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Microstructural White Matter Integrity in HIV-Infected Individuals in the HAART Era: A Diffusion Tensor Imaging Study

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Clinical Psychology

by

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ABSTRACT OF THE DISSERTATION

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Approximately half of HIV-infected people exhibit cognitive impairment, which has been related to cerebral white matter damage. Despite the effectiveness of antiretroviral (ARV) treatment, cognitive impairment remains common even in

individuals with undetectable viral loads. One explanation for this may be subtherapeutic concentrations of some ARVs in the CNS.

We utilized diffusion tensor imaging (DTI) to investigate the relationship of white matter integrity to cognitive impairment and ARV treatment variables, including CSF viral load and an index of the CNS penetration of ARVs. Participants included 39 HIV-infected (HIV+) individuals and 25 seronegative subjects. DTI indices were mapped onto a common whole-brain white matter tract skeleton, allowing between-subject voxelwise comparisons.

The total HIV+ group exhibited abnormal white matter in the internal capsule, inferior longitudinal fasciculus, and optic radiation; while HIV+ with AIDS exhibited more widespread damage, including in the internal capsule and the corpus callosum. Cognitive impairment in HIV+ was related to white matter injury in the internal capsule, corpus callosum, and superior longitudinal fasciculus. White matter injury was not found to be associated with HIV viral load or estimated CNS penetration of ARVs.

DTI 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 DTI 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 ARV treatment.

INTRODUCTION

HIV INFECTION

It is estimated that human immunodeficiency virus (HIV) has caused more than 20 million deaths worldwide since 1981, with about 3 million occurring in 2004. About 40 million people are currently living with the infection, while about 5 million were newly infected in 2004. Lacking adequate access to effective treatment, it appears that the developing world is disproportionately affected by the virus. Estimated numbers at the end of 2004 indicate that North America accounted for about 2.5% (1 million cases) of infected cases worldwide, while Sub-Saharan Africa accounted for about 64.5% (25.4 million) of infected cases, 63.3% (3.1 million) of newly infected cases, and 74.2% (2.3 million) of deaths in that year (UNAIDS/WHO, 2004).

HIV is a retrovirus that produces progressive immunodeficiency, primarily through profound CD4 depletion associated with a chronic immune activation and fatigue of homeostatic immune responses (McArthur, Brew and Nath, 2005). Lacking effective antiretroviral (ARV) treatment, most infected individuals progress over a number of years to a life-threatening condition termed acquired immunodeficiency syndrome (AIDS). The clinical diagnosis is defined by a decline in immunocompetence reflected by CD4 level of fewer than 200 cells per cubic millimeter of blood, and the presence of AIDS-associated opportunistic infections and neoplasms.

HIV primarily infects a type of cells crucial for immune function known as CD4+ helper T-cells. The virus enters into cells by way of the interaction between viral gp120 molecules and the host cell CD4 receptor, resulting in a gp41-modulated fusion into the host cell. The retroviral RNA is then converted into DNA in the host cell, which is then replicated, enabling the spreading of infection to other cells. The reverse transcriptase and protease enzymes are necessary components in the replication process. The modulation of their activities forms the basis of the majority of current ARV treatment of HIV.

ANTIRETROVIRAL THREAPY

Since the mid 1990's, the use of potent combination ARV regimens has resulted in dramatic changes in the course of illness in HIV-infected individuals. Commonly referred to as highly active antiretroviral therapy (HAART), such treatment regimens have the goals of lowering plasma viral RNA to an undetectable level and restoring immune function as reflected by increasing CD4 level. Dramatic reductions in plasma HIV RNA usually occur within weeks of starting HAART regimens, while immunologic responses usually occur over a few months (McArthur et al., 2003). A 60% decrease in mortality rate in the US from 1996 to 1998 was attributed to the use of combination ARV regimens (Palella et al., 1998). The use of HAART has also been associated with reduction in mother-infant HIV transmission rates, decreased incidence of HIV-related complications, including those of the central

nervous system (CNS), and generally improved quality of life for infected individuals (Sacktor et al., 2001).

Effective ARV treatment of HIV became available in 1987 with the introduction of zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI). This class of drugs works by suppressing the viral replication by interfering with the reverse transcriptase enzyme after intracellular phosphorylation. NRTIs also act as chain terminators for viral DNA replication. In a double-blind placebo-controlled trial, Schmitt et al. (1988) reported improved performance on a brief neuropsychological battery, and reduced "intensity of symptomatic distress" in HIV-infected individuals who received zidovudine. Another double-blind placebo-controlled clinical trial (Sidtis et al., 1993) reported improved neuropsychological performance and reduced neurological symptoms in individuals who received high doses of zidovudine. These findings demonstrated the effectiveness of zidovudine, particularly on the CNS. Other drugs in this class later introduced include abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zalcitabine.

Protease inhibitors were introduced in 1995. This class of drugs work by inhibiting the functioning of the protease enzyme required in the process of viral maturity before HIV can infect other cells. These drugs include amprenavir, atazanavir, fosamprenavir, indinivir, lopinavir, nelfinivir, ritonavir, and saquinivir. Non-nucleoside reverse transcriptase inhibitors (NNRTI), first approved for use in 1997, represent an additional class of ARV drugs. They act as non-competitive inhibitors of reverse transcriptase at a site distinct from NRTIs, not requiring

phosphorylation for activity. Drugs in this class include delavirdine, efavirenz, and nevirapine. An additional class of ARV drug works by preventing the fusion of HIV to host cells by binding to the viral gp41 envelopes. Enfuvirtide is the only current FDA-approved fusion inhibitor (Duffalo and James, 2003). Clinical trials of enfuvirtide have shown improved immunologic function and reduced viral RNA associated with twice daily subcutaneous injection (Barbaro et al., 2005; Oldfield et al., 2005).

The use of protease inhibitors and NNRTIs in combination with previously introduced NRTIs marked the beginning of the so-called HAART era in the mid 1990's. Potent ARV regimens consisting of 3 or more drugs have become the current standard of care. Comparing the effects of a triple ARV therapy regimen and alternating dual therapy regimens, Price et al. (1999) showed a higher survival rate and better performances on a quantitative neurological battery in subjects who received a 3-drug regimen. Ferrando et al. (1998) demonstrated higher general neuropsychological performances associated with lower plasma viral RNA in HIV-infected individuals treated with protease inhibitor-containing HAART regimens. Sacktor et al. (2000) reported improvement in psychomotor slowing in patients initiating similar ARV regimens. In addition, Deutsch et al. (2001) compared the time to initial incidence of cognitive impairment in individuals with AIDS in the pre- and post-HAART era (using the end of 1995 as the cut-off period), and reported a reduced hazard for AIDS-related cognitive impairment in post-HAART individuals.

The most recent guideline for treatment of HIV infection by the United States Department of Health and Human Services (DHHS) was issued on October 10, 2006

(Panel on Antiretroviral Guidelines for Adults and Adolescents, 2006). Based on a panel of expert and current scientific information, the guideline recommends ARV therapy for all patients with AIDS-defining illnesses or severe symptoms of HIV infection regardless of CD4 level. For asymptomatic patients, ARV therapy is recommended for individuals whose CD4 levels are below 200 cells/mm^3; and it is recommended that treatment be considered for patients with CD4 levels between 200 and 350, and patients with CD4 levels higher than 350 but with plasma HIV RNA higher than 100,000 copies/mm^3. Table 1 shows treatment recommendations for ARV-naïve patients, with regimens constructed by choosing one component (NNRTI or protease inhibitor) from columns A, and an NRTI combination from column B.

Despite the demonstrated efficacy of current ARV therapy, successful treatment may be hindered by the complex medication regimens and numerous side effects, which may in turn affect treatment adherence. In a study of ARV treatment adherence, Paterson et al. (2000) showed that complete viral suppression occurred in 81% of subjects with adherence level of more than 95%, while only 6% of subjects with less than 70% adherence showed complete viral suppression. This dramatic difference was attributed partly to HIV drug resistance. Rapid replication of the virus results in a high error rate of RNA to DNA transcription, which allows the genome to readily mutate to drug-resistant forms. King et al. (2005) demonstrated that increased probability of ARV resistance is associated with low treatment adherence. Other factors that promote the development of drug-resistant strains of HIV include under-dosing and pharmacokinetic interactions with other drugs (Condra et al., 2000). These

findings underline the importance of both careful monitoring by the physician, and strict adherence by the patient.

CENTRAL NERVOUS SYSTEM EFFECTS

HIV-related neuropathology may result from direct effects of the virus on the CNS characterized by HIV encephalitis (HIVE). CNS complications may also arise from reactivated latent infections, or infections with organisms that normally are not pathogenic to immunocompetent individuals. Common examples of these opportunistic infections are cytomegalovirus, JC papovavirus, herpes simplex, toxoplasmosis, and cryptococcosis (Marra, 1999). Additionally, AIDS-related neoplasms, including primary CNS lymphomas, metastatic systemic lymphomas, and Karposi's sarcoma can also result in CNS damage.

From early in the HIV epidemic, it was apparent that the CNS is extensively involved in the course of the disease progression (McArthur et al., 1993). In a review of 390 AIDS autopsy cases, Masliah et al. (2000) found CNS complications, including HIVE and opportunistic infections, in 63% of cases. These were second in frequency only to complications of the lung, which occurred in 84% of cases. CNS complications, especially in advanced disease, may lead to HIV-associated dementia (HAD), a debilitating illness characterized by significant cognitive impairment usually accompanied by motor symptoms. Neurological conditions, including HAD and sensory neuropathy, have played major roles in AIDS-associated illnesses and mortality. HAD has an estimated lifetime prevalence of 15% in infected individuals

(McArthur et al., 1993), and account for about 5% of new AIDS-defining illnesses in the US (McArthur et al., 2003).

HIV-associated opportunistic infection can produce various effects on the CNS, which are usually dependent on the location of infection, including cystic lesions, meningitis, and encephalitis. In addition, JC papovavirus infection is associated with progressive multifocal leukoencephalopathy, a condition characterized by extensive and widespread white matter damage associated with infection of the oligodendrocytes and failure of myelin maintenance (Marra, 1999). AIDS-related CNS neoplasms are associated with focal neurological deficits, commonly in subcortical white matter (Pfefferbaum, Rosenbloom and Sullivan, 2001).

NEUROPATHOGENESIS OF HIVE

Although neurological conditions are usually not apparent prior to the development of systemic immunosuppression, especially associated with of AIDS, converging evidence suggests that HIV enters the CNS shortly after the initial infection, possibly around the time of seroconversion. For instance, meningitis and meningo-encephalitis have been reported at the time of HIV seroconversion. HIV antigens and antibodies have also been found in cerebrospinal fluid (CSF) during the asymptomatic phase of HIV (Brew et al., 1989; Morris et al., 1998). In addition, autopsy studies have found positive polymerase chain reaction (PCR) results for HIV in brains of individuals in early/asymptomatic stages of the disease (Bell et al., 1993; Sinclair et al., 1994).

The CNS is separated from the rest of the body by the blood-brain barrier (BBB), which regulates the traffic of cells and substances form the bloodstream to the CNS. The BBB is a selectively permeable, continuous cellular layer of brain microvascular endothelial cells. HIV infection of cells within the brain provides evidence of the virus's ability to cross the BBB. At least 2 types of mechanisms of HIV neuroinvasion have been proposed. Using *in situ* hybridization of a sheep-specific lentivirus as a model for HIV, Peluso et al. (1985) and Haas (1986) proposed a "Trojan Horse" mechanism, in which HIV enters the CNS as passengers in cells that are trafficking to the brain, especially monocytes that migrate across the BBB to replenish the population of perivascular macrophages. Supporting evidence for this model includes findings of viral accumulation in the perivascular region CD14+ and CD16+ cells (Fischer-Smith et al., 2004), the limited pathological evidence of infection of endothelial cells consistent with their lack of conventional HIV receptors, and the presence of activated bone-marrow-derived cells in individuals with advanced illnesses (reviewed in Gonzalez-Scarano and Martin-Garcia, 2005). Alternatively, using *in vitro* models, some investigators have proposed that HIV crosses the BBB through the endothelial cells by transcytosis, a process of transporting across an epithelium by uptake into and release from coated vesicles (Bomsel, 1997; Banks et al., 2001, Liu et al., 2002).

Following its entry into the CNS, HIV comes in direct contact with several types of cells. Although infections of astrocytes, oligodendrocytes, and neurons have been reported, primary targets of HIV infection appear to be macrophages and

microglia. Microglia and macrophages are bone marrow-derived cells that function as primary immunocompetent cells of the brain. Immunohistochemistry and *in situ* hybridization studies have shown that perivascular macrophages represent the cell population most infected by HIV (Williams et al., 2001; Fischer-Smith et al., 2004). *In vitro* experiments have demonstrated that microglia isolated from the CNS can support replication of HIV (Watkins et al., 1990; Strizki et al., 1996; Shieh et al., 1998). Microglia also possess a high level of expression of the CD4 and HIV CCR5 co-receptors (Gonzalez-Scarano and Martin-Garcia, 2005). Conflicting opinions still exist regarding whether microglia can be infected by HIV (Cosenza et al., 2002). However, most investigators regard macrophages and microglia as the only types of cells capable of supporting productive HIV infection in the CNS. Utilizing PCR/*in situ* hybridization and immunocytochemistry, Takahashi et al. (1996) reported productive HIV infection of macrophages and microglia focused within the perivascular region. Additionally, using postmortem immunohistochemical methods, Kure et al. (1991) and Brew et al. (1995) reported high levels of productive HIV infection involving macrophages and microglia within the basal ganglia, subcortical white matter, and brainstem.

Macrophages and microglia are also implicated in the formation of multinucleated giant cells (MNGC), the presence of which is a hallmark pathological feature of HIV encephalitis. MNGC's are found particularly in the subcortical white matter and the basal ganglia and are believed to represent the fusion of infected and uninfected macrophages and/or microglia, mediated by HIV-envelope glycoproteins

present at the surface of the infected cells and CD4 and chemokine receptors at the surface of uninfected cells (Budka, 1986).

Astrocytes are responsible for maintaining homeostasis in the CNS, including neurotransmitter regulation, and maintenance of the BBB through their interaction with endothelial cells. Evidence for HIV infection of astrocytes has been reported (Conant et al., 1994; Tornatore et al., 1994; Brack-Werner, 1999). In the absence of detectable levels of CD4 or the main HIV co-receptors, however, the mechanism for viral attachment to astrocytes remains unknown (Bell, 2004). More limited evidence for HIV infection of neurons, oligodendrocytes, and endothelial cells also exists (Pang et al., 1990).

