Mapping cerebellar degeneration in HIV/AIDS

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Progressive brain atrophy in HIV/AIDS is associated with impaired psychomotor performance, perhaps partly reflecting cerebellar degeneration; yet little is known about how HIV/AIDS affects the cerebellum. We visualized the three-dimensional profile of atrophy in 19 HIV-positive patients (age: 42.9 ± 8.3 years) versus 15 healthy controls (age: 38.5 ± 12.0 years). We localized consistent patterns of subregional atrophy with an image analysis method that automatically deforms each patient's scan, in three dimensions, to match a reference image. Atrophy was greatest in the posterior cerebellar vermis (14.9% deficit) and correlated with depression severity (P=0.009, corrected), but not with dementia, alcohol/substance abuse, CD4 + T-cell counts, or viral load. Profound cerebellar deficits in HIV/AIDS (P=0.007, corrected) were associated with depression, suggesting a surrogate disease marker for antiretroviral trials. *NeuroReport* 00:000–000 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

HIV/AIDS is the fourth leading cause of death worldwide, and one in every 100 adults aged 15–49 years is HIV-infected [1]. Antiretroviral therapy has greatly increased life expectancy for many of those infected with HIV, but as patients survive longer, there is increasing concern that chronic viral neurotoxicity can lead to progressive brain atrophy and associated neuropsychological/neurocognitive impairment in some patients, which is not resisted by current treatments. Only 15% of those infected have overt dementia, but around 35% of patients exhibit neurocognitive deficits affecting concentration, psychomotor skills, and information processing. Cognitive impairments range from minor cognitive motor disorders to HIV-associated dementia, often with a progressive trajectory leading to death [2]. Other studies suggest that, paradoxically, although antiretroviral treatments have limited penetration of the blood–brain barrier, there may be little change in cognitive function over several years so long as peripheral levels of therapeutic drugs are maintained [3]. Cognitive deficits are thought to result from the effect of HIV on the brain, as they commonly occur in the absence of opportunistic infections, which take advantage of the progressively declining immune system.

HIV infection is associated with progressive striatal, hippocampal, and white matter volume loss, starting in the medically asymptomatic stage, and accelerating later [4,5]. The few MRI-based studies of HIV/AIDS reveal that atrophy reaches 10–15% in selective brain regions, with a predilection for systems involved in motor control such as the basal ganglia [6,7], and primary, supplementary motor, and prefrontal cortices [8]. The caudate and adjacent ventricles are enriched in the virus, and therefore atrophy may progress as the virus spreads out radially into cortical projection areas [9].

Classically defined as a motor control center, the cerebellum is increasingly recognized as contributing to general cognitive processing and emotional control [10]. Studies in rats have associated HIV infection with increased cerebellar neuronal death [11], but little is known regarding the extent of atrophy in HIV patients and how it relates to cognition, although one earlier case study found a speech disorder associated with cerebellar dysfunction after HIV infection [12]. To further elucidate the connection between HIV and neuropsychological impairment, we mapped the profile of cerebellar atrophy in three dimensions (3D), and correlated atrophy with neuropsychological measures, depression and dementia ratings, T-cell counts, viral load, and cerebellar function measures. We used a recently validated analysis that fluidly deforms MRI scans onto a common template [6]. The applied deformations were analyzed statistically to gauge the level of atrophy, visualizing systematic differences between patients and controls. We hypothesized that the cerebellar vermis would show greatest atrophy, and therefore we also hand-traced cerebellar subregions using a standardized protocol, for additional anatomic validation.

Patients and methods

Patients

All 34 patients were identically scanned with 3D volumetric spoiled gradient echo T1-weighted brain MRI ($256 \times 256 \times 124$ matrix; 24 cm field-of-view; 1.5 mm slices, zero gap; flip angle, 40 degrees, echo/repetition time: TE=5 ms, TR=25 ms). Nineteen of them were HIV-positive AIDS patients (mean age 42.9 ± 8.3SD years, three females/16 males) and 15 were matched HIV-seronegative healthy control participants with similar HIV-related risk factors (age: 38.5 ± 12.0 years, 5 females/10 males). All patients met the Centers for Disease Control criteriafor AIDS, and none had HIV-associated dementia (patients' mean CD4 + T-cell count was 448.0 + /320.9 SD; patients' mean $\log_{10}$ viral load was 2.53 ± 1.17 RNA copies per milliliter blood plasma). AIDS patients seen in the sentinel offices and clinics of an Allegheny County (Pennsylvania, USA) health care provider network were approached for participation by their treating physician. All AIDS patients were eligible to participate, excluding only those with a history of central nervous system opportunistic infections, lymphoma, or stroke. Demographic data for this cohort are reported in ref. [13]. The study was approved by the Institutional Review Board and all participants gave informed consent.

