Diffusion tensor imaging with quantitative fibre tracking in HIV infection and alcoholism comorbidity: synergistic white matter damage

Adolf Pfefferbaum,1,2 Margaret J. Rosenbloom,1,2 Elfar Adalsteinsson3 and Edith V. Sullivan2

1Neuroscience Program, SRI International, 2Department of Psychiatry and Behavioral Sciences and 3Harvard-MIT Division of Health Sciences and Technology and Department of Electrical Engineering and Computer Science, MIT, Stanford University School of Medicine, Stanford, CA, USA

Correspondence to: Edith V. Sullivan, PhD, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine (MC5723), 401 Quarry Road, Stanford, CA 94305-5723, USA
E-mail: edie@stanford.edu

A substantial proportion of individuals infected with human immunodeficiency virus (HIV) also abuse alcohol. Given that each condition can disrupt brain structural integrity, with a predilection for white matter, we used MR diffusion tensor imaging (DTI) and quantitative fibre tracking to examine the separate and combined effects on the microstructure of the corpus callosum. Subjects were men and women with alcoholism alone (n = 87), HIV infection alone (n = 42), alcoholism and HIV infection comorbidity (n = 52) and non-affected controls (n = 88). The two alcoholism groups had similar lifetime alcohol consumption histories; the two HIV-infected groups had similar CD4+ counts and viral loads; all groups were matched in body mass index, and no participant was demented. Compared with controls, all patient groups had lower fractional anisotropy (FA) and higher mean diffusivity (MD) in callosal regions and fibre bundles coursing through the genu and splenium, but these effects were only significant in the two groups with alcoholism, which exhibited 0.65–1.2 SD abnormalities in FA and MD. The callosal regions were differentially affected by alcoholism, with the genu more affected than the splenium, a pattern even more pronounced in the fibre tracks. When the HIV-infected groups were divided by disease severity defined as an acquired immunodeficiency syndrome (AIDS)-defining event or low CD4+ counts (<200) and alcoholism comorbidity, the HIV-infected subgroup with AIDS and alcoholism exhibited ~2 SD FA and MD abnormalities in the callosal sectors and fibres, abnormalities that were more than twice the effect sizes observed in the other three HIV-infected subgroups. Degradation of the callosal microstructure was consistently associated with alcoholism, with evidence for compounded alcoholism-HIV effects. Functional relevance of the microstructural abnormalities was supported by associations between motor deficits and low FA or high MD within the diagnostic groups. The high prevalence of alcoholism in HIV-infected individuals and the interfering effect of alcohol on HIV pharmacological response and therapy compliance underscore the need to recognize the independent and synergistic contributions of each condition to brain structure and function.

Keywords: AIDS; alcoholism; diffusion tensor imaging; neuroimaging; white matter

Abbreviations: DTI = diffusion tensor imaging; FA = fractional anisotropy; MD = mean diffusivity


Introduction

It is currently estimated that more than 40 million adults and children world-wide are infected with the human immunodeficiency virus (HIV; UNAIDS/WHO AIDS Epidemic Update, December 2005). In addition to a profoundly higher mortality rate in infected than non-infected individuals, HIV causes significant morbidities, notably
Alcohol, HIV and white matter microstructure

those that affect the CNS, contributing to diminished quality of life and productivity. Although HIV-associated dementia typically develops late in the disease and is marked by disruption of neuronal integrity (e.g. Chang et al., 2002, 2003, 2004), less severe motor and cognitive signs of HIV infection can occur early in the course of infection, likely arising from affected white matter (Wu et al., 2006). A major risk factor for HIV infection is alcoholism, which itself is widespread, with 70 million people estimated to have alcohol use disorders (WHO, December 2001). The rate of heavy drinking among HIV-infected individuals is almost twice that of the general population (Galvan et al., 2002). One study noted that of the HIV-positive clinic patients (45 out of 111) 41% met criteria for alcoholism (LeFevre et al., 1995). Further, those who abuse alcohol have a 5–10% higher risk of acquiring HIV than the general population (Meyerhoff, 2001).