POSTMORTEM FINDINGS

Postmortem studies of HIVE have commonly found general brain atrophy associated with reduction in brain weight and ventricular dilation (Bell, 1998). Diffuse cerebral white matter damage is one of the most frequent pathological features, especially at later disease stages and in individuals presented with dementia (Price et al., 1988; Gray et al., 1996). Studies have shown that white matter of the frontal lobes and subcortical grey matter structures, notably the basal ganglia, appear to be disproportionately affected (Navia et al., 1986; Neuen-Jacob et al., 1993). Autopsy studies have also reported HIV-related brain abnormalities in the corpus callosum (Neuen-Jacob et al., 1993); the pons (Miller et al., 1998), and the cerebellum (Tagliati et al., 1998).

Dendritic and synaptic damages appear to contribute to HIV-related brain injury. Masliah et al. (1997) reported a negative correlation between dendritic simplification, based on microtubule-associated protein 2 immunohistochemical staining, and antemortem neuropsychological performance in AIDS patients with varying degree of cognitive impairment. Utilizing a combination of stereology and confocal microscopy, Everall et al. (1999) also found a reverse relationship between synaptic density and ante-mortem neuropsychological performance in a similar population. Evidence for myelin loss and axonal damage associated with HIV infection has also been reported. HIVE has been associated with a widespread myelin breakdown and loss, resulting in accumulation of lipid macrophage (Bell, 1998). Axonal damage, as evidenced by immunopositivity for beta-ameloid precursor protein in white matter, has been demonstrated both in pre-AIDS cases (An et al., 1997), AIDS cases (Raja et al., 1997), and individuals with confirmed HIVE (Giometto et al., 1997).

Based on postmortem findings of individuals with AIDS, it appears that brain white matter plays a major role in HIV-related brain injury. This is further demonstrated in a study by Bell et al. (1998) of 31 postmortem brains with HIVE, in which more than 90% showed abnormalities in white matter of the frontal lobes, while grey matter abnormities were evident in about 50%.

Postmortem studies have the advantage of being able to conclusively characterize the etiology and anatomical location of CNS pathology. Its obvious shortcoming, however, is in *in vivo* examination, which is especially important in

understanding pathology at earlier stages of the disease. Postmortem studies are also cross-sectional by nature, preventing the longitudinal tracking of neuropathological changes. In addition, postmortem study samples may be selective for individuals with high risk of morbidity, including those with comorbid conditions that may not necessarily be identified, and individuals not responsive to ARV treatments. Results from these studies, therefore, may not generalize to other HIV-infected individuals.

MAGNETIC RESONANCE NEUROIMAGING FINDINGS

Magnetic resonance imaging (MRI) presents *in vivo* techniques for examining the HIV-infected brains, allowing the examination of pathology at earlier stages of the disease. Repeated examinations also allow tracking of longitudinal changes. Currently available MRI techniques can provide multiple types of information that yield corroborating information for detecting, diagnosing, and monitoring HIV-related CNS complications.

STRUCTURAL MRI.

General cerebral atrophy both from radiological and pathological examinations, especially at later stages of the disease, has been a common clinical observation from early in the HIV epidemic (Bell, 1998). Structural MRI (SMRI) allows the characterization of cerebral atrophy and other abnormal structural changes associated with HIV infection via both within- and between-subject comparisons. SMRI studies can generally be divided into 2 categories. A number of studies, especially earlier ones, utilize clinical evaluation for visually apparent neurological

abnormalities. This methodology has been criticized for the possible differences between the criteria used in each study to define abnormalities, which can prevent direct between-study comparisons. Alternatively, brain morphology may be quantitatively examined by utilizing high-resolution magnetic resonance (MR) images to demarcate structures within the brain and estimate their volumes. Such technique presents an objective way of characterizing abnormal brain structures.

Consistent with results from postmortem findings, abnormalities within the basal ganglia and white matter have been extensively demonstrated in SMRI studies. Generally utilizing clinical neuroradiological evaluation, earlier studies tended to focus on individuals in later disease stages, especially those with AIDS. Post et al. (1986) demonstrated the utility of SMRI in detecting AIDS-related structural brain abnormalities, especially white matter lesions and small lesions surrounded by edema. Grant et al. (1987) reported general atrophy and white matter hyperintensity both in individuals with AIDS, and pre-AIDS individuals with AID-related illnesses. Similar findings were reported by Olsen et al. (1988) and Flowers et al. (1990). Utilizing quantitative volumetric examination of brain morphology, Jernigan et al. (1993) reported a reduction in the volume of cerebral white matter and cerebral grey matter, including the basal ganglia, in non-demented medically symptomatic HIV-infected men.

Although some investigators (see Manji et al., 1994) have proposed that pre-AIDS HIV-related neurological changes are not detectable by MRI, abnormal cerebral structural changes have been reported in numerous MRI studies of individuals in

earlier disease stages. Post et al. (1991) found cerebral atrophy and white matter hyperintensities in both medically asymptomatic individuals (13% of cases) and symptomatic individuals (46%). Di Sclafani et al. (1997) reported global atrophy and atrophy of the caudate nuclei in both medically asymptomatic and symptomatic HIV seropositive individuals, which were more pronounced in the latter group. In a longitudinal study, Stout et al. (1998) reported progressive decline in white matter and basal ganglia volumes and general cerebral atrophy in both medically asymptomatic and symptomatic/AIDS individuals.

HIV-related cerebral atrophy in similar regions has also been related to cognitive impairment, especially at later disease stages. Using ratio indices of cerebral atrophy, Dal Pan et al. (1992), reported a general cerebral atrophy and atrophy of caudate nuclei in HIV-infected individuals, which were more pronounced in those with HAD. Using similar indices of cerebral atrophy in a 30-month longitudinal study, Hall et al., (1996) reported a relationship between the decline in neuropsychological performance and the presence of caudate atrophy, which was more pronounced in individuals with AIDS than in asymptomatic individuals. Aylward et al. (1993) reported a reduction in basal ganglia volume in individuals with HAD, in comparison to non-demented HIV-infected individuals and healthy controls. The latter 2 groups, however, were comparable regarding basal ganglia volume. Following up on the previous study, Aylward et al. (1995) reported more pronounced white matter volume loss, as well as less grey matter volume loss in the basal ganglia and posterior cortex, in individuals with HAD than in non-demented individuals. Again, the differences

between non-demented and healthy control individuals were not statistically significant. In a longitudinal study, Pedersen et al. (1991) reported an associated between the development of HAD and the presence of MRI white matter lesions.

Studies utilizing SMRI have demonstrated its ability in detecting HIV-related brain abnormalities. These studies, however, tend to be labor intensive, especially when volumetrically characterizing specific structures of interest. Additional, they tend in general to be less sensitive in individuals at earlier disease stages. Magnetic resonance spectroscopy (MRS) presents an additional MRI modality in examining the HIV-infected brains.

PROTON MAGNETIC RESONANCE SPECTROSCOPY.

Proton MRS (referred to here as MRS) is an MR technique that allows the quantitation of the concentrations of neurochemical compounds within regions of interest (ROI) in the brain. Brain metabolites quantifiable by MRS that are of clinical relevance include *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho), and myo-inosital (mI). Apart from water, the largest relevant proton signal detected by MRS is NAA. NAA is found almost exclusively in neurons, and is generally considered a measure of neuronal integrity. Since Cr signal tends to be relatively constant across clinical populations, its concentration is often used as a reference for other signals for the purpose of standardizing the concentration unit. The Cho signal is generated by water-soluble Cho-containing compounds, including free Cho, phosphocholine, and glycerophosphocholine. Cho concentration has been associated with cell membrane synthesis and turnover, cellular density, and glial density, and usually increases with

normal aging. mI is found in glial and not neurons, and is a component in the process of cell volume maintenance. mI has been described as a glial marker, an intracellular osmolyte, a precursor of myelin phosphatidyl inositol, a progenitor of the widespread inositol polyphosphate messenger cascade, and the breakdown product of phosphatidyl inositol. (See Pfefferbaum, Rosenbloom and Sullivan (2002) for a review of relevant compounds associated with MRS.)

Consistent with postmortem and SMRI findings, HIV-related changes in the CNS detected by MRS tend to focus in the white matter, especially in the frontal lobes, and in subcortical grey matter structures, especially the basal ganglia. In one of the first MRS studies of HIV, Menon et al. (1992) reported decreased NAA/Cr and increased Cho/Cr ratios in individuals with "moderate to severe" HAD, in comparison to those with "early HAD" and healthy controls. In 3 separate group comparisons, Chong et al. (1994) reported decreased NAA/Cr ration in a parietooccipital white matter ROI associated with 1) AIDS vs. non-AIDS clinical diagnosis, 2) HIV-associated cognitive/motor complex vs. neurologically asymptomatic, and 3) those with abnormal SMRI findings vs. otherwise. Meyerhoff et al. (1993) found reduced NAA/Cr ratio in ROI's in the white matter regions within the centrum semiovale and the mesial cortex in cognitively impaired HIV-infected individuals, only 20% of whom had apparent SMRI abnormalities.

The latter finding of abnormal MRS signals associated with normal SMRI suggests that MRS may be a more sensitive technique in detecting HIV-associated CNS complications. Other studies reviewed below have reported similar results. In

addition, MRS appears to be more sensitive in detecting brain abnormalities in neurologically asymptomatic individuals. Moller et al. (1999) found decreased NAA/Cr and increased Cho/Cr ratios within the basal ganglia and the insula of HIV-infected individuals, including those who were neurologically asymptomatic and/or had no SMRI-indicated abnormalities. Suwanwelaa et al. (2000) reported decreased NAA/Cr and NAA/Cho ratios in the centrum semiovale and the thalamus in neurologically asymptomatic HIV-infected individuals.

Earlier findings cited above tended to use standardized ratio measures of brain metabolite concentrations, with Cr as the reference. This practice, however, has been criticized due to findings of fluctuation of Cr concentration in some clinical populations (Pfefferbaum et al., 1999). By eliminating the possible statistical noise due to the fluctuating standardization denominator (Cr), absolute measure of metabolite concentrations may provide a more sensitive measure of brain abnormalities. In general, more recent studies utilizing absolute measures have yielded similar results. Chang et al. (1999) found increased NAA and decreased mI and Cho concentrations in ROI's within the frontal grey matter, frontal white matter, and basal ganglia in individuals with HAD, while these changes were observed only in the frontal white matter in individuals with cognitive impairment who did not meet criteria for HAD. Stankoff et al. (2001) reported decreased NAA concentration in a frontal white matter ROI in individuals with HIV-associated cognitive impairment. Chang et al. (2002) found elevated mI and Cho levels and decreased Cr level in ROI's

in the frontal lobes, and decreased basal ganglia Cr in individuals with HAD. MRS changes were related to the severity of dementia.

DIFFUSION TENSOR IMAGING.

Diffusion tensor imaging (DTI) presents an additional MRI modality for *in vivo* examination of HIV-related brain abnormalities. Utilizing novel pulse sequences in diffusion-weighted MRI, a technology routinely implemented in clinical MR scanners, and diffusion tensor data analysis techniques, DTI allows the quantitation of free water diffusion in the brain. The clinical utility of DTI is based on the fact that water mobility is dependent on the surrounding tissues. Any deviation from the typically assumed Gaussian distribution of unrestricted water can therefore be attributed to water's interactions with barriers such as cell membranes and organelles (Horsfield and Jones, 2002).

Using a diffusion tensor, both the magnitude and directionality of water diffusion can be quantified (Parker, 2004). These values can be expressed as anisotropy, which is indicative of restricted water diffusion, typically in a regularly organized region such as along an axonal fiber tract. A typical index of anisotropy is a rotationally invariant scalar quantity referred to as fractional anisotropy (FA), scaled to values between 0 and 1. Lower FA values reflect free water diffusion such as in cerebrospinal fluid (CSF), and higher values reflect restricted diffusion such as within the white matter tracts of the corpus callosum. Let λ_1, λ_2, and λ_3 be the eigenvalues for the diffusion tensor.

$$FA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_1 - \lambda_3)^2}}{\sqrt{2}\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

An additional common measure of diffusion, referred to as mean diffusivity (MD), reflects the mean square displacement of water. MD values, therefore, generally are inversely related to FA, i.e., higher MD reflects unrestricted water movement, and vice versa.

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

Given DTI's ability to quantify water diffusion within brain tissues, microstructural neurological changes associated with clinical conditions may be detected by characterization of water diffusion within the tissues of interest. DTI has been demonstrated to be sensitive to white matter disruptions, including breakdown of myelin, microtubule structure, and axon density (Sullivan and Pfefferbaum, 2003). The utility of DTI in detecting neurological changes has been demonstrated in many populations, including normal aging (Sullivan et al., 2001), multiple sclerosis (Miller et al., 1998), stroke (Spielman et al., 1996), schizophrenia (Lim et al., 1999), and chronic alcoholism (Pfefferbaum and Sullivan, 2002). In addition, a number of investigators have utilized DTI to examine HIV-related changes in the brain.

Filippi et al. (2001) examined 10 HIV-infected individuals characterized on plasma HIV RNA. The author reported no abnormalities apparent on SMRI, except for "age-appropriate" atrophy (mean age = 42 years, age range was not reported). All subjects were HAART-treated, while 4 were not on protease inhibitors. Elevated viral

load was associated with decreased FA in the genu and splenium of the corpus callosum, and increased MD in subcortical white matter.

Pomara et al. (2001) examined 6 HIV-infected individuals and 9 age-matched seronegative controls. Four subjects met the criteria for AIDS, 3 had peripheral neuropathy, and 5 subjects were receiving HAART. Average CD4 count was 289. No group differences were apparent on qualitative reviews of white matter hyperintensities. The authors reported decreased FA in frontal white matter and internal capsules in the HIV-infected group. No group differences in MD were found.

Cloak, Chang and Ernst (2004) examined 11 seropositive individuals and 14 age-matched controls. All subjects were under 50 years of age, and were characterized with proton MRS and a neuropsychological battery designed to assess brain functions associated with the frontal lobe and/or the basal ganglia. Six of the seropositive subjects were receiving HAART, while 5 were ARV-naïve. Only MD was used in the analysis. The authors reported that MD was elevated in the frontal white matter of HIV+ subjects. Elevated frontal white matter MD was associated with elevated mI concentration based on MRS in the same region, and lower composite cognitive performance score.

Ragin et al. (2004a) examined 9 seropositive individuals and 9 demographically matched controls, using both DTI and magnetization transfer imaging (MTI). “Peak whole brain” DTI and MTI measures were used as response variables. FA and MTI index were both significantly related to HIV serostatus and

severity of HIV-associated dementia. MD was not found to be related to serostatus, but was related to MTI index and psychomotor ability.