Neurobehavioral assessment

Briefly, each participant underwent a detailed neurobehavioral assessment within 4 weeks before their MRI scan, involving a neurological exam, psychosocial interview, and neuropsychological testing, including (i) a semistructured psychiatric interview, modified from the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorder*, 3rd edition revised [14] administered by a trained interviewer; (ii) the Brief Symptom Inventory [15] and the Neuropsychiatric Inventory [16] to assess subclinical psychiatric symptoms; (iii) Heaton's Patient's Assessment of Own Function questionnaire [17] and the Modified Instrumental Activities of Daily Living scale [18]; and (iv) measures from multiple cognitive domains, sensitive to AIDS-related cognitive impairments [13]. Detailed neurocognitive test data from a partially overlapping set of patients is summarized in ref. [8], and included tests of psychomotor speed, estimated intelligence quotient [19], and the Wechsler Adult Intelligence Scale-Revised [20]. Depression and substance abuse were established based on *Diagnostic and Statistical Manual of Mental Disorder*, 4th edition criteria applied to the results from the structured interview. A diagnosis of HIV-associated dementia was established after a review of study results (J.T.B., H.J.A., O.L.L.) using the current NIMH/NINDS Consensus Criteria [21].

Image processing

All MRI scans were aligned through linear (9-parameter) scaling to the standardized coordinate system of the ICBM-53 average brain to correct for global intersubject differences in brain scale. The resulting images were hand-edited to include only the cerebellum, and aligned using a 9-parameter global scaling transform to a cerebellum-only ICBM-53 template to further improve intersubject alignment. Tensor-based morphometry was performed, as detailed in [6] Chiang *et al*. Briefly, cerebellar images were fluidly deformed in 3D to match a Mean Deformation Template, that is, a standard brain image created by applying the average deformation field of all 34 patients warped onto one randomly selected temporary target to that temporary target. Such a customized template avoids bias toward one group in the level of any registration errors.

Statistics

In tensor-based morphometry, maps of the local expansion factor (sometimes called the Jacobian determinant) between the mean template and each individual encode the local differences in volume between each individual and the template, after global volume differences are discounted (these global differences are examined in the region of interest analysis below). At each image voxel in the cerebellum, a linear statistical model assessed whether local volume at that point depended on (i) diagnosis, (ii) cognitive impairment, (iii) depression scores, (iv) CD4 + T-cell counts, (v) viral load (after logarithmic transformation), (vi) cerebellar function, and (vii) alcohol and substance abuse. The *P* value describing the significance of this linkage was plotted at each point in the cerebellum using a color code to produce a significance map. Color-coded statistical maps also visualized local volume differences between AIDS patients and controls. The spatial maps (uncorrected) visualize the spatial patterns of cerebellar degeneration (Fig. 1). Permutation testing and positive false discovery rate methods (pFDR; [22]) were used to compute their overall significance (implemented as in [6] Chiang *et al*., 2007). Each of these two commonly used alternative methods gives overall *P*- and pFDR values for maps of observed effects, corrected for multiple comparisons, and represents the likelihood of the observed pattern of group differences that are found by chance. The overall *P* value in permutation testing was computed by comparing the number of voxels of the largest suprathreshold cluster (the suprathreshold cluster was defined as voxels with a significant *P* value of less than 0.01) in the true labeling to the permutation distribution. Finally, to create simple summary measures of volumetric deficits, manually traced delineations of the anterior, posterior, and inferior vermis were performed following a standardized protocol, by a rater blind to diagnosis and other demographic data (S.E.L.; Fig. 2) [23].