Consequently, alcoholism is a prevalent concomitant of HIV infection (Petry, 1999; Cook et al., 2001; Samet et al., 2003, 2004a, b), and, like HIV, is associated with significant brain pathology and dysfunction, also damaging brain white matter—in vivo (Pfefferbaum et al., 1992, 2000a, 2001; Shear et al., 1994; Hommer et al., 1996; O’Neill et al., 2001) and post-mortem (Harper and Kril, 1988, 1993; Kril et al., 1997). With extended life-spans made possible by highly active antiretroviral therapy (HAART), HIV-infected individuals remain at high risk for continuing or reverting to hazardous behaviour, including excessive alcohol consumption (Stein et al., 2005), which can reduce medication compliance (Braithwaite et al., 2005) and exacerbate the progression of the infection by contributing to immune suppression (Wang and Watson, 1995; Wang et al., 2002). These effects place dually affected individuals at compounded risk for brain functional and structural insult even prior to developing clinically-defined dementia, thereby highlighting the relevance of examining groups affected by both conditions (cf. Fein et al., 1995; Heaton et al., 1995; Meyerhoff, 2001; Pfefferbaum et al., 2002; Green et al., 2004; Rothlind et al., 2005).

HIV and alcoholism affect brain white matter through different mechanisms. In HIV, infected monocytes invade the white matter perivascularly (Langford et al., 2002). This infiltration results in activation of microglia, compromised immunosuppression (Langford and Masliah, 2001), and diffuse white matter degradation (Budka, 1997), which, in turn, may be the genesis of neuronal injury as HIV does not directly affect neurons (Heindel et al., 1994; Masliah et al., 2000). Langford and colleagues (2002) proposed that in HIV-infected patients who failed HAART, HIV-infected monocyte infiltration damages brain endothelial cells and is followed by myelin loss, axonal damage and astrogliosis. Cloak et al. (2004) found increased diffusivity in frontal white matter in HIV-seropositive patients, suggesting that increased brain water diffusion may reflect glial activation or inflammation. Neuropathological studies of alcoholism indicate that brain white matter, including the corpus callosum (Harper and Kril, 1988; Tarnowska-Dziduszko et al., 1995), is especially affected (De la Monte, 1988; Harper and Kril, 1990; Badsberg-Jensen and Pakkenberg, 1993) regardless of sex (Harper et al., 1990). Alcoholism-related white matter pathology has been observed as volume reduction, demyelination, loss of myelinated fibres and axonal deletion possibly arising from regional neuronal loss (Alling and Bosstrom, 1980; Harper and Kril, 1989; Kril et al., 1997).

In vivo evidence of these white matter signs of HIV and alcoholism may be detectable with MR diffusion tensor imaging (DTI), which is exquisitely sensitive to water diffusion and is used to quantify the magnitude of diffusivity and the orientation and linearity (that is, anisotropy) of water motility in white matter at a microstructural level (Moseley et al., 1990; Pierpaoli and Basser, 1996; Jones et al., 1999a; Le Bihan, 2003). In normal ageing, for example, anisotropy (typically expressed as fractional anisotropy, FA) declines (Pfefferbaum et al., 2000b; Sullivan et al., 2001; Bhagat and Beaulieu, 2004; Head et al., 2004; Salat et al., 2005), whereas diffusivity (typically expressed as mean diffusivity, MD) increases (Pfefferbaum and Sullivan, 2003; Pfefferbaum et al., 2005). These age-related changes in DTI metrics, which are exacerbated in alcoholism (Pfefferbaum et al., 2000a, 2006b; Pfefferbaum and Sullivan, 2002, 2005b), have been interpreted as in vivo signs of perturbation of the axon’s cytoskeleton or myelin microstructure (cf. Arfanakis et al., 2002) and trapping of fluid between thin or lysed sheathes and between fibres and bulbous swelling of oligodendrocytes noted post-mortem (Peters et al., 2001; Peters and Sethares, 2002, 2003).