Ragin et al. (2004b) examined 6 HAART-treated advanced HIV seropositive individuals and 8 demographically matched controls. CD4 counts for seropositive subjects ranged from 10 to 187, while $\log_{10}$ of plasma viral load ranged from 1.9 to 2.6. Seropositive subjects were also characterized on a dementia scale rating. The authors used whole-brain FA as the dependent variable in the study. FA level was found to be decreased in HIV-infected subjects, although no difference in MD was reported. FA level was also found to be negatively correlated with dementia severity.

Ragin et al. (2005) examined 11 seropositive individuals and 11 demographically matched controls. All HIV+ individuals were ARV-experienced, and had CD4 count ranging from 24 to 427, and plasma HIV RNA level from undetectable to 154938. ROI's were in the basal ganglia (caudate and putamen), and centrum semiovale. The authors reported no main effects of HIV on FA or MD, although the DTI indices were significantly correlated with deficits in working memory, verbal memory, visual memory, and visuoconstruction ability. It should be noted that no type I error control for multiple comparisons was attempted despite the 8 analyses performed on neuropsychological variables for each of the two DTI indices, with significant *p*'s ranging from .017 to .048.

Thurnher et al. (2005) examined 60 HIV seropositive individuals and 30 demographically matched controls. ROI's were in the genu and splenium of the corpus callosum, frontal white matter, and hippocampus. Significant main effects of HIV on

FA and MD in the expected directions were reported only in the genu. This appears to be the largest published study to date.

Ragin et al. (2006) examined 11 seropositive individuals who appear to be the same HIV+ sample as reported in Ragin et al. (2005). The authors focused on the relationships between DTI indices and biomarkers of HIV CNS disorders, namely monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF-α), and hematocrit. ROI's were in the caudate, putamen, and centrum semiovale. Significant relationships in the expected directions were found in the caudate (MD and MCP-1, $p = .007$), putamen (MD and MCP-1, $p = .05$; FA and hematocrit, $p = .05$), and centrum semiovale (MD and MCP-1, $p = .03$; FA and TNF-α, $p = .03$).

Wu et al. (2006) examined 11 seropositive individuals and 11 controls. The sample appears to be the same as that reported in Ragin et al. (2005). ROI's were in the genu and splenium of the corpus callosum. Significant main effects of HIV on FA and MD were reported in the splenium. DTI indices were also related to HIV-related dementia severity, and deficits in verbal memory, visual memory, and visuoconstruction ability.

It therefore appears that HIV-related white matter abnormalities may be demonstrated by DTI, as apparent by reduced FA and increased MD, especially in later disease stages and in individuals with cognitive impairment. The findings in the frontal lobes, basal ganglia, and corpus callosum also appear to be consistent with those from autopsy studies and other neuroimaging studies.

HIV-ASSOCIATED COGNITIVE IMPAIRMENT

HIV infection is frequently accompanied by cognitive and behavioral complications, especially in later stages of illness (McArthur et al., 1993). The most advanced form of these complications is referred to as HIV-associated dementia (HAD). HAD is characterized by severe impairments in cognitive functioning with marked interference in social-occupational performance, and is diagnosed following marked neuropsychological impairment in multiple ability domains, as well as marked interference in day-to-day functioning (Grant and Martin, 1994). HAD is a common AIDS complication, and can present as the first or only manifestation of AIDS (Navia and Price, 1987). HAD seldom develops before severe immunosuppression accompanying advanced HIV infection. For instance, Miller et al. (2000) reported a 0.4% prevalence of HAD in medically asymptomatic HIV-infected individuals in a pre-HAART cohort, while McArthur (1987) reported 16% prevalence among symptomatic individuals. In an early cohort, the lifetime cumulative risk of a seropositive individuals developing HAD was estimated to be 15-20% (McArthur et al., 1993).

HAD usually presents as an insidious decline in cognitive and psychomotor functioning, most notably reflected by psychomotor slowing, impaired concentration and attention, memory disturbances, and executive dysfunction, usually accompanied by changes in affect and social functioning (Navia et al., 1986; McArthur et al., 2003). In addition, HAD has been associated with a verbal memory profile characterized by a retrieval deficit, with relatively spared retention (White et al., 1997). Neurobehavioral

disturbances commonly reported in HAD include bradyphrenia, bradykinesia, extrapyramidal signs, and apathy (Grant et al., 1995). This neurobehavioral picture appears to resemble those of conditions with subcortical neuropathology such as Parkinson's and Huntington's diseases. Together with converging evidence from neuropathological and neuroimaging studies, HAD has often been described as a subcortical dementia associated with preferential disruption of the frontal-basal ganglia systems.

In addition to HAD, a milder form of HIV-related neurobehavioral disorders has been described. Minor cognitive motor disorder (MCMD) is diagnosed following observed neuropsychological impairment in at least 2 ability domains, in addition to disruption in everyday living, including work, home life, and social activities (Grant & Martin, 1994). MCMD patients usually present with difficulty in concentrating, unusual fatigability when required to engage in demanding mental tasks, and subjective feelings of "slowing down" and forgetfulness. Prevalence rates of about 30% in symptomatic individuals have been reported (Janssen et al., 1989; Sacktor et al., 2002). Diagnosis of MCMD has been associated with a worse prognosis for AIDS (Sacktor et al., 1996), and subsequent detection of HIVE at autopsy (Cherner et al., 2002). Despite the similarity in their clinical presentations, MCMD only occasionally progresses to HAD, and it remains unclear whether they represent levels of severity of one disorder, or are separate entities that also differ in clinical course and pathogenesis (Heaton et al., 1995).

Cognitive impairment also appears to exist to a more limited extent in medically asymptomatic seropositive individuals. Earlier studies, however, presented inconsistent results. White et al. (1995) reviewed 57 studies of neuropsychological impairment in asymptomatic individuals, and reported that 32% found significant HIV-related impairment, while 21% had inconclusive results, and 47% found no impairment. Despite the findings, however, the authors found a strong association between the comprehensiveness of the test batteries and the likelihood of a positive finding of group difference. Utilizing a comprehensive neuropsychological test battery in 249 medically asymptomatic HIV-infected individuals and 111 seronegative controls, Heaton et al. (1995) found no group difference based on mean comparisons of test scores. However, using a global rating of neuropsychological impairment, a larger proportion of seropositive subjects than controls appeared to be impaired. The authors stressed the importance of comprehensive neuropsychological batteries, and data analysis methods that permit detection of the variable patterns of mild cognitive impairment, such as exhibited in medically asymptomatic HIV-infected individuals.

CNS EFFECTS OF HAART

Among the dramatic improvements attributed to modern ARV treatment is the reported decline in the incidence of HAD. In a cohort of 2,734 men, Sacktor et al. (2001) reported a 53% decline in HAD incidence from 21.3:1000 person-years (PY) from 1990 to 1992, to 10.0:1000 PY from 1996 to 1998. This picture, however, is complicated by a recent report by McArthur (2004), in another large cohort, that

despite a similar finding in earlier years following the introduction of HAART, the incidence of HAD has begun to increase again in 2003. The data on the prevalence rates of HAD is also problematic. Sacktor et al. (2002) reported comparable prevalence of HAD between a 1994-1995 cohort and a 1998-1999 cohort. In contrast, Dore et al. (2003) reported a 30.8% increase in HAD prevalence from 5.2% in 1993-1995 to 6.8% in 1996-2000. McArthur et al., (2003) reported a 53% increase in prevalence of HAD from 6.6:100 PY in 1994 to 10.1:100 PY to 2000. The apparent rise in the prevalence rate of HAD may be attributable to the decreased mortality rate and the associated increase in the number of people living with HIV/AIDS.

Neuropathological studies also present an interesting picture of the HIV epidemic in the HAART era. In a review of 390 AIDS autopsy cases from 1982 to 1998, Masliah et al. (2000) reported a downward trend in the frequency of CNS opportunistic infections. Despite an initial decrease from 1987-1989, however, there was an overall trend towards an increase in HIVE, which was found in 26.3% of all examined cases. In addition, Gray et al. (2003) reported a rise in the number of variants of HIVE, probably associated with prolonged survival and ARV exposure.

Despite somewhat uncertain results from many quantitative studies of the CNS effects of HAART, the clinical impression appears to be that people with HAD are currently more neurologically stable, and tend to show reversal of neurological deficits (McArthur et al., 2003). Findings from MRS studies also show improved neuronal and glial integrity associated with ARV treatment. Wilkinson et al. (1997) reported an initial increase in a ratio measure of NAA in the parietooccipital white matter,

associated with improvement in neurological status, in subjects receiving ARV therapy. Following the initiation of combination ARV treatment, Chang et al. (1999) found increased Cho/Cr ratios in the midfrontal cortex and the basal ganglia, and decreased mI concentrations in the basal ganglia and the frontal white matter.

A number of studies have also documented improved neuropsychological performance associated with HAART. Ferrando et al. (1998) reported better neuropsychological performance in attention, concentration, learning, memory, and psychomotor speed associated with HAART. Sacktor et al. (1999) reported improved psychomotor speed, while Tozzi et al. (1999) reported improved concentration and speed of mental processing, mental flexibility, memory, fine motor functioning, and visuospatial and constructional abilities. In addition, Deutsch et al. (2001) found a reduced risk of developing cognitive impairment in individuals with AIDS in the HAART era.

Despite the apparently common findings of the benefit of HAART on HIV-associated neuropsychological impairment, Cysique, Maruff, and Brew (2004) noted that these improvements might not be maintained in the long run, due to factors such as age and drug resistance associated with prolonged ARV exposure. Indeed, in a cohort of 141 non-demented HIV-infected individuals, the authors reported no difference in the prevalence of cognitive impairment between those in the ARV monotherapy era and HAART era. However, it was noted that the pattern of impairment between the 2 eras appeared to be different, with improvement in the

HAART era in attention, verbal fluency, and visuoconstructional ability; and deterioration in learning efficiency and complex attention.

The fact that many medically stable HAART-treated individuals may be cognitively impaired suggests that ARV treatment may have limited effectiveness in the CNS in some cases. This has been attributed to the limited penetration of ARV drugs through the blood-brain barrier, or to divergent evolution of the virus in the CNS leading perhaps to more neuroadaptive and neurovirulent strains or divergence in ARV resistance. HIV replication, therefore, may persist within the CNS even with successful systemic ARV treatment. For example, Ellis et al. (2002) found that HIV RNA level may occasionally be higher in CSF than in plasma; and that CSF viral RNA better predicted progression to neuropsychological impairment than plasma RNA. In addition, since the introduction of HAART, people with HAD have exhibited higher CD4 counts (Dore et al., 1999), and an altered relationship between plasma HIV RNA and neurological status (McArthur et al., 2004). These findings indicate the contrast between the systemic and CNS effects of HIV in the context of current ARV treatment. Strain et al. (2005) reported that the viral populations found within the CNS were distinct from those found in plasma and lymphoid tissues. Lowered plasma and CSF HIV RNA level associated with initiation of a HAART regimen has also been associated with improved cognitive functioning (Marra et al., 2003; Robertson et al., 2004). In addition, Letendre et al. (2004) demonstrated that ARV regimens focused on CSF virologic suppression were associated with improved neuropsychological functioning. CNS-penetrating drugs were defined as those with reported CSF

concentrations exceeding the level needed to inhibit HIV replication, and included NRTIs (stavudine, zidovudine, abacavir), NNRTIs (efavirenz, nevirapine), and a protease inhibitor (indinivir). HIV infection within the CNS, therefore, may be protected from the effects of ARV and allowed to produce additional infection. In this context, the CNS has been referred to as both a sanctuary and a reservoir for the virus (McArthur, 2003).

PROPOSED STUDY AND HYPOTHESES

CNS complications are frequently observed in HIV-infected individuals. Corroborating lines of evidence suggest that white matter, particularly of the frontal lobes and the basal ganglia, are preferentially affected. Alteration of the corpus callosum has also been implicated by neuropathological and DTI studies. As an extension to current findings, this study proposes to use DTI to examine HIV-associated white matter injury. A methodologically novel aspect of the current study is the use of a voxelwise whole-brain approach in DTI data processing. Abandoning the ROI approach (utilized in all DTI studies of HIV infection to date) enables the examination of HIV-related white matter injury without specifying *a priori* ROI's. This approach is therefore particularly appropriate in the HIV-infected population, in which diffused white matter damage is implicated. Other methodological difficulties associated with the ROI approach are also avoided, as further discussed in the next section.

One goal of this study is to provide additional corroborating data to the previously reported association between HIV infection and alterations of DTI index values, with the benefit of a large and well-characterized sample relative to those reported in the current literature. In addition, participants in this study received a comprehensive neuropsychological assessment, allowing the examination of the relationship between white matter injury and cognitive impairment.

It is apparent that modern ARV treatment has altered the manifestation of HIV infection in the brain. Evidence of decreased white matter damage associated with HAART has been reported, although a limited number of neuroimaging studies have directly addressed the issue, and none utilizing DTI. Therefore, this study also attempts to examine the effect of ARV treatment on HIV-related white matter injury, i.e., whether successful suppression of HIV in the CNS is related to decreased white matter injury. To this end, CSF HIV viral load is used to reflect ARV treatment efficacy in the CNS (Ellis et al., 2002).

ARV drugs differ on their degrees of penetration into the CNS. In light of the association between ARV regimens including drugs with high CNS penetration and improved cognitive performance (Letendre et al., 2004), we hypothesize that the inclusion of such drugs in a treatment regimen would be related to decreased HIV-related white matter injury.

Thus, the current study is driven by the following hypotheses.

Hypothesis 1: HIV-infected individuals, especially those diagnosed with AIDS, will exhibit white matter injury (i.e., lower FA and higher MD) when compared to seronegative controls.

Hypothesis 2: Controlling for pre-treatment immunosuppression level (i.e., nadir CD4 level), the extent of white matter injury in HIV-infected individuals will be related to ARV treatment efficacy in the CNS (i.e., CSF HIV RNA).

Hypothesis 3: Controlling for pre-treatment immunosuppression level, individuals on ARV regimens with better CNS penetration will exhibit less white matter injury.

Hypothesis 4: The extent of white matter injury in HIV-infected individuals will be related to neuropsychological impairment.

METHODS

PARTICIPANTS

Participants include 39 individuals with HIV infection (HIV+) and 25 HIV seronegative healthy controls (CON). HIV infection was indicated by enzyme linked immunosorbent assays and a Western Blot confirmatory test. Thirty-six of the HIV+ were recruited as part of the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study, an NIH-funded multi-site study of the CNS effects of HAART. In addition to neuroimaging examinations, participants in the CHARTER study received detailed laboratory, medical history, and neuropsychological examinations. The majority of these participants (83%, N = 30) were undergoing ARV treatment at the time of the study. As a result, 22 participants had undetectable CSF viral load. In addition, CSF samples were not acquired for 8 participants, yielding a total of 6 participants with detectable CSF HIV RNA. Since this is a primary variable of interest in this study, 3 additional individuals with detectable CSF HIV RNA were recruited from ongoing studies at the HIV Neurobehavioral Research Center in San Diego, CA. These additional participants received comparable neuroimaging, laboratory, medical history, and neuropsychological examinations as above.