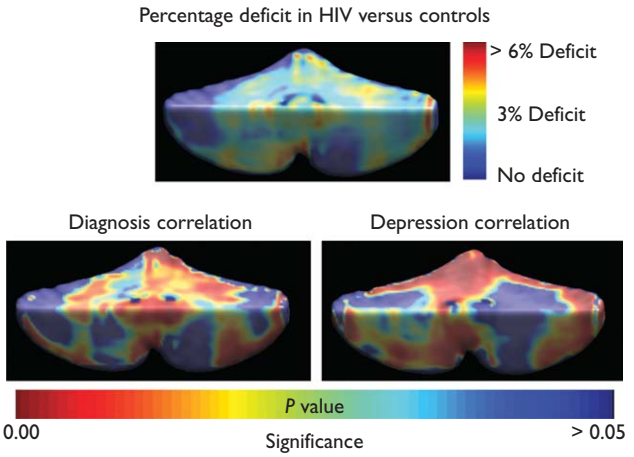

Fig. 1 Mapping cerebellar atrophy. Tissue volumes are reduced by 3–6%, on average, in the HIV group versus controls (top map). The level of atrophy is associated with diagnosis (bottom left; *P*=0.007, corrected) and with depression severity in the patient group (*P*=0.009, corrected, bottom right).

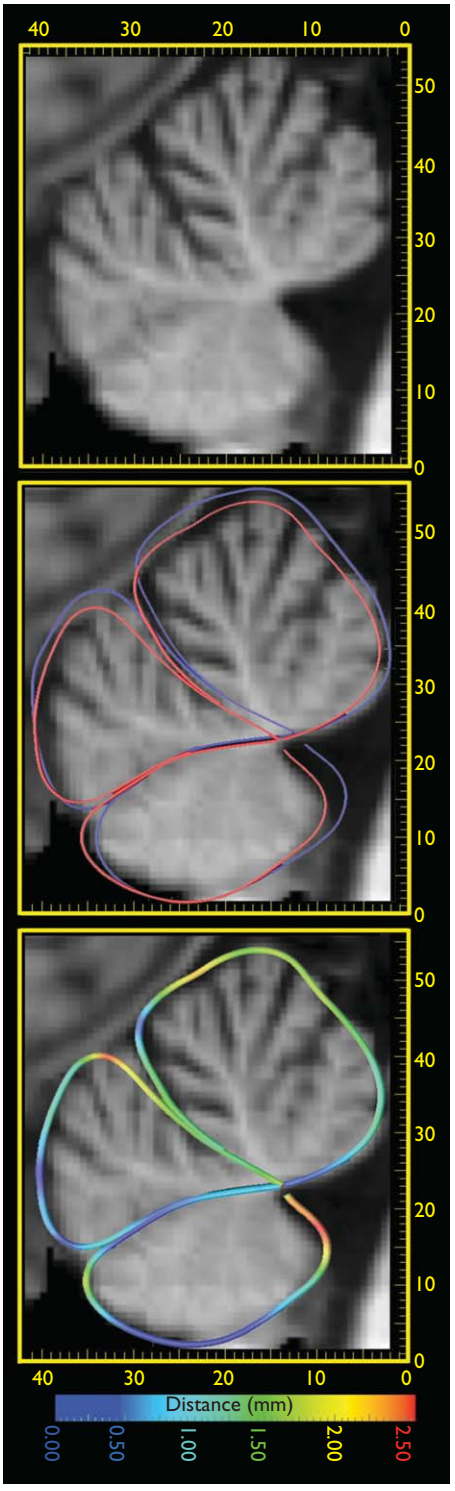

Fig. 2 Atrophy in the cerebellar vermis. Here a sagittal section through a patient's MRI scan is shown (left panel; scale is in millimeters). Outlines of the anterior, posterior, and inferior vermis are shown (middle panel), as average shape contours for the HIV group (red) and controls (blue). After global registration of all images, the distance was plotted (as in ref. [22]) between the mean HIV and mean normal traces. As this distance is less than 2.5 mm everywhere (color-coded, right panel), this level of atrophy would be hard to appreciate by visual inspection of MRI in a single sagittal section, without additional computational analysis.