Conventional neuroimaging of brain macrostructure has identified some abnormalities, typically occurring in symptomatic HIV-infected individuals (The HNRC group, 1993; Aylward et al., 1995; Symonds et al., 1999) and increasing with worsening clinical and cognitive status (Heindel et al., 1994; Heaton et al., 1995; Di Scalfani et al., 1997; Stout et al., 1998; Thompson et al., 2006). Non-specific white matter hyperintensities (WMHI) may be present in HIV patients, especially those with progressive multifocal leucoencephalopathy (Ernst et al., 1999) and presence of large volumes of WMHI has been predictive of length of survival (Thurnher et al., 2001) and alcoholism comorbidity (Pfefferbaum et al., 2006c). To the extent that WMHI observed in vivo can herald degradation of white matter constituents measured post-mortem (Langford et al., 2002; Archibald et al., 2004), DTI should be particularly sensitive to the untoward effects of HIV infection on white matter microstructure (Pfefferbaum and Sullivan, 2005a). Despite its likely utility, only a few quantitative DTI studies have been published, and all but one study (Thurnher et al., 2005) have been based on small samples, and none has used quantitative fibre tracking (Pierpaoli and Basser, 1996; Tang et al., 1997; Basser, 1998; Conturo et al., 1999; Jones et al., 1999b; Mori et al., 2002; Pajevic et al., 2002; Masutani et al., 2003; Jones and Pierpaoli, 2005; Lazar and Alexander, 2005; Sullivan et al., 2006a).
Initial DTI studies reported that abnormally high apparent diffusion coefficient (ADC) or low FA was detectable in normal-appearing periventricular white matter and corpus callosum of HIV-infected subjects and that these DTI metrics correlated with low CD4+ count and high viral load (Ulug et al., 2000). Other studies reported that whole-brain FA was predictive of dementia severity, whereas diffusivity was predictive of psychomotor deficits (Ragin et al., 2004). A later study by this group (Wu et al., 2006) identified the splenium as a locus of abnormal FA in HIV-infected patients and that FA and MD in the splenium were predictive of motor speed. In addition, FA in the genu and frontal white matter correlated with performance on visual memory and visuoconstruction tasks (Wu et al., 2006). Further, FA and MD in selective subcortical structures and white matter correlated in expected directions with measures of memory components, visuospatial abilities, and general cognitive impairment (Ragin et al., 2005). White matter regions showing such microstructural changes and relations with HIV disease progression were the genu and splenium of the corpus callosum and frontal and parietal subcortical white matter in mixed groups of HIV-infected patients with and without acquired immunodeficiency syndrome (AIDS)-defining events (Filippi et al., 2001; Pomara et al., 2001). Contradicting findings are also reported: one study showed lower FA and higher MD values in the splenium of HIV-infected individuals, but another found lower FA, with no difference in MD, in the genu but not splenium of 60 patients with HIV infection and AIDS or cognitive impairment compared with 30 age-matched controls (Thurnher et al., 2005). Whether comorbidity for alcoholism was a factor in any of these studies is unknown and its occult presence could contribute to observed group differences or lack of them (cf. Pfefferbaum et al., 2006c).

Fibre tracking provides visual depiction of white matter fibre systems (Stieltjes et al., 2001; Xu et al., 2002; Lehericy et al., 2004) and can be used to quantify FA and ADC along the length of identified fibre bundles (Gerig et al., 2005; Sullivan et al., 2006a). This approach, referred to as quantitative fibre tracking, does not actually identify anatomically specific fibres or fibre bundles as detected histologically. Rather, it is a statistical representation of the voxel-to-voxel coherence of MRI-detectable water diffusion in white matter that is, nonetheless, increasingly being shown as representative of the underlying anatomy.