The 25 HIV seronegative CON were recruited as part of a neuroimaging study of HIV infection (N = 12), and another study of alcoholism (N = 13). CON and HIV+ were comparable regarding age, $t(62) = .93$, $p > .1$, years of education, $t(62) = .31$, $p > .1$, proportions of gender, $\chi^2(1, N = 64) = 1.05$, $p > .1$, and proportions of ethnicity

(White/non-White), $\chi^2(1, N = 64) = 2.49$, $p > .1$. Table 2 shows demographic information and relevant lab results of study participants.

Potential participants were excluded if they met any of the following criteria: 1) a history of head injury with loss of consciousness greater than 10 minutes; 2) a history of neurological or psychiatric illness that would adversely impact cognitive functioning (e.g., seizure disorder, toxoplasmosis, or schizophrenia); 3) a diagnosis of substance use disorder (except marijuana) within the past 6 months.

As indicated above, CSF data were available for 31 of the 39 HIV+. Plasma data were available for 38 of the 39 participants. Available CD4 level of HIV+ ranged from 18 to 1223 (M = 529, SD = 243). Nadir CD4 data were available for all HIV+, and ranged from 0 to 600 (M = 219, SD = 159). Ten participants had detectable plasma HIV RNA, with $\log_{10}$ values ranging from 2.92 to 5.71 (M = 4.40, SD = 0.75). Nine participants had detectable CSF HIV RNA, with $\log_{10}$ values ranging from 2.42 to 3.79 (M = 3.06, SD = 0.46). In these 9 individuals, CSF and plasma HIV RNA values were not significantly related, $r(7) = .50$, $p > .1$. CSF HIV RNA detectability is directly related to plasma HIV RNA detectability, $\chi^2(1, N = 31) = 30.85$, $p < .0001$, and inversely related to CD4 level $t(28) = 2.28$, $p < .05$.

Approximately half (49%, N = 19) of all HIV+ had an AIDS diagnosis. HIV+ with and without AIDS were comparable regarding age, $t(37) = .26$, $p > .1$, years of education, $t(37) = 1.97$, $p > .1$, proportions of gender, $\chi^2(1, N = 39) = .43$, $p > .1$, and proportions of White ethnicity, $\chi^2(1, N = 39) = 3.63$, $p > .05$. Participants with AIDS exhibited lower CD4 level, $t(36) = 3.17$, $p < .0005$, and nadir CD4 level, $t(37) = 6.79$,

$p < .0001$. No group differences were found, however, in proportions of individuals with detectable plasma HIV RNA, $\chi^2(1, N = 38) = .871$, $p > .1$, and proportions with detectable CSF HIV RNA, $\chi^2(1, N = 31) = .261$, $p > .1$. The lack of significant relationships between disease stage and detectability of virologic markers was likely due to ARV treatment: 84% of individuals with AIDS were receiving treatment. A comparable proportion of HIV+ without AIDS (80%) also received treatment, $\chi^2(1, N = 39) = .118$, $p > .1$. Table 3 shows demographic information and relevant lab results of HIV+ with and without the AIDS diagnosis.

CNS PENETRATION OF ARV DRUGS

Hypothesis 3 of the current study concerns the relative abilities of ARV drugs to enter the CNS. To quantify this, a scoring system for CNS penetration of ARV drugs introduced in a preliminary study by Letendre et al. (2006) was used. In this system, each ARV drug is assigned a score of 0, 0.5, or 1, based on published data on CSF concentrations and/or chemical properties. For each participant's treatment regimen, the scores for all drugs are summed to represent the degree of CNS penetration. The authors also showed a significant relationship between the penetration score and CSF viral load in a large sample of HIV-infected persons (N = 374). Table 4 shows the scores assigned to ARV drugs currently in use for the treatment of HIV. Calculated in this way, CNS penetration scores for HIV+ ranged from 0 to 3.5 (M = 1.44, SD = 1.07). Due to the high number of participants with undetectable plasma and/or CSF viral load, these variables cannot be directly

correlated with the CNS penetration scores. Participants were therefore divided into 2 groups based on viral load detectability. Neither of these grouping variables for plasma nor CSF viral loads were related to the CNS penetration scores, although there were trends towards lower penetration scores in the detectable groups for both plasma, $t(37) = 1.44$, $p = .16$, and CSF, $t(37) = 1.53$, $p = .13$.

NEUROPSYCHOLOGICAL EXAMINATION

NP data are available for HIV+. All testing was performed by trained psychometrists using standardized procedures. The standard battery examined 7 neurocognitive ability domains most implicated in HIV-associated cognitive disorders (Heaton et al., 1995): Speed of Information Processing, Learning, Delayed Recall, Abstraction/Executive Functioning, Verbal Fluency, Attention/Working Memory, and Motor Skills. Table 5 shows the tests within each ability domain.

A statistically derived summary score were used to reflect the severity of NP impairment. This system of scoring, proposed and validated by Heaton et al. (1995 & 2004), focuses on impaired performances, and gives no credits for above-average performances, i.e., it minimizes the chance that good performance on some component test measures will obscure impairments on others. In this scoring system, the demographically corrected T-score for each test is converted to a deficit score (DS), representing normal to severe impairment, as follows: DS = 0 (normal) for $T > 39$, DS = 1 (mildly impaired) for $35 \leq T \leq 39$, DS = 2 (mildly to moderately impaired) for $30 \leq T \leq 34$, DS =3 (moderately impaired) for $25 \leq T \leq 29$, DS = 4 (moderately to

severely impaired) for $20 \leq T \leq 24$, and DS =5 (severely impaired) for $T < 20$. As a summary measure of cognitive impairment, the global deficit score (GDS) is subsequently calculated by averaging the DS across all tests in the battery. Using case conference diagnoses of cognitive impairment as the gold standard, the authors demonstrated that a GDS cutpoint of 0.5 reliably yields approximately 15% specificity in detecting of cognitive impairment. (This GDS value can be thought of as being equivalent to having mildly impaired, DS = 1, ratings on half of the tests in the battery.)

Calculated in this way, GDS values ranged from 0 to 3.47 (M = 0.38, SD = .62). Due to the floor effect apparent in the variable values (51% of HIV+ had GDS < .1), participants were divided into 2 groups based on cognitive impairment, defined as GDS ≥ 0.5 (i.e., the previously established cutpoint for 15% specificity of detecting cognitive impairment). In this manner, 10 participants exhibited cognitive impairment (NPI+), while 29 were cognitively normal (NPI-). The 2 groups were comparable in age, $t(37) = .55$, $p > .1$, years of education, $t(37) = .68$, $p > .1$, and proportions of gender, $\chi^2(1, N = 39) = .10$, $p > .1$, and White ethnicity, $\chi^2(1, N = 39) = .47$, $p > .1$. Likewise, no group differences were found in proportion with AIDS, $\chi^2(1, N = 39) = .009$, $p > .1$, CD4 level, $t(36) = .08$, $p = .94$, nadir CD4 level, $t(37) = .67$, $p > .1$, proportions of participants with detectable plasma HIV RNA, $\chi^2(1, N = 38) = .105$, $p > .1$, or proportions with detectable CSF HIV RNA, $\chi^2(1, N = 31) = .087$, $p > .1$. However, a significantly higher proportion of NPI+ are on ARV than NPI-, $\chi^2(1, N = 39) = 4.65$, $p < .05$. Table 6 shows demographic information and relevant lab results of

HIV+ with and without cognitive impairment.

DTI DATA ACQUISITION AND PROCESSING PROTOCOLS

All neuroimaging scans were performed on a GE 1.5T imager at the San Diego VA Medical Center. DTI data for all HIV+ and 12 CON participants were collected through a single-shot spiral spin-echo acquisition with TE = 120 ms, TR = 6000 ms, field of view = 250 mm, slice thickness = 3.9 mm, image matrix = 64x64, and b-value = 2416 s/mm^2. Diffusion-weighted images were acquired in 42 diffusion directions, in addition to the normalization image with no diffusion encoding (Frank, 2001). Four images were acquired and averaged for each direction (and for the normalization image). The spirally acquired DTI images were reconstructed at 128x128 in-plane resolution, with slice thickness remaining at 3.9 mm. For 13 CON participants, data were collected with the same pulse sequence except for the following parameters: TE = 100 ms, TR = 6000 ms, field of view = 240 mm, slice thickness = 3.8 mm, and b-value = 1745 s/mm^2. Post-acquisition processing procedures were identical for all participants.

For the current study, a voxelwise whole-brain approach was utilized for the examination of HIV-related white matter damage, which provides several advantages over ROI-based approaches. Examining whole-brain white matter changes eliminates the necessity of a priori hypotheses regarding white matter changes in specific regions, making this approach particularly suitable for examining the generally diffused HIV-related white matter damage. Other complications associated with ROI-based

techniques are also avoided, including the determination of optimal ROI shapes and sizes across subject groups (a special challenge in the studies of clinical populations with significant neuroanatomical changes, such as the general white matter atrophy associated with advanced HIV-infection), the reliability of ROI placement, and the comparability of ROI's across subjects (i.e., whether they represent identical neuroanatomical regions in all subjects).

A common voxelwise whole-brain approach for analyzing MR neuroanatomical data is generally referred to as voxel-based morphometry (VBM; see Ashburner and Friston, 2000, for an overview). VBM was originally developed for finding local changes in grey matter density in T1-weighted structural brain images. A typical processing scheme involves initially registering anatomical images to a template (generally either a standard space template, or a study-specific template typically created by averaging all subjects' images after registering to a standard-space template). Registered images are then segmented for the tissue type(s) of interest; and the segmented images are spatially smoothed. Smoothing is generally performed for several reasons, including to ameliorate imperfect registration, and to increase sensitivity (which is achieved only if the extent of smoothing matches the spatial extent of the structure of interest). Smoothing also renders the data more Gaussian distributed, which improves the validity of Gaussian random field (GRF) theory, a statistical thresholding approach commonly used for VBM data to account for Type I error level inflation associated with multiple comparisons.

Primary limitations of VBM approach relevant to the analysis of diffusion data

can be categorized into issues related to alignment, and those related to spatial smoothing. Alignment issues have to do with the comparability across individual brains among corresponding voxels within the tissue of interest, i.e., how can one guarantee that any given voxel in the tissue of interest contains data from anatomically corresponding regions across subjects, especially considering the typically imperfect registration of individual brains to the template. Such problem is especially apparent when the registration process utilizes low to medium degrees of freedom, which is typical in VBM studies. For example, in a study of children with chromosome 22q11.2 deletion syndrome, Simon et al. (2005) found apparent alteration of FA in the patient group. However, the authors also noted that, due to imperfect registration, changes in ventricle size may actually be driving the FA results (i.e., "white matter" voxels may in fact have situated in the ventricles, thus representing the more isotropic water diffusion of the CSF). An obvious solution for minimizing residual misalignment after registration is the use of non-linear high-degrees-of-freedom registration approaches. In extreme cases, such approaches can render target images virtually identical to template. However, this procedure is performed at the risk of excessive distortion of anatomical topology, such as splitting an actual fiber bundle into two after registration, or vice versa, thus compromising the validity of the analysis.

Another limitation of VBM approach is related to the spatial smoothing procedure generally performed for reasons indicated above. Typically, there is no principled way of determining the appropriate smoothing extent; and different smoothing extents can yield significantly different results and conclusions from the

data. For example, in a VBM dataset of FA images in schizophrenia, Jones et al. (2005) demonstrated that group differences appeared and disappeared at different levels of smoothing extents ranging from 0 to 16 mm FWHM. In addition, depending on the extent of application, spatial smoothing can also increase the partial volume effect (i.e., the blurring of tissue classes at the border of two tissues), further complicating the interpretation of the results.

With these issues related to voxelwise whole-brain DTI approach in mind, the current study utilized a data processing methodology introduced by Smith et al. (2006). The so called Tract-Based Spatial Statistics (TBSS) approach allows voxelwise investigation of whole-brain white matter, while minimizing the necessity of near-perfect registrations required in VBM. In addition, the spatial smoothing process is altogether eliminated. These methodological issues are circumvented by the reduction of the investigated white matter tissue to a whole-brain "skeleton" of only the centers of white matter fiber tracks. The one-voxel-wide skeleton is derived from the average image of all subjects' FA images after registration to a common template. This average skeleton is used as the basis for a search algorithm for optimal white matter skeletons in individual subjects' brains. By eliminating partial voluming issues (by focusing on the narrow skeleton within white matter), and by individually fitting the skeleton for each subjects, the issues of smoothing and perfect registration are avoided in this type of analysis. However, due to the nonlinear registration procedure, and lacking the spatial smoothing procedure, the assumption of Gaussian distribution necessary for GRF analysis cannot be made for DTI images processed in this manner.

An alternative permutation-based nonparametric statistical analysis method is described in the next section.

The steps involved in DTI data processing in this study are described as follow. The computational algorithms were performed using ready-to-use executables included in the Analysis of Functional NeuroImages package (AFNI; Cox, 1996), and the TBSS tools of the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Package (FSL; Smith et al., 2004).

1. FA and MD images were derived from the acquired diffusion-weighted (and normalization) images via the *3dDWItoDT* program in the AFNI package. First, the normalization (b = 0) image was skull-stripped with a built-in AFNI algorithm (identical to default stripping parameters by the AFNI *3dAutomask* program). The stripped normalization image was subsequently used as a mask for the diffusion-weighted images in 42 directions, rendering the shape and size of each image slice in each subject identical across all diffusion encoding directions. The masked diffusion data were then fitted to a 3x3 symmetric tensor model. The orthogonal eigenvectors and corresponding eigenvalues were derived for the 3 axes of the ellipsoid fitted to the tensor model, enabling the calculations of FA and MD based on the standard formulas included above.

(The following steps were performed using tools included with FSL.)

2. For convenience of later data handling, FA and MD values were linearly scaled to roughly $0 \leq x \leq 10000$. For FA, which theoretically ranges from 0 to 1, all values were multiplied by 10000. For MD, which ranges approximately from 0 to 5,

all values were multiplied by 2000. No negative FA or MD values were apparent using the current processing procedures.