Results

As shown in Fig. 1, significant mean volume reductions (around 3–6%) were detected throughout the cerebellum in the HIV group versus controls, with both permutation-corrected and pFDR values of $P=0.007$ and $P=0.014$, respectively, for the one-tailed test of atrophy in HIV. Within the HIV cohort, significant correlations were found between the level of atrophy and depression scores ($P=0.009$, permutation test; pFDR=0.035). Maps of these associations implicated regions that overlapped with the disease effect, with the greatest effect sizes at the cerebellar midline (Fig. 2). No significant correlations were detected between cerebellar atrophy and the presence of alcohol abuse, substance abuse, measures of dementia or cerebellar function, or with CD4 + T-cell counts or viral load. Average hand-traced contours at midline showed that the posterior cerebellar vermis in HIV was contracted by approximately 2.5 mm relative to the mean contour in controls. Consistent with this, in the region-of-interest analyses (Fig. 3), the posterior cerebellar vermis showed deficits (approximately 14.9%; $P<0.02$). Disease-related differences showed greater effect sizes in the maps than in the volumetric analyses, perhaps partly because the effects were not completely uniform within traditionally defined regions of interest (as shown in the maps).

Discussion

HIV/AIDS-related atrophy was prominent in midsagittal cerebellar regions, including the posterior vermis (14.9%; $P<0.02$), even though the mean cerebellar volume was only 5.1% smaller. These tissue reductions correlated with depression ratings.

The cellular basis of these volumetric differences cannot be inferred from MRI, but they likely reflect neuronal loss secondary to the neurotoxic effects of HIV viral proteins and the toxic products of infected macrophages, as well as secondary effects of associated white matter degeneration. Several case reports describe cerebellar syndrome as a rare initial manifestation of HIV infection, and asymptomatic cerebellar atrophy has been reported in several neuroimaging studies of HIV [24]. HIV enters the brain within 2 weeks of initial infection [25], and damages neurons primarily by stimulating the production of cytokines that are toxic to neurons, leading to excitotoxic cell death, dendritic simplification, and neuronal loss [26]. Virus-encoded proteins are directly toxic to glutamate-containing neurons [27,28], and neuronal degeneration is also observed when infected macrophages, lymphocytes, and microglia release lymphokines and other neurotoxic substances *in vitro* (e.g. tumor necrosis factor, oxidative radicals, proteases, quinolinic acid, etc.; [29]). In postmortem studies of HIV-positive patients with cerebellar degeneration, Tagliati *et al.* [30] found diffuse loss of neurons in the granule cell layer, white matter degeneration, and cerebellar atrophy. As potential mechanisms for these deficits, Kwakwa and Ghobrial [31] suggested that, in addition to direct neurotoxic effects of the virus, autoimmune destruction of the Purkinje cells may account for the changes, or an as-yet-unrecognized opportunistic infection (although the patients in this study were free from opportunistic infections).

It is not clear why the largest volume reduction was found in the vermis. Postmortem studies show diffuse attrition of