In light of the high prevalence of the comorbidity of HIV infection and alcohol use disorders, we examined the contribution of the separate and combined diseases to the microstructure of the corpus callosum using separate analysis of DTI metrics in geometrically-defined regions of the corpus callosum and fibre bundles coursing through the corpus callosum. All regional and fibre tracking measures were quantitative and were based on 269 men and women from the four age-matched subject groups: HIV only, HIV with alcoholism, alcoholism only and age-matched controls. We tested the hypothesis that groups with only one of the two conditions would exhibit mild to moderate effects on anisotropy and diffusivity but that the comorbid group would exhibit a compounded effect that would be further exacerbated by presence of AIDS-defining events. We also examined the clinical and functional relevance of the DTI metrics by testing correlations between DTI metrics and measures of disease stage or severity and measures of upper and lower limb motor speed and performance.

**Material and methods**

**Subjects**

Construction of the subject groups was based on the largest dataset from a common DTI protocol available in our laboratory (n = 312). Accordingly, subjects were drawn from multiple recruitment efforts for longitudinal studies: the first targeted chronic alcoholism (66 controls, 54 alcoholics), the second Alzheimer’s disease (12 controls) and the third the separate and combined effects of alcoholism and HIV infection on brain structure and cognitive and motor abilities (180 subjects from four diagnostic groups). MRI and DTI data from the first study have appeared in other reports (Schulte et al., 2003, 2005, 2006; Pfefferbaum et al., 2006a, b); structural MRI data from the full sample appear in another report (Pfefferbaum et al., 2006c). Clinical and demographic descriptions and analysis of the first (Rosenbloom et al., 2005) and third (Rosenbloom et al., 2006) cohorts are described elsewhere; 84% of the comorbid group of Cohort 3 met DSM-IV criteria for alcohol dependence, on average, 10 years before being infected with HIV (Rosenbloom et al., 2006). The DTI data used to determine group differences were based on subjects who spanned the age range of the HIV patients. The resulting sample included all subjects between ages 19–60 years: 91 subjects from the first cohort, 2 from the second and 176 from the third. This sample comprised men and women with alcoholism alone (n = 87), HIV infection alone (n = 42), alcoholism and HIV infection comorbidity (n = 52) and non-affected controls (n = 88). The demographic characteristics of these 269 men and women are presented in Table 1.

Men and women in the patient groups were recruited by referral from several San Francisco Bay Area outpatient HIV/AIDS and alcohol and substance abuse treatment centres, presentations by project staff, and distribution of flyers at the AIDS Walk and similar events. Control subjects were recruited by referral from patient participants, by Internet posting, newspaper advertisements, flyers and word of mouth. Referrals and inquiries were followed up with a brief screening interview designed to identify subjects who would be ineligible for the study by virtue of a diagnosis of schizophrenia, bipolar disorder, neurological disease not related to alcohol use or HIV infection, recent drug abuse or dependence, or inability to undergo MRI. Those who met initial criteria were invited for a more detailed assessment at our laboratory (Cohorts 1 and 2) or the AIDS Community Resource Center (ACRC; Cohort 3), where a trained nurse informed them about the full scope of the study and obtained informed consent. The nurse obtained a medical history including itemization of current medications, HIV treatment history, and HIV-related symptoms. Only HIV-infected patients with CD4+ count >100 and Karnofsky score (Karnofsky, 1949) >70 (can care for self but unable to carry out normal activity) were further considered for enrolment in the HIV/alcoholism study.
Clinical evaluation