3. Prior to computing the mean FA image necessary for subsequent generation of the white matter skeleton, individual subjects' FA images were nonlinearly registered to a common target image using the Image Registration Toolkit (Rueckert et al., 1999), which performs nonlinear registration based on free-form deformations and B-Splines. This target image was chosen from among the study subjects in a way that minimized the amount of warping necessary across all subjects in the registration process. To identify this "most typical" registration target, Smith et al. (2006) recommended registering every subject to every other subject, in order to calculate the mean displacement of the corresponding warping. The registration target is the subject with the minimum mean displacement to all other subjects. The authors also noted that this ideal procedure may be impractical when performed on a single-processor microcomputer, and showed that selection of the registration target with alternative procedures yielded similar results. To illustrate this point, estimating 15 minutes for registering each pair of brains, the full sample in the current study (N = 64) would require 42 days (i.e., 64P2 x 15 minutes) of uninterrupted processing time to complete this procedure. Consequently, a representative subject was visually selected, considering both image quality and anatomical similarity to other subjects in the sample.

4. The registered images from step 3 were affine-transformed into 1x1x1 mm^3 MNI152 space. The purpose of the upsampling is to avoid partial voluming.

Transformed images were then averaged to create a mean FA image.

5. A white matter tract skeleton was generated from the mean FA image. The goal of this process was to identify the one-voxel-thick lines or surfaces with maximum FA values within white matter tracts, representing that center of the tracts. The identified white matter tract skeletons were then thresholded to eliminate noises. In this study, an FA value of 0.25 (= 2500 following the scaling in step 2) was used as the lower threshold.

6. To account for residual misalignment from the registration procedures up to this point, subjects' FA images were individually "projected" on to the mean FA skeleton. Essentially, this process involves superimposing the skeleton on an FA image, then searching perpendicular to the tracts for maximum FA values (i.e., tract center), which are subsequently assigned to the corresponding tract location. Individual skeletons after projection remain anatomically identical to the mean FA skeleton, with only the values assigned to each voxels altered, allowing direct voxelwise comparisons.

7. Step 6 was repeated to generated individual projected MD skeletons, using the same mean FA skeleton as above, superimposed on individual MD images, as the basis for the searching algorithm.

STATISTICAL ANALYSIS

A voxelwise whole-brain statistical analysis, by nature, is complicated by Type I error inflation due to multiple comparisons. Studies using VBM approach typically

utilize GRF theory to account for this issue. TBSS approach, however, involves nonlinear registration, deviating the data from a Gaussian distribution. The spatial smoothing procedure utilized in VBM, which renders data more Gaussian distributed, is also lacking in TBSS. Consequently, Smith et al. (2006) recommend the use of a nonparametric permutation-based approach for the statistical analysis of TBSS data (see Nichols & Holmes, 2001, for an introduction to the approach with PET and fMRI examples). Briefly, this nonparametric statistical technique involves creating a sampling distribution of test statistic based on a large number of random permutations of the data. The critical value of the test statistic can then be determined from the rank orders. For the current analyses of TBSS data, the suprathreshold cluster test was used for the analysis of DTI index values. This technique involves thresholding the statistic image at a predetermined primary threshold, and assessing the resulting pattern of suprathreshold activity. The procedure therefore requires the construction of a permutation distribution of cluster sizes of voxels with values above the primary threshold. The critical suprathreshold cluster size is then the $\lfloor \alpha N \rfloor + 1$ largest member of the permutation distribution, where α is the desired Type I error level, and N is the number of permutations (5000 in this study).

Before performing the suprathreshold test, an arbitrary choice of the primary threshold has to be made. The selection of a threshold value involves a tradeoff between detecting larger clusters of small-magnitude differences, and vice versa. It is generally recommended that a threshold be chosen that yields the most meaningful results in a given dataset (Nichols & Holmes, 2001). This should also be chosen prior

to statistical analyses, ideally in a separate dataset from the one under investigation, to avoid further inflating Type I error level. For the current study, an analysis investigating the effect of HIV infection on FA (i.e., comparing HIV+ and CON), and another analysis investigating the effect on MD were examined for threshold values yielding reasonable cluster sizes that appeared meaningful for the interpretation of results. For both FA and MD, a threshold of $t > 2$ was chosen for all subsequent analyses.

All statistical analyses were performed using the *randomise* program included with FSL, using a threshold of $t > 2$, and 5000 permutations to construct the sampling distributions. The possible number of permutations is directly proportionate the size of the dataset. For the current study, these numbers in all analyses approached infinity. The number 5000 was chosen because it was recommended by the *randomise* program; and increasing the value to 10000 did not alter the results in initial analyses.

As noted above, 13 of the 25 CON participants received a slightly different DTI protocol than the other CON and HIV+ participants. Since FA values are automatically scaled to range from 0 to 1, all CON participants were treated as equivalent. A permutation-based test with 5000 permutations confirmed that the 2 CON subgroups were comparable in FA values, $N = 25$, $t > 2$, $p > 0.1$. MD values, however, are not scaled, preventing a comparison between the 13 CON participants in this subgroup and HIV+ participants without the confounding effect of the difference in protocols. Confirming this, a similar permutation-based test showed statistically significant differences in the majority of skeleton voxels, $N = 25$, $t > 2$, maximum $p <$

.01. These 13 CON participants were therefore excluded from statistical analyses involving MD.

In this manner, the following statistical analyses were performed in this study. All analyses were performed separately for FA and MD.

Hypothesis 1 analyses involve examining white matter injury associated with HIV infection, and whether AIDS contributes to additional injury. Two comparisons were performed for each DTI index. To examine the effect of HIV infection, FA and MD of CON and all HIV+ were compared. To examine the effect of disease stage, HIV+ with and without AIDS were compared. The Bonferroni-corrected α-level for these comparisons was .025. In addition, exploratory analyses were performed to compare FA and MD between CON and HIV+ with AIDS, and between CON and medically asymptomatic HIV+.

Hypothesis 2 analyses involve examining the relationship between white matter injury and a CSF marker of ARV treatment efficacy in the CNS, i.e., CSF HIV RNA. Two analyses were performed. First, HIV+ with detectable CSF HIV RNA were compared with those who were undetectable. Additionally, for individuals with detectable CSF HIV RNA, this marker variable was correlated with FA and MD, after covarying for nadir CD4 level.

Hypothesis 3 analysis involves examining the relationship between white matter injury and the degrees of CNS penetration of participants' ARV regimens. CNS penetration scores of HIV+ were correlated with FA and MD, after covarying for nadir CD4 level.

Hypothesis 4 analysis involves examining the relationship between white matter injury and HIV-associated NP impairment. FA and MD were compared between NPI- and NPI+.

RESULTS

All permutation-based inferences on suprathreshold cluster size were performed with a threshold of $t > 2$, using 5000 permutation to construct the null distribution of the cluster-size statistic. The findings below are summarized in Table 7.

HYPOTHESIS 1: HIV INFECTION

Using a nonparametric permutation-based suprathreshold cluster test, voxelwise FA was found to be significantly higher in CON than HIV+ in the right posterior limb of the internal capsule, the right inferior longitudinal fasciculus, and the right optic radiation, $N = 64$, $p < .025$ (Figure 1). No significant differences were found in MD of CON 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, $N = 51$, $p = .0642$ (Figure 2).

No significant differences in FA were found between medically asymptomatic HIV+ and those with AIDS, $N = 39$, $p > .1$. However, significantly increased MD in the AIDS group was apparent in the bilateral posterior corona radiata, the left anterior and right posterior superior longitudinal fasciculus, the bilateral inferior longitudinal fasciculus, the left anterior and bilateral posterior limbs of the internal capsule, the corpus callosum body and the splenium, and the bilateral optic radiation, $N = 39$, $p < .025$ (Figure 3).

Additional exploratory analyses comparing CON and HIV+ with AIDS

showed AIDS-associated FA decrease in the right posterior limb of the internal capsule, the right inferior longitudinal fasciculus, and the right optic radiation, $N = 44$, $p < .025$ (Figure 4). Significantly increased MD in the AIDS group was apparent in the bilateral corona radiata, the bilateral superior longitudinal fasciculus, the right inferior longitudinal fasciculus, the bilateral internal capsule, the corpus callosum body and the splenium, and the right optic radiation, $N = 31$, $p < .05$ (Figure 5).

No significant diffusion differences were found between CON and medically asymptomatic HIV+, $N = 45$ (FA) and 32 (MD), $p > .1$.

HYPOTHESIS 2: ARV TREATMENT EFFICACY

No significant diffusion differences were found between HIV+ with detectable and undetectable CSF HIV RNA, $N = 31$, $p > .1$. Similarly, no significant relationships were found between FA/MD and detected CSF HIV RNA, $N = 9$, $p > .1$, regardless of whether nadir CD4 was used as a covariate.

HYPOTHESIS 3: CNS PENETRATION OF ARV REGIMEN

No significant relationships were found between FA or MD, and the ARV CNS penetration score, $N = 39$, $p > .1$, regardless of whether nadir CD4 was used as a covariate.

HYPOTHESIS 4: COGNITIVE IMPAIRMENT

Decreased FA in the NPI+ group was apparent in the genu and anterior corpus

callosum body, N = 39, $p < .05$ (Figure 6). NPI+ exhibited significantly higher MD in the right posterior superior longitudinal fasciculus, the right posterior internal capsule, and the genu, body, and splenium of the corpus callosum, N = 39, $p < .05$ (Figure 7).

DISCUSSION

Cognitive impairment is observed in up to half of people infected with HIV (Heaton et al., 1995). In addition, up to 20% of infected individuals are estimated to develop HIV-associated dementia (HAD), a diagnosis characterized by severe cognitive, motor, and neurobehavioral dysfunction, accompanied by significant disruption in instrumental activities of daily living (McArthur, 2004). HAD has also been associated with shorter life span (Tozzi et al., 2005). Although these symptoms are typically not apparent until more advanced disease stages, evidence points to CNS involvement early following HIV infection. For example, an iatrogenic case study reported virus in the brain as soon as 15 days after an accidental intravenous inoculation (Davis et al., 1992). MR spectroscopy (MRS) studies have also reported abnormalities in brain metabolite concentrations in clinically and cognitive asymptomatic HIV-infected individuals (Moller et al., 1999; Suwanwelaa et al., 2000). It has also been suggested that the accumulation of CNS insults in earlier disease stages may explain the development of HAD (McArthur, 2004). These findings point to the importance of understanding the mechanisms and manifestations of HIV infection in the CNS at all stages of the disease, in addition to the need for additional sensitive markers of early HIV involvement in the CNS.

White matter abnormalities are among the hallmark pathological features of HIV encephalitis (Masliah et al., 2000). To better understand the disease processes of HIV within the CNS, the current study attempted to examine white matter injury

associated with HIV infection via diffusion tensor imaging (DTI). DTI is a relatively novel neuroimaging technique for the examination CNS abnormalities, the utility of which is derived from its sensitivity to differential degrees of restriction of the diffusion of water molecules within tissues. Since microscopic water diffusion is more uniform in fibrous tissues, such as white matter tracts within the brain, DTI is especially sensitive in detecting changes in white matter microstructures. Two typically used scalar diffusion indices are fractional anisotropy (FA), and mean diffusivity (MD). These values both reflect water movement along the 3 primary axes of the diffusion ellipsoid, formally represented by the eigenvalues associated with the 3 orthogonal eigenvectors of the symmetrical 3x3 tensor matrix fitted to the diffusion-weighted image data (Parker, 2004). FA is a summary measure of the differences among the eigenvalues, and thus provides information regarding the orientation specificity of water diffusion. White matter injury is typically associated with decreased FA values, i.e., decreased orientation specificity or "anisotropy" of water diffusion. MD is derived by averaging the 3 eigenvalues, and thus reflects the total degree of water diffusion along the ellipsoid axes. White matter abnormalities are typically associated with increased MD values.

A number of studies have reported HIV-associated alterations in FA and MD in regions of interest (ROI) placed in the frontal white matter, basal ganglia structures, the internal capsule, and the genu and splenium of the corpus callosum (reviewed above). A goal of the current study is to provide additional supporting information regarding alterations in water diffusion within brain white matter in a large and well-

characterized group of participants relative to currently published studies. In addition, a methodologically novel aspect of this study is the use of a data processing technique that allows voxelwise examination of major white matter tracts in the whole brain. This enables the identification of affected white matter regions without the need for a priori hypotheses regarding anatomical specificity, as has been the case for all existing DTI studies of HIV.

Since the introduction of highly active antiretroviral therapy (HAART) of HIV infection, antiretroviral (ARV) treatment has been largely successful at achieving the treatment goals of suppressing viral replication and restoring immunologic functioning. This has resulted in significant improvements in clinical symptoms and quality of life of infected individuals (Tozzi et al., 1999). Data on the effect of ARV treatment on cognitive functioning, however, have been less conclusive. For instance, conflicting accounts regarding the pre-/post-HAART change in HAD prevalence in either direction have been reported (McArthur, 2004). It should be noted these prevalence comparisons may be confounded by factors such as the prolonged survival associated with effective ARV treatment. Moreover, epidemiological studies typically do not focus on qualitative changes in HIV-associated cognitive impairment. In fact, alterations in the cognitive domains affected by HIV in the HAART era have been demonstrated (Cysique, Maruff, & Brew, 2004). However, again, the different neuropsychological batteries across time may have confounded the results. Leaving aside these issues, however, the fact remains that many medically stable HAART-treated individuals are still cognitively impaired. This may be partly explained by

evidence indicating that HIV replication may persist in the CNS even with successful systemic treatment (Ellis et al., 2002). These findings have been attributed to the limited CNS penetration of ARV drugs, and/or divergent evolutions of HIV in and outside of the CNS. The former theory is supported by findings associating improved performance on neuropsychological testing, and ARV regimens including drugs known to have good CNS penetration (Letendre et al., 2004).

To further clarify this issue, this study also attempted to examine the relationship between diffusion alteration and 2 ARV treatment variables: 1) CSF HIV RNA, a marker representing ARV treatment efficacy in the CNS, and 2) an index of CNS penetration of ARV regimens (Letendre et al., 2006). In addition, in an attempt to better understand the cognitive correlates of HIV CNS infection, the relationship between white matter tract integrity and cognitive impairment was also examined in this cohort of primarily ARV-treated individuals (83% of HIV+ had ongoing ARV treatment at the time of the DTI examinations).

