction in volume within select subcortical regions including the amygdala, caudate, and corpus callosum. A reduction in brain volumetrics was seen for HIV+ participants regardless of the presence or absence of HAART. Observed changes in brain volume due to HIV infection were independent of aging. Our results suggest that HIV+ individuals continue to have volumetric loss even in the HAART era.

Changes in brain volume due to HIV were primarily seen within subcortical areas such as the amygdala, caudate, and corpus callosum. Observed changes correspond with previous volumetric studies that showed significant decreases in particular subcortical regions such as the caudate, amygdala, and hippocampus.[9,10,20,21,23] Changes have also been previously noted within the corpus callosum using volumetrics.[21,46] In contrast, other subcortical areas such as the thalamus, putamen, or parahippocampus were not affected.[17,24,25] The precise reason why specific subcortical areas are affected remains unknown, but may reflect the close proximity of these structures to the ventricles.[47] This proximity may allow for easier viral penetration by HIV-infected mononuclear cells trafficking into the brain as well as increased HIV toxic products, such as gp120 and Tat.[48–50] In particular, the highest concentrations of HIV have been observed in the corpus callosum and caudate compared with other brain regions.[20,48,51] Our results suggest that pathological changes continue to persist, primarily in subcortical regions, despite HAART.[11]

In this analysis, we observed no significant differences in total cerebral cortex and gray matter or white matter volume for HIV+ participants compared with who are HIV−. A number of studies have observed a decrease in gray matter because of HIV infection,[17,20,24,52] whereas others also observed no significant changes.[12,53–56] Discrepancies between the various studies may be because of differences in the degree of impairment in HIV+ participants as well as the inclusion of matched HIV− participants.

Interestingly within the HIV+ participants, similar volumetric values were observed for HIV+/HAART+ and HIV+/HAART− subjects. Furthermore, when assessing HIV+/HAART− participants both before and after starting on medications, no significant changes were observed in subcortical and cortical volumes despite good virological control. Although no deleterious structural alterations could be specifically attributed to HAART, this cross-sectional study was not designed to determine with certainty if HAART is toxic to brain structures. Larger longitudinal studies with structured assessment end points are required for HIV+ participants receiving HAART.

In this study, we also observed no significant correlations between laboratory values such plasma HIV values (CD4 current, CD4 nadir, or $\log_{10}$ plasma HIV VL) and structural neuroimaging measures. A number of studies have observed no correlation between current CD4 or nadir CD4 and brain volumes.[12,22,25,57] However, other investigators have observed a strong correlation between laboratory values, especially CD4 nadir, and brain volumes.[17,21,46,53–56,58] A complex relationship may exist with a reduction in brain volumetric loss occurring not only at a lower CD4 nadir but also at a higher current CD4 cell count because of significant inflammation associated with reconstitution.[55] Observed differences between various groups may reflect differences (1) in the sample size studied, (2) when a subject was scanned in relation to starting medications, (3) in methods used for brain segmentation,[46] and (4) in regions investigated. Additional longitudinal studies of larger cohorts of HIV+ participants on stable HAART need to be performed.

Our exploratory analysis using a broken stick model demonstrated that brain atrophy may slowly develop over time. Based upon the self-reported duration of infection, our model estimates that a period of approximately 13 years is required for atrophy within the caudate to reach levels observed in this study. These changes can occur despite initiation of HAART because most HIV+ participants were prescribed medications when they met the current guidelines (CD4 cell count <350 cells/mm^3 or an AIDS-defining illness). A wide variability existed for this effect. Our exploratory results suggest that a chronic subclinical process continues to occur within the caudate despite peripheral control of viral replication.[24] Observed atrophy within the caudate could reflect low-level inflammation and neuronal loss.[10,21] Initiation of adjunctive therapies soon after initial infection could be beneficial in an attempt to preserve brain integrity within the caudate.[16] Additional longitudinal studies are required in acute and early HIV+ participants to confirm the effects of HIV on this brain structure.

Both HIV and aging independently affect brain volume regardless of HAART status. Previous studies using structural imaging,[24] magnetic resonance spectroscopy,[10,33] and arterial spin labeling[8] have shown that age and HIV independently affect the brain. Our results are similar to these previous studies. In our cohort, HIV led to approximately 17 years of aging of the brain. No interaction occurred between HIV and aging. However, in each of the previous studies that assessed HIV and aging, HIV+ participants both on and off HAART were combined into a single group and compared with HIV− HIV−. In this study, HIV+ individuals were specifically divided into 2 groups (HIV+/HAART− and HIV+/HAART+) to identify the possible effects of HAART on structural brain changes as they relate to HIV. No significant differences were seen between HIV+/HAART− and HIV+/HAART− with regard to caudate volume as a function of age. Overall, our results suggest that a decrease in brain integrity within older HIV+ participants, even those taking HAART, may indicate that these individuals have increased vulnerability for subsequently developing neurodegenerative disorders.

In terms of neuropsychological function, the HIV+/HAART− group performed worse than HIV− group. The HIV+/HAART+ also had mild cognitive disturbances but did not perform significantly worse than HIV− individuals. These changes in neuropsychological performance were not correlated with volumetric measures. The HIV+/HAART+ group had lower mean volumetric measures for most ROIs despite having better NPZ-4 test performances. The etiology of this discordance between neuroimaging and neuropsychological performance remains unknown. Previously, HAART has been shown to lead to mild improvements in neuropsychological performance.[26] Although a slight improvement in neuropsychological performance was seen after starting HAART, these changes were not significant in the subgroup of HIV+ participants assessed longitudinally. These changes may be influenced by practice effects or the test battery used. A rather limited number of neuropsychological tests were administered in this study and therefore may not be sensitive to the possible subtle changes in cognition. Previous work has described the relationships between neuropsychological performance and neuroimaging in greater detail.[18]

A limitation of this study was that the 2 HIV+ groups differed significantly in their self-reported duration of HIV infection. Participants may not have accurate recall of the time they seroconverted or may initially present to a health care provider at different stages of the disease. Larger longitudinal studies that follow-up HIV+ participants soon after documented seroconversion are needed to assess changes in brain volume due to HIV. In addition, this study was unable to assess if particular regimens could further modify brain atrophy. The effects of CPE on brain volumetric measures could not be assessed because most participants were prescribed only a limited number of regimens. On average, most HIV+ participants were taking regimens with relatively good brain penetration (average CPE = 7). Further studies comparing brain volumetric measures between HIV+ participants on high and low CPE regimens are needed.

In summary, this study has demonstrated that HIV continues to be associated with brain atrophy within subcortical regions in the HAART era. It is possible that this volume change occurs slowly over time even with the initiation of HAART. It is therefore important that HIV is diagnosed early with additional neuroprotective agents considered for preventing volume loss with subcortical areas. In addition, both HIV and age could independently decrease brain volume, but no significant interaction was observed. Additional studies of older HIV+ (>50 years old) are needed to determine if they are at increased vulnerability for subsequently developing neurodegenerative diseases.

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Author Contributions: B. Ances proposed the project, performed statistical analyses, drafted the manuscript, and supervised the project, M. Ortega analyzed the data and revised the manuscript, F. Vaida performed statistical analyses, J. Heaps analyzed the data and revised the manuscript, and R. Paul revised the manuscript.

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