All controls and patients from all three study cohorts underwent a series of structured interviews designed to characterize history of HIV illness and treatment, alcohol history, and other pertinent medical and psychiatric information. Clinical psychologists administered the structured clinical interview for DSM-IV (First et al., 1992) to identify patients who met criteria for alcohol dependence or abuse; exclude subjects who met lifetime criteria for schizophrenia or bipolar disorder, or for non-alcohol substance dependence or abuse within the prior 12 months for Cohort 1 and 3 months for Cohort 3 (to match the demographics of the HIV infected samples); identify any patients who met criteria for a depressive or anxiety disorder; and confirm that prospective controls did not meet DSM-IV criteria for any Axis I disorder. A history of alcohol consumption (Skinner, 1982; Skinner and Sheu, 1982; Pfefferbaum et al., 1992) yielded quantitative lifetime consumption of alcohol, time since last drink, number of days drinking >8 drinks per day for women or >12 for men, and number of days since drinking that amount. Participants were assigned to one of four groups on the basis of this assessment: (i) HIV-infected patients whose lifetime alcohol use did not include any 30-day period when they exceeded 6 drinks per day for men or 4 per day for women and who had never met criteria for alcohol dependence or abuse (HIV); (ii) HIV-infected patients who also met criteria for alcohol dependence or abuse (HIV+ALC) within the past 3 years; (iii) patients who met lifetime criteria for alcohol dependence within the past 3 years but were not HIV-infected (ALC); and (iv) a control group who were neither HIV-infected nor met criteria for alcohol abuse, dependence or other Axis I diagnoses.

All subjects from the third cohort, that included HIV, ALC, HIV+ALC, and controls, underwent a panel of blood tests to determine HIV status. Interviews and questionnaires assessed global medical and psychiatric information. Clinical psychologists administered the structured clinical interview for DSM-IV (First et al., 1998) to identify patients who met criteria for alcohol dependence or abuse; exclude subjects who met lifetime criteria for schizophrenia or bipolar disorder, or for non-alcohol substance dependence or abuse within the prior 12 months for Cohort 1 and 3 months for Cohort 3 (to match the demographics of the HIV infected samples); identify any patients who met criteria for a depressive or anxiety disorder; and confirm that prospective controls did not meet DSM-IV criteria for any Axis I disorder. A history of alcohol consumption (Skinner, 1982; Skinner and Sheu, 1982; Pfefferbaum et al., 1992) yielded quantitative lifetime consumption of alcohol, time since last drink, number of days drinking >8 drinks per day for women or >12 for men, and number of days since drinking that amount. Participants were assigned to one of four groups on the basis of this assessment: (i) HIV-infected patients whose lifetime alcohol use did not include any 30-day period when they exceeded 6 drinks per day for men or 4 per day for women and who had never met criteria for alcohol dependence or abuse (HIV); (ii) HIV-infected patients who also met criteria for alcohol dependence or abuse (HIV+ALC) within the past 3 years; (iii) patients who met lifetime criteria for alcohol dependence within the past 3 years but were not HIV-infected (ALC); and (iv) a control group who were neither HIV-infected nor met criteria for alcohol abuse, dependence or other Axis I diagnoses.

All subjects from the third cohort, that included HIV, ALC, HIV+ALC, and controls, underwent a panel of blood tests to determine HIV status. Interviews and questionnaires assessed global assessment of functioning (GAF); Beck Depression Index (Beck et al., 1996), a quantitative measure of depressive symptoms; Socioeconomic Status Scale (SES; a two-factor scale based on education and occupation; Hollingshead and Redlich, 1958); handedness (Crovitz and Zener, 1962); and body mass index (height/weight in cm/kg²), an index of nutritional status. General cognitive status was assessed with the Peabody Picture Vocabulary Test (PPVT-III) (Dunn and Dunn, 1997). Means ± SD or frequency counts of these and other demographic values are presented in Table 1. At the outset of the study, no patient was clinically demented.

The incidence of hepatitis C was higher in the HIV+ALC group (52%) than the HIV (17%) or ALC (29%) groups ($\chi^2 = 13.482$, $P = 0.0012$), but the incidence of self-reported neuropathy was