The current study cohort consisted of 30 HIV-infected individuals (HIV+) and 25 seronegative controls (CON). As discussed above, data from only 12 CON were used in the analyses of MD data, while all 25 were used in FA analyses. Both medically asymptomatic individuals and those with AIDS were well represented, each comprising about half of the HIV+ sample. Results from Hypothesis 1 analyses confirm previous findings regarding white matter abnormalities associated with HIV infection. DTI as implemented here appears to be sensitive to HIV-related microstructural white matter abnormalities in the overall HIV+ group, consisting of

individuals in both disease stages. Statistically significant findings, however, were limited to white matter tracts within the right posterior region, and only with the FA measure; while a trend ($p = .06$) towards significant group differences in MD was apparent. The smaller sample size in the MD analysis should be noted. This suggests that the lack of significant finding may be attributable to a statistical power issue.

Within the HIV+ group, more advanced infection, represented by the AIDS diagnosis, was associated with more widespread white matter damage. These include bilateral findings in most significant regions, more widespread damage to the internal capsule (including the anterior limb), and findings in the corpus callosum and other regions. These findings, however, were limited to the MD measure. Additional exploratory pairwise group comparisons showed similar effects of AIDS in relation to the CON group on MD. CON/AIDS group differences in FA, however, were also apparent, suggesting a larger effect size in the comparison between these groups over the AIDS/non-AIDS comparison. It may therefore be inferred that medically asymptomatic infected individuals exhibited white matter injury relative to CON. A direct comparison between the 2 groups, however, failed to find any group differences. The current DTI implementation, therefore, appears insensitive to white matter tract injury in subclinical HIV. Table 7 summarizes findings from the above group comparison.

Diffuse cerebral white matter damage, apparent in neuropathological and structural neuroimaging studies, is a hallmark feature of HIV encephalitis. This is confirmed by the demonstrated widespread white matter injury in medically

symptomatic HIV-infected individuals. The significant diffusion alterations within the corpus callosum are also consistent with current evidence. Using immunohistochemistry and in situ hybridization in AIDS patients with HIV encephalitis, Gosztonyi et al. (1994) found heavy labeling of HIV DNA and RNA in the corpus callosum. A simian model of AIDS indicated significant correlations between motor impairment and accumulation of beta-amyloid precursor protein in the corpus callosum, indicating axonal injury (Weed et al., 2003). Moreover, callosal thinning in individuals with AIDS was reported in a recent MRI study using computational anatomy techniques (Thompson et al., 2006).

In addition, injury to the internal capsule demonstrated here also corroborates existing data. Histopathological data indicated heavy presence of HIV DNA and RNA in the internal capsule (Gosztonyi et al., 1994). Pomora et al. (2001) also reported decreased FA in a DTI study of individuals primarily with more advanced infection. Similar to the finding here, the latter study reported a preferential damage to the posterior limb in the internal capsule. Situating directly adjacent to the internal capsule, the basal ganglia has been extensively implicated in HIV CNS disorders. Brew et al. (1995) reported a high level of productive HIV infection within the basal ganglia. Multinucleated giant cells, another hallmark pathological feature of HIVE, are also found primarity within these structures (Budka, 1986). Structural compromises of basal ganglia structures have also been demonstrated in a number of autopsy (e.g., Neuen-Jacob et al., 1993) and structural MRI (e.g., Stout et al., 1998) studies.

Specific HIV-related injuries to the superior/inferior longitudinal fasciculi, the optic radiation, or the corona radiata, however, have not been reported in the literature in the absence of opportunistic infections or secondary conditions. For example, Fabricius, Moller, and Prantl (1991), reported a high incidence of optic neuritis in AIDS patients in the course of cytomegalovirus retinitis. Apparent white matter injuries to these regions, therefore, may reflect the diffuse white matter pathology associated with HIV encephalitis. Whether the current findings in these regions represent anatomically specific HIV-related CNS damage remains a question for future investigation.

In the second set of analyses (Hypotheses 2 and 3) involving ARV treatment variables, the lack of association between diffusion alterations and either the measure of CNS treatment efficacy (i.e., CSF HIV RNA) or the degree of CNS penetration of ARV drugs was in contrary to expectation. This is especially remarkable in light of reported association between elevated CSF HIV RNA and subsequent development of HAD (Ellis et al., 2002). The degree of pretreatment immunosuppression level, indicated by nadir CD4 count, was used as a covariate in examining the white matter effects of both variables. However, it is possible that these analyses were confounded by other factors, including history of medication regimens, response, and adherence; time since seroconversion; and structured treatment interruption (which may be associated with detectable viral loads despite history of good treatment response and adherence). Moreover, only 9 HIV-infected individuals (23%) in this study had detectable CSF viral load, suggesting a possible statistical power issue in the analyses

of the variable. Currently, no other published studies have examined diffusion alterations in the context of these variables. The relationship between ARV treatment and HIV-related white matter injury therefore remains to be investigated.

The current study also showed abnormal diffusion alterations in cognitively impaired HIV-infected individuals (Hypothesis 4). Table 8 summarizes findings from these analyses involving cognitive impairment. White matter injury has been widely implicated in HIV-related cognitive impairment. A number of DTI studies have reported similar findings. Cloak, Chang, and Ernst (2004) found an association between elevated frontal white matter MD and lower composite cognitive performance. Ragin et al. (2004a & b) reported correlations between average whole-brain DTI measures and HAD severity. Ragin et al. (2005) reported correlations between DTI measures in the centrum semiovale and basal ganglia (grey matter) structures and neuropsychological deficits. Wu et al. (2006) reported associations between DTI measure in the frontal white matter and the genu and splenium of the corpus callosum, and neuropsychological deficits. In additional to DTI studies, structural MRI studies of individual with HAD have demonstrated white matter volume loss in both pre- (Pedersen et al., 1991; Aylward et al., 1995) and post-HAART cohorts (Chiang et al, 2007). A number of MR spectroscopy studies also reported abnormal metabolite concentrations in HIV-infected individuals with cognitive impairment, particularly in the frontal white matter (Chang et al., 1999 & 2002; Stankoff et al., 2001)

In this study, white matter damage in the genu, body, and splenium of the corpus callosum was found in cognitive impairment individuals. The corpus callosum contains fiber bundles interconnecting the cerebral hemispheres, and has been implicated in a wide and complex range of cognitive functions, particularly those involving interhemispheric interactions. Symptoms indicating interhemispheric disconnection are therefore common in patients with callosal dysgenesis or damage. Left-ear suppression during verbal dichotic listening tasks associated with callosal damage has been reported in various populations, including patients with multiple sclerosis (Gadea et al., 2002), and partial section of the corpus callosum (Sugishita et al., 1995). In addition, individuals with callosal agenesis typically exhibit slowed simple reaction time and a range of motor dysfunctions (Sauerwein & Lassonde, 1983). Although frank disconnection symptoms tend to be associated with extensive callosal dysfunction, and have not been associated with HIV infection, general cognitive impairment marked by frequent motor dysfunctions has been reported in individuals who exhibited callosal thinning on MRI exams (Thompson et al., 2006). Considering previous data indicating extensive HIV involvement in the corpus callosum, the finding here provides promising evidence for the use of DTI in monitoring the development of HIV-related cognitive disorders.

Cognitive impairment was also associated here with white matter injury in the posterior limb of the internal capsule, which has been described to contain corticospinal fibers (Kretschmann, 1988). Although lacking specific empirical evidence, the damage to such white matter tracts would be consistent with the motor

deficits commonly observed in people with HIV-related cognitive disorders. The single study of focal injury to the internal capsule reported motor weakness in patients with damage to the posterior limb and the genu of the internal capsule (Tredici et al., 1982).

The superior longitudinal fasciculus, a major intrahemispheric fiber tract, contains fibers connecting the dorsolateral frontal and posterior associative areas. The region is believed to subserve a wide range of complex cognitive functions, including sensorimotor coordination, higher aspects of motor behaviors, and coordination of visuospatial and audiospatial information (Makris et al., 2005). FA reduction in the region has been related to impaired performance on the Trail Making Test B in patients with frontotemporal dementia (Borroni et la., 2007). The superior longitudinal fasciculus has not been specifically implicated in HIV-related cognitive impairment. The current finding in this region is novel and remains to be corroborated.

It should be noted that the findings discussed above were based on a global measure of cognitive impairment, which additively takes into account impairments on any of the assessed neuropsychological domains. The significant regions reported here, therefore, represent white matter tracts most commonly affected in cognitively symptomatic HIV-infected individuals, who generally exhibit “spotty” neuropsychological impairment profiles (Heaton et al., 1995). Similar analyses focusing on individual neuropsychological domains may provide more specific information regarding cognitive correlates of white matter damage.

HIV-associated CNS insults have been shown to occur through a number of pathways. Direct neuronal infection is not commonly reported; and HIV-associate neuronal damage appears to be secondary to neurotoxic cascades associated with the virus itself and/or infection or activation of other CNS cell types. The virally encoded HIV envelope protein gp120 has been implicated in the overactivation of glutamate receptors, leading to neuronal excitotoxicity (Tenneti & Lipton, 2000). Such process typically results in alterations in dendritic morphology and neuronal physiology, rather than neuronal apoptosis (Garden et al., 2002). Reactive astrocytosis and microgliosis, increased activation of microglia, and loss of large pyramidal neurons have also been reported (Kaul & Lipton, 2005). HIV infection of astrocytes results in the production of the viral protein transactivator of transcription (Tat), which has been associated with dendritic loss, mitochondrial dysfunction, microgliosis, and neuronal death (Chauhan et al., 2003). HIV-associated CNS injury has also been attributed to neurotoxic products of infected or activated monocytes, macrophages, microglia, and astrocytes. Among the consequences of such neurotoxic mechanisms, abnormal activation of cytokine and chemokine receptors has been associated with inflammatory cascades leading to abnormal dendritic morphology and failure of long-term potentiation (Kaul & Lipton, 2006).

As delineated above, HIV-associated neuronal injury is related primarily to changes in pre- and postsynaptic structures, often without substantial neuronal loss. This is corroborated by the relationship between HIV-related synaptodendritic degenerative changes and the presence and severity of cognitive impairment. For

instance, histopathological evidence in AIDS patients demonstrated inverse relationships between antemortem neuropsychological performance, and dendritic simplification (Masliah et al., 1997) and synaptic density (Everall et al., 1999). Animal model of AIDS has also suggested that neuronal damage might initially occur in synapses and dendrites, before spreading to the rest of the neuron and activating apoptotic cascades (Garden et al., 2002). In addition, gliosis and multinucleated giant cells, pathological hallmarks of HIV encephalitis, are consistent with the neurotoxic mechanisms outlined above.

Considering the nature of HIV neurotoxicity, therefore, the relative sensitivity of MD to HIV-related white matter injury demonstrated here is not surprising. Synaptodendritic injury structurally manifests as pruning of synaptic network, retraction of dendritic spines, dendritic beading, and aberrant sprouting (Masliah et al., 1997). Such alterations may result in a general increase in water diffusivity (i.e., MD). Diffusion anisotropy (i.e., FA), however, may be more associated with frank axonal or neuronal losses, which are less evident in HIV infection. Following this speculated dissociation between the microstructural alterations underlying changes in FA and MD then, the significant AIDS-related FA alterations found here in the posterior internal capsule, the superior longitudinal fasciculus, and the optic radiation, may reflect a distinctive nature of white matter injury in these regions, possibly involving more white matter tract breakdown. The same may apply to the relative sensitivity of FA to white matter injury in the genu and body of the corpus callosum associated with HIV-related cognitive impairment.

Despite the utility of both FA and MD, the underlying biological phenomena driving these indices remain incompletely understood (Beaulieu, 2002). For example, the FA measure is conceptualized to reflect the orientation specificity (i.e., anisotropy) of intracellular water diffusion though restrictive fibers such as axons. However, it has been observed that the contribution of diffusion signals from extracellular water, which comprises about 82.5% of water volume within biological tissue, is unlikely to be negligible. Studies using biexponential diffusion models have corroborated the existence of 2 ("fast" and "slow") diffusion signal pools (Cohen & Assaf, 2002). The physical origin of these signals, however, has not been adequately explained, leaving a rigorous description of FA to remain the goal for future studies.

Regardless of the exact biological meaning of FA and MD alterations, their demonstrated sensitivity to white matter abnormalities confirms that the eigenvalues of the diffusion tensor, which constitute these indices, do reflect some microstructural changes associated with disease processes. Their usefulness has thus been firmly established. Beyond these commonly used scalar indices, however, the diffusion tensor formulation of diffusion-weighted images also provides additional multidimensional data that are potentially useful for clinical research. The primary eigenvector for the diffusion tensor, for example, is formulated to provide information regarding the average direction of water diffusion within a voxel. Mapping such information over 2- or 3-dimensinal spaces, therefore, may provide data regarding anatomical connectivity within the brain. However, the validation of such fiber track

mapping results against actual anatomical data still remains methodologically problematic (Mori & van Zijl, 2002).

The current study is the first to use DTI to investigate HIV-related white matter alteration in a voxelwise manner, in addition to focusing exclusively on major white matter tracts. For this reason, and also due to the novelty of the data processing technique (Smith et al., 2006), the results here should be regarded as preliminary, pending future corroborations. As discussed above, possibly due to uncontrolled confounding factors and/or statistical power issues, the hypothesized relationship between white matter alterations and CNS ARV treatment was not found here. In light of the still significant prevalence of HAD, this remains an important issue to be investigated. In addition, the relationships between impairment on specific cognitive domains and diffusion alteration also warrant investigation. This may provide valuable information regarding the neural substrates of HIV-related cognitive impairment. Other avenues for DTI investigation of HIV infection include the use of supporting information from other neuroimaging modalities. For example, white matter signal intensity abnormalities on MRI examinations are frequently observed in HIV/AIDS (Filippi et al., 1998). The use of such technique to initially identify affected white matter regions may enhance the utility of DTI.

TABLES

Table 1. DHHS treatment recommendations (October 2006) for ARV-naïve HIV-infected patients.

	Column A		Column B
	NNRTI	PI	2-NRTI
Preferred (alphabetical order)	Efavirenz	Atazanavir + ritonavir Fosamprenavir + ritonavir BID Lopinavir/ritonavir BID	Tenofovir/emtricitabine Zidovudine/lamivudine
Alternative (alphabetical order)	Nevirapine	Atazanavir (unboosted) Fosamprenavir (unboosted) Fosamprenavir + ritonavir once daily Lopinavir/ritonavir once daily	Abacavir/lamivudine Didanosine + lamivudine

Table 2. Participants' demographic information and relevant lab results.

	CON (N = 25)	HIV+ (N = 39)
Age (years)	38.64 (12.97)	42.35 (8.35)
Education (years)	13.76 (2.52)	13.56 (2.48)
% male	84%	92%
% Caucasians	88%	72%
% AIDS	-	49%
CD4 level (N = 38)	-	529 (243)
Nadir CD4 level	-	219 (159)
% with detectable plasma HIV RNA	-	29%
% with detectable CSF HIV RNA	-	26%

Table 3. Demographic information and relevant lab results of HIV+ participants with and without the diagnosis of AIDS. * denotes $p < .0005$.