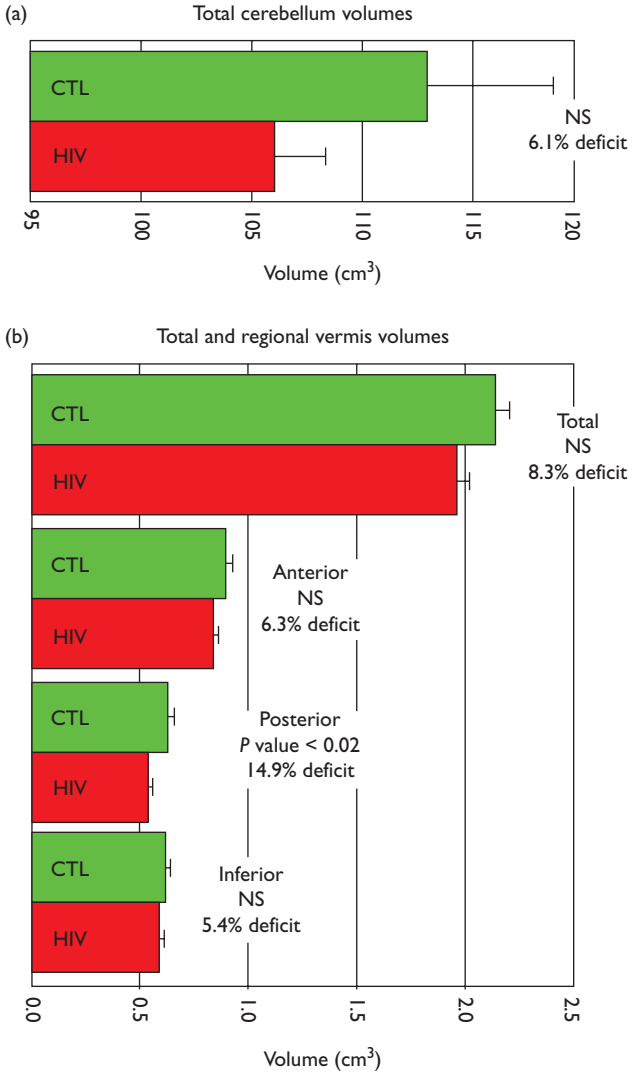

Fig. 3 Volumetric deficits in HIV/AIDS. In a post-hoc volumetric analysis, overall cerebellar volumes were only 5.1% lower on average in HIV/AIDS (a), but cerebellar subregion deficits reached 14.9% ($P < 0.02$) in the posterior vermis. Strictly speaking, the posterior vermis deficits would only be considered a trend, if a strict Bonferroni correction were applied for the number of regions assessed. The better mod maps detected atrophy more powerfully – the disease effect was significant after rigorous multiple comparisons correction (either by permutation or using false discovery rate methods). CTL, cytotoxic T lymphocytes.

granule cells, and some of the volume reduction may reflect white matter reduction secondary to neuronal degeneration, as cerebellar granule cells account for almost half of the neurons in the central nervous system. Cerebellar atrophy may also correlate with concomitant degeneration of limbic and other cortical areas involved in affective regulation, and therefore the correlation with depressive symptoms may be mediated by atrophy in other, distinct brain systems, occurring at the same time as cerebellar atrophy. For example, in Alzheimer's disease, the severity of apathy is associated with atrophy of the cingulate gyrus, which is involved in emotional regulation, but also with atrophy of the supplementary motor cortices, which typically degenerate at the same time [32].

Autopsy studies of AIDS patients with minor cognitive motor disorder reveal widespread loss of synapses [26] and reduced dendritic complexity without overt neuronal loss. As the cerebellum is part of a key motor control network, cerebellar atrophy may also contribute to the mild-to-moderate psychomotor impairments found in 35% of HIV/AIDS patients, and to their risk for impaired cognition in the future.

The hallmark of AIDS/HIV infection is progressive immunosuppression, particularly the depletion of CD4+ T-lymphocytes. Highly active antiretroviral therapy (HAART) restores immune function in most patients, reducing opportunistic infections, but whether HAART can prevent neuropathological progression is controversial. The pathophysiological parameters measured in this study (CD4+ T-lymphocyte counts and viral load) were not correlated with the level of cerebellar atrophy. We may have failed to detect a true association because of limited statistical power in a small sample, or the correlation may be low because of the waxing and waning pattern of plasma viral RNA level and T-cell counts in patients undergoing treatment. A rising prevalence of a 'burnout' form of HIV encephalopathy in which neuronal degeneration may persist or worsen even in patients with undetectable viral load [33]. Although cerebellar degeneration and T-cell depletion may both result from viral infection, treatment may resist one more vigorously than the other, so that the two measures become uncorrelated.

Conclusion

3D cerebellar maps such as these may help in assessing how HIV affects the brain, and may be useful for gauging the extent of atrophy and treatment efficacy. In the more advanced stages of the disease, approximately 15% of AIDS patients have HIV-associated dementia, a complex disorder consisting of psychomotor slowing, behavioral abnormalities, and Parkinsonian features such as bradykinesia and gait disturbance [34]. Current HAART medications fail to significantly permeate the blood–brain barrier [35], and therefore cerebellar degeneration may be present and be associated with depressive symptoms, even in patients treated with antiretroviral therapy, whose viral load is low. T-cell counts were not linked with cerebellar atrophy (although they associate with cortical and deep nuclear atrophy), suggesting that HIV neurotoxic effects may proceed unchecked when immunosuppression is largely contained.

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