<table>
<thead>
<tr>
<th>Table 1 Demographic characteristics of the four study groups (n = 269)</th>
<th>Control (C)</th>
<th>Alcohol (A)</th>
<th>HIV (H)</th>
<th>HIV+ALC (HA)</th>
<th>Group differences [$\chi^2$ or ANOVA ($P \leq 0.05$)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: M/F</td>
<td>42/46</td>
<td>59/28</td>
<td>29/13</td>
<td>41/11</td>
<td>$P = 0.0011$</td>
</tr>
<tr>
<td>Age (years) [mean (SD)]</td>
<td>44.5 (9.9)</td>
<td>46.6 (9.0)</td>
<td>42.5 (9.8)</td>
<td>45.4 (6.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Education (years) [mean (SD)]</td>
<td>15.8 (2.3)</td>
<td>14.2 (2.3)</td>
<td>13.8 (3.0)</td>
<td>12.8 (2.1)</td>
<td>C $&gt;$ A $=$ H $=$ HA</td>
</tr>
<tr>
<td>Handedness score [mean (SD)]</td>
<td>24.2 (13.7)</td>
<td>26.2 (15.1)</td>
<td>25.2 (11.2)</td>
<td>25.5 (12.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>(RH = 14–30; LH = 50–70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index [mean (SD)]</td>
<td>25.8 (4.7)</td>
<td>26.1 (4.4)</td>
<td>26.1 (3.9)</td>
<td>26.1 (4.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>(lower score = higher status)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index [mean (SD)]</td>
<td>103.7 (15.4)</td>
<td>95.9 (12.7)</td>
<td>100.0 (15.0)</td>
<td>90.9 (14.4)</td>
<td>C $&gt;$ A = HA; H $&gt;$ HA</td>
</tr>
<tr>
<td>[mean (SD)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beak Depression Index</td>
<td>2.6 (2.9)</td>
<td>9.8 (8.2)</td>
<td>10.8 (8.6)</td>
<td>12.3 (8.3)</td>
<td>C $&lt;$ A = A = H</td>
</tr>
<tr>
<td>(cohort 3 only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[mean (SD)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAF [mean (SD)]</td>
<td>81.6 (8.9)</td>
<td>55.6 (11.5)</td>
<td>71.6 (11.7)</td>
<td>59.1 (9.5)</td>
<td>C $&gt;$ H $&gt;$ A</td>
</tr>
<tr>
<td>[mean (SD)] years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV onset age [mean (SD)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T cell count [mean (SD)]</td>
<td></td>
<td>33.7 (9.0)</td>
<td>34.5 (8.1)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Viral load [mean (SD)]</td>
<td>10412.9 (21319.0)</td>
<td>15590.8 (25206.3)</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days since drink ≥4 or 6 drinks</td>
<td>359.6 (746.8)</td>
<td>747.8 (1718.0)</td>
<td>P = 0.0705</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4 drinks for women; 6 for men)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days since drink ≥8 or 12 drinks</td>
<td>750.2 (1787.9)</td>
<td>1311.8 (2448.0)</td>
<td>P = 0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8 drinks for women; 12 for men)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker (0/1/2) (0 = never; 1 = current; 2 = past)</td>
<td>66/4/18</td>
<td>23/43/21</td>
<td>13/14/15</td>
<td>7/34/11</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>[mean (SD)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-defined ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>61</td>
<td>70</td>
<td>28</td>
<td>16</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>African American</td>
<td>8</td>
<td>16</td>
<td>12</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
similar in the HIV+ALC (27%) and HIV groups (22%; \( \chi^2 = 0.304, \) n.s.). More subjects in the HIV+ALC (42%) tended to be medication-free than in the HIV only (21%) group (\( \chi^2 = 4.795, \) \( P = 0.05909 \)). Among the medicated, the HIV only and HIV+ALC were equally represented by medication type (Efavirenz 7/9; protease inhibitors 15/15; non-nucleoside reverse transcriptase inhibitors 16/11; nucleoside reverse transcriptase inhibitors 40/37).

A history of non-alcohol substance abuse or dependence was as follows: 45 of the 52 HIV+ALC subjects, 15 of the 42 HIV only subjects and 29 of the 41 alcoholics in Cohort 3 and 15 of 46 alcoholics in Cohort 1.