	Non-AIDS (N = 20)	AIDS (N = 19)
Age (years)	42.70 (9.43)	42.00 (7.29)
Education (years)	14.30 (2.30)	12.79 (2.49)
% male	95%	89%
% Caucasians	85%	58%
% on ARV	80%	84%
CD4 level* (N = 38)	635 (212; N = 20)	411 (225; N = 18)
Nadir CD4 level*	333 (121)	100 (90)
% with detectable plasma HIV RNA	20%	33%
% with detectable CSF HIV RNA	25%	33%

Table 4. CNS penetration scores for antiretroviral drugs.

	0	0.5	1
NRTI's:	Tenofovir	Stavudine	Zidovudine
	Didanosine	Lamivudine	
	Zalcitibine	Emtricitabine	Abacavir
NNRTI's:		Efavirenz	Delavirdine
			Nevirapine
Protease Inhibitors:	Nelfinavir	Amprenavir	Amprenavir-r
	Saquinavir	Fosamprenavir	Fosamprenavir-r
	Saquinavir-r	Atazanavir	Atazanavir-r
	Ritonavir	Indinavir	Indinavir-r
	Tipranavir-r		Lopinavir-r
Fusion Inhibitors:	Enfuvirtide		

Note. NRTI (nucleoside reverse transcriptase inhibitor); NNRTI (non- nucleoside reverse transcriptase inhibitor); -r (drugs boosted with ritonavir)

Table 5. Neuropsychological tests within each ability domain.

Neuropsychological Ability Domain	Tests
1. Speed of Information Processing	WAIS-III Digit Symbol
	WAIS-III Symbol Search
	WAIS-III Processing Speed Index
	Trail Making Part A
	Paced Auditory Serial Addition Test (PASAT)
2. & 3. Learning & Delayed Recall	Hopkins Verbal Learning Test – Revised
	Brief Visuospatial Memory Test – Revised
	Story Memory
	Figure Memory
4. Abstraction/Executive Functioning	Wisconsin Card Sorting Test
	Trail Making Part B
5. Verbal Fluency	Letter Fluency
	Category Fluency
6. Attention/Working Memory	Letter-Number Sequencing
	PASAT 1st channel
7. Motor Skills	Grooved Pegboard

Table 6. Demographic information and relevant lab results of HIV+ participants with and without cognitive impairment. * denotes $p < .05$.

	NPI- (N = 29)	NPI+ (N = 10)
Age (years)	42.79 (8.54)	41.10 (8.06)
Education (years)	13.74 (2.23)	13.10 (3.18)
% male	93%	90%
% Caucasians	69%	80%
% AIDS	48%	50%
% on ARV*	76%	100%
CD4 level (N = 38)	530 (267; N = 28)	523 (171; N = 10)
Nadir CD4 level	229 (168)	190 (131)
% with detectable plasma HIV RNA	28%	22%
% with detectable CSF HIV RNA	30%	25%

Table 7. 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[a]	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	231		1817	1045	1369		262
	Effect size (d)	0.94	1.28		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	303		57	79	464		85
	Effect size (d)	0.38	1.24		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. Voxels are isometric 1 mm^3.

[a] $p = .06$

FIGURES

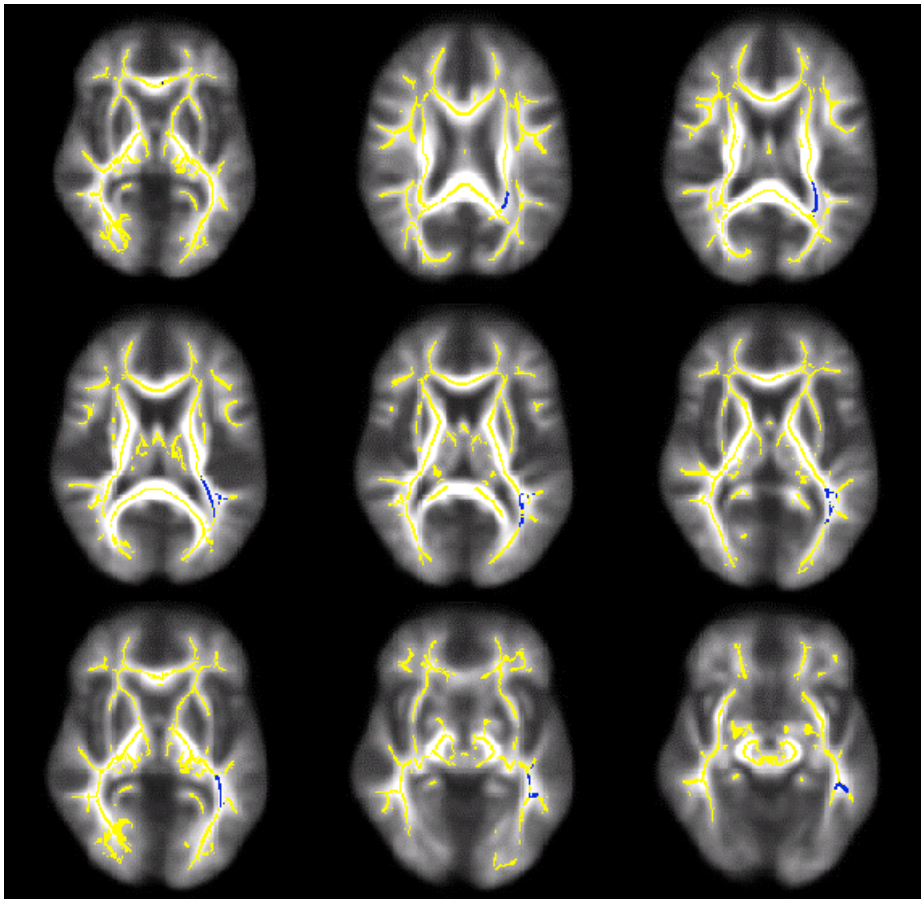

Figure 1. Axial view of white matter tract voxels with significantly decreased FA (shown in blue) in HIV+ relative to CON ($p < .025$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

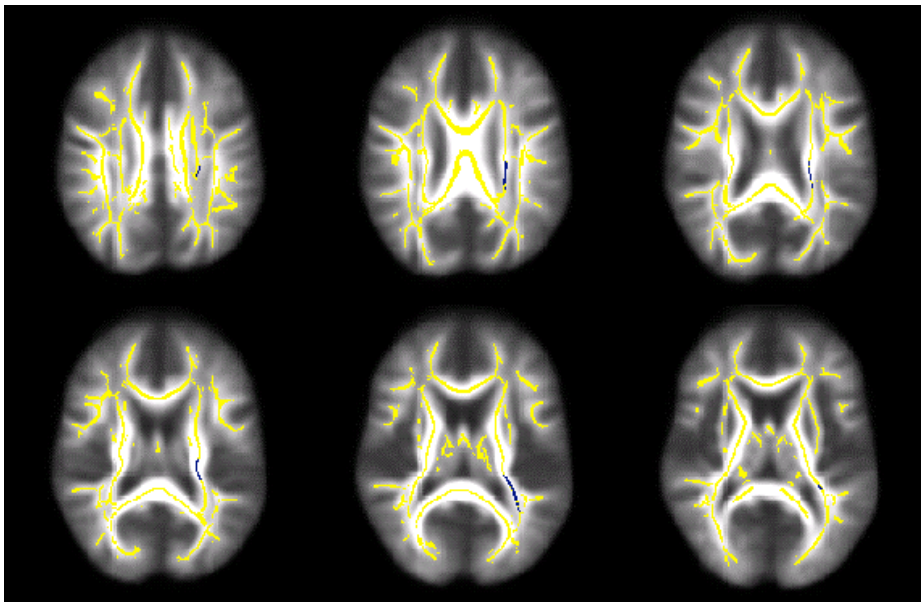

Figure 2. Axial view of white matter tract voxels with a trend towards increased MD (shown in blue) in HIV+ relative to CON ($p = .0642$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

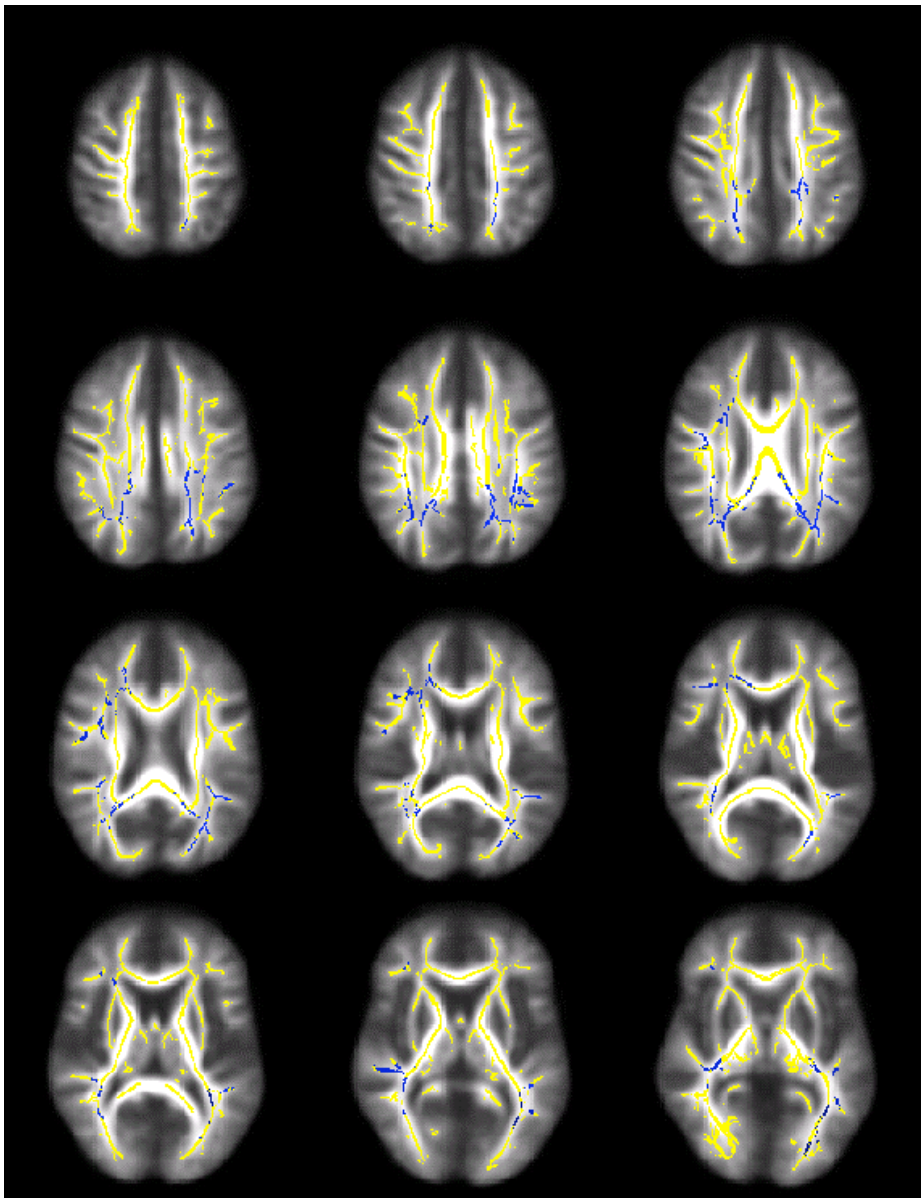

Figure 3. Axial view of white matter tract voxels with significantly increased MD (shown in blue) in HIV+ with AIDS relative to those without AIDS ($p < .025$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

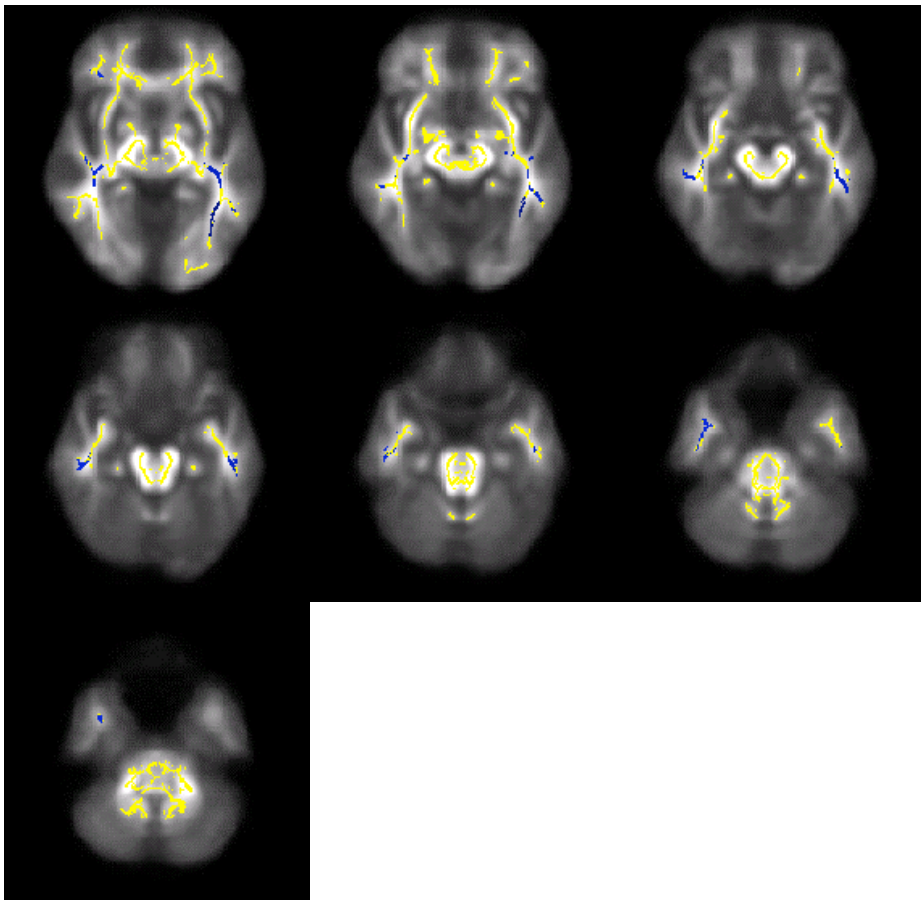

Figure 3. Axial view of white matter tract voxels with significantly increased MD (shown in blue) in HIV+ with AIDS relative to those without AIDS ($p < .025$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale), Continued.

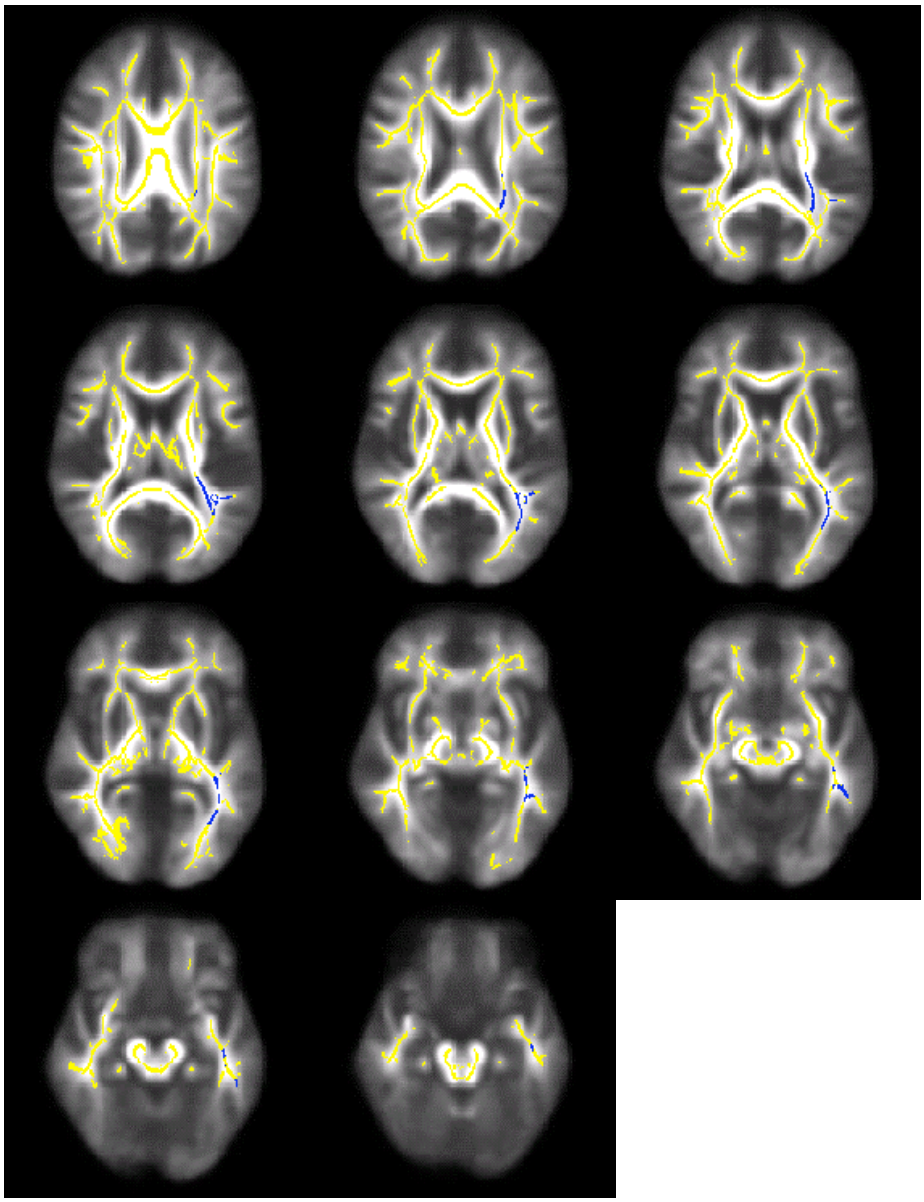

Figure 4. Axial view of white matter tract voxels with significantly decreased FA (shown in blue) in HIV+ with AIDS relative to CON ($p < .025$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale)

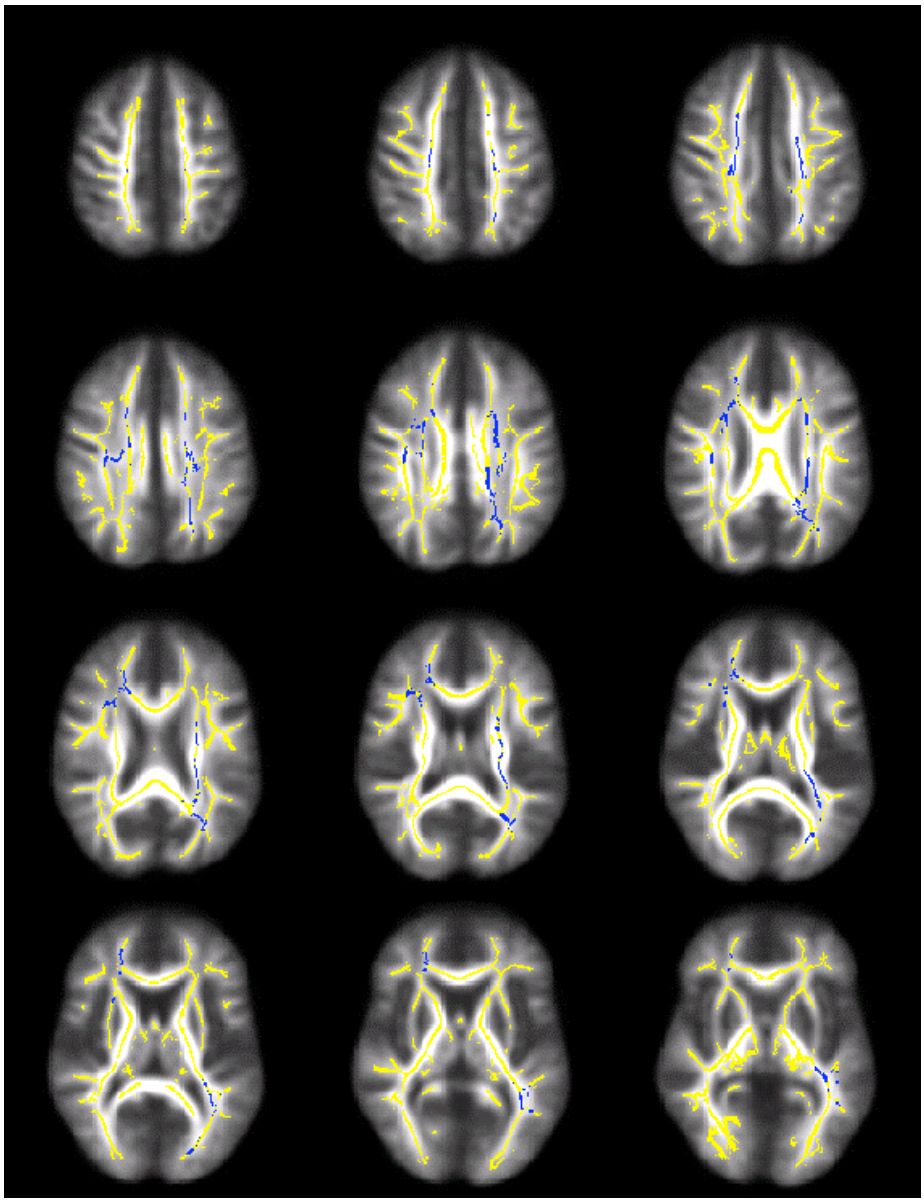

Figure 5. Axial view of white matter tract voxels with significantly increased MD (shown in blue) in HIV+ with AIDS relative to CON ($p < .05$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

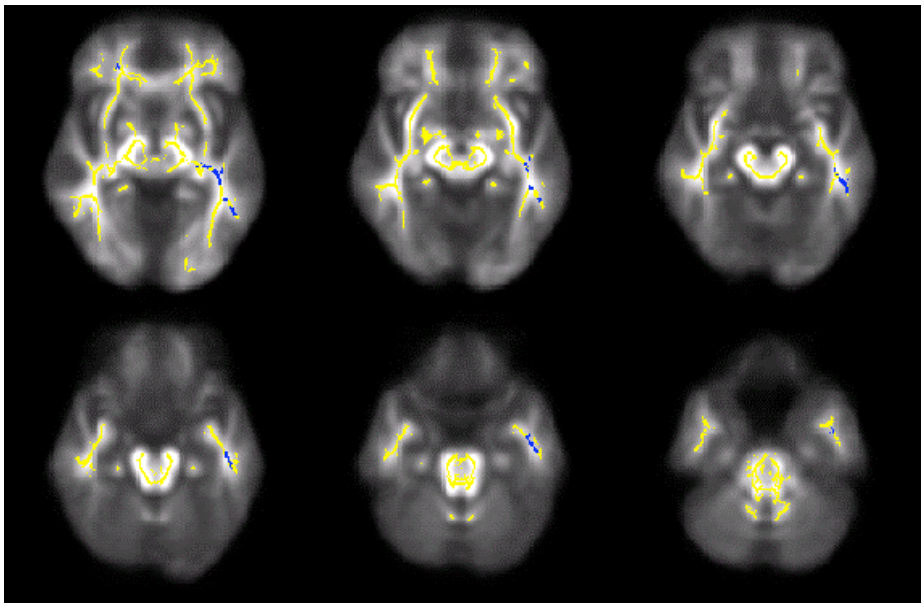

Figure 5. Axial view of white matter tract voxels with significantly increased MD (shown in blue) in HIV+ with AIDS relative to CON ($p < .05$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale), Continued.

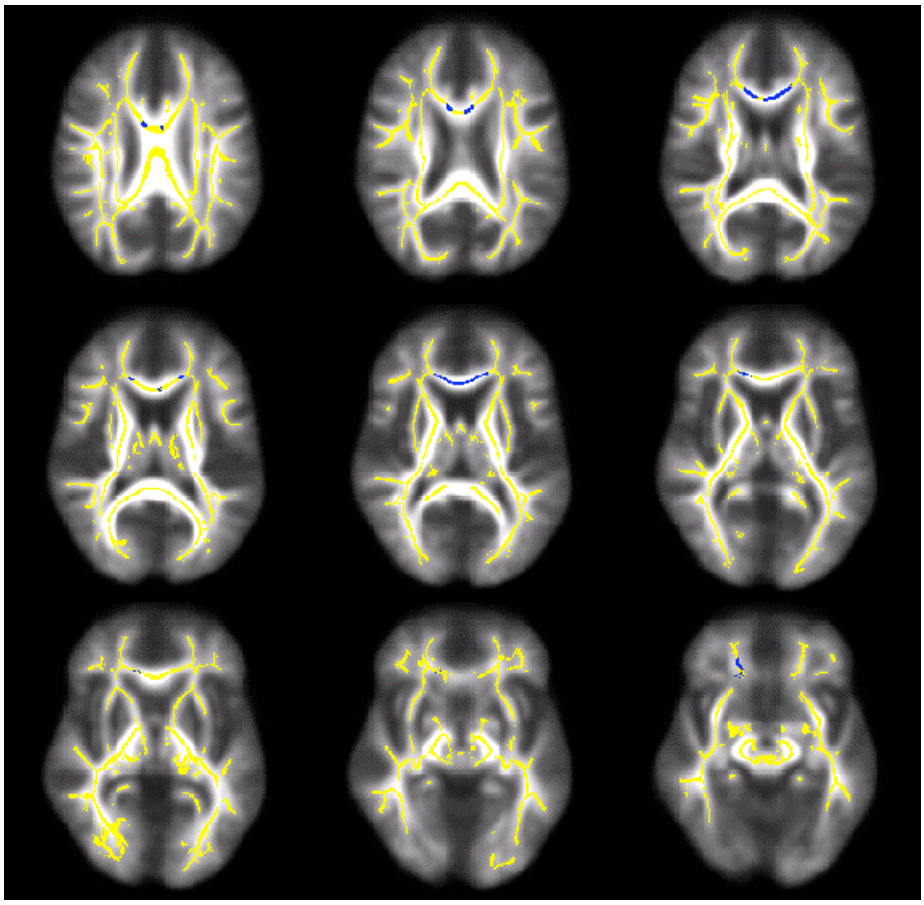

Figure 6. Axial view of white matter tract voxels with significantly decreased FA (shown in blue) in NPI+ relative to NPI- ($p < .05$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

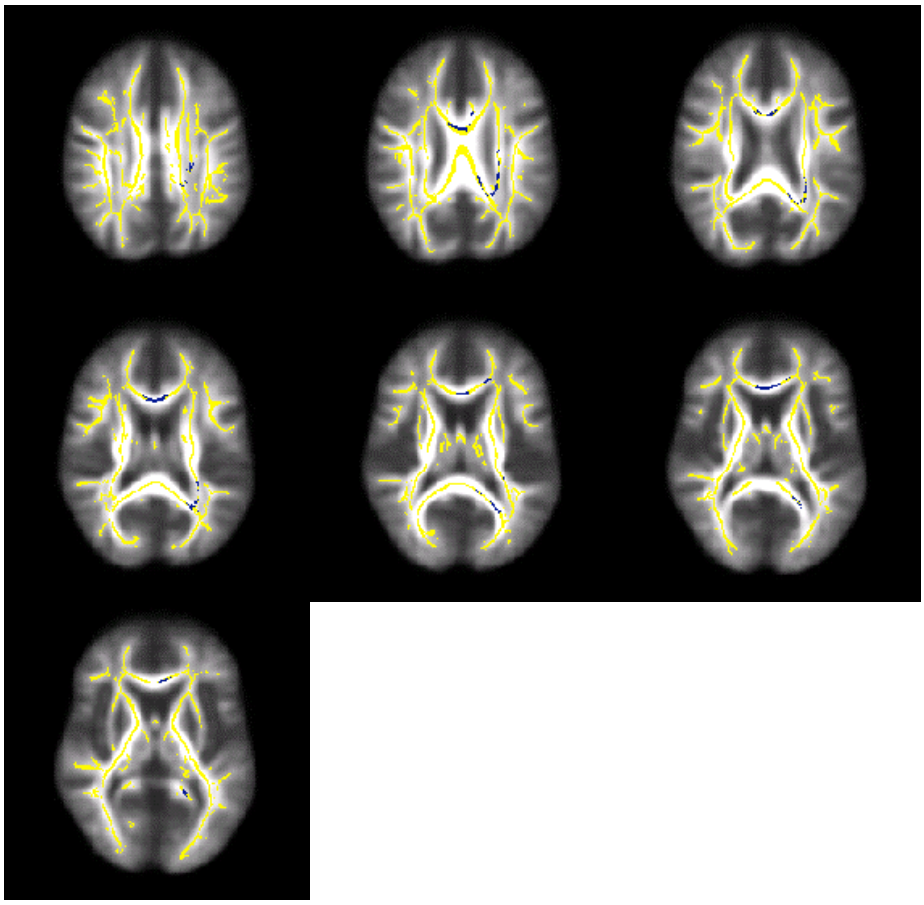

Figure 7. Axial view of white matter tract voxels with significantly increased MD (shown in blue) in NPI+ relative to NPI- ($p < .05$), overlaid on white matter tract skeleton (yellow) and mean FA image (grayscale).

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