MRI acquisition protocol
An initial spin-echo midsagittal localizer scan [13 contiguous, 4 mm thick, slices; TR/TE = 300/14 ms; matrix = 256 × 256, field of view (FOV) = 24 cm] was used to identify landmarks for prescription of all subsequent coronal scans. The superior/inferior (SI) centre position of the coronal acquisitions was chosen as the most inferior extent of the midpoint of the isthmus of the corpus callosum, and the extent of the prescription in the anterior/posterior (AP) orientation subtended the entire brain for all subjects.

Two coronal structural sequences used in this analysis were acquired with a 24 cm field of view: (i) a dual-echo fast spin-echo (FSE) sequence (47 contiguous, 4 mm thick slices; TR/TE1/TE2 = 7500/14/98 ms; matrix = 256 × 192); and (ii) a SPoiled Gradient Recalled Echo (SPGR) sequence (94, 2 mm thick slices; TR/TE = 25/5 ms, flip angle = 30°, matrix = 256 × 192). All images were zero-filled to 256 × 256 pixels in-plane by the scanner reconstruction software.

DTI was performed in the coronal plane with the same slice location parameters as the dual-echo FSE, using a single shot spin-echo echo-planar imaging technique with a 24 cm field of view (47 contiguous, 4 mm thick slices, TR/TE = 10 000/103 ms, matrix = 128 × 128, in-plane resolution = 1.875 mm\(^2\)). The amplitude of the diffusion-sensitizing gradients was 1.46 gauss/cm with 32 ms duration and 38 ms separation, resulting in a \( b \)-value of 860 s/mm\(^2\). Diffusion was measured along six non-collinear directions with alternating signs to minimize the need to account for cross-terms between imaging and diffusion gradients (Neeman et al., 1991). For each gradient direction, six images were acquired and averaged. For each slice, six images with no diffusion weighting (\( b = 0 \) s/mm\(^2\)) were also acquired. The coronal MRI and DTI acquisitions produced either 2 or 4 mm thick slices and were prescribed for consistent slice locations so that each 4 mm slice encompassed a pair of 2 mm thick slices.

Image processing
The 47-slice, dual-echo FSE images were passed through the FSL Brain Extraction Tool (BET; Smith, 2002) to extract the brain and exclude dura, skull, scalp and other non-brain tissue, and the mask was also used to extract the brain from the SPGR data.

Eddy-current-induced image distortions due to the large diffusion encoding gradients cause spatial distortions in the diffusion-weighted DTI images that vary from one diffusion direction to the next. These artefacts were minimized by alignment with an average made of all 12 diffusion-weighted images with a 2D 6-parameter affine correction on a slice-by-slice basis (Woods et al., 1998a) to un warp the eddy-current distortions in the diffusion-weighted DTI images for each direction. After eddy-current correction, the DTI data were aligned with the FSE data with a non-linear 3D warp (3rd order polynomial), which provided in-plane and through-plane alignment.

Using the averaged images with \( b = 0 \) and 860 s/mm\(^2\), six maps of the ADC were calculated, each being a sum of three elements of the diffusion tensor. Solving the six equations with respect to ADCxx, ADCxy, etc. yielded the elements of the diffusion tensor. The diffusion tensor was then diagonalized, yielding eigenvalues \( \lambda_1, \lambda_2, \lambda_3 \), as well as eigenvectors that define the predominant diffusion orientations. Based on the eigenvalues from the tensor, FA was calculated on a voxel-by-voxel basis. The trace of the tensor matrix (the sum of the eigenvalues) and bulk MD (the mean of the eigenvalues), like FA, were calculated on a voxel-by-voxel basis. Thus, each diffusion-weighted study was reduced to a set of three images for each slice (FA, diffusivity and \( b = 0 \)) to be used for analysis in conjunction with the anatomical images. FA was expressed as a percent, and MD was expressed in units of \( 10^{-3} \) mm\(^2\)/s.

Warping to common coordinates
To place the images for all subjects into a coordinate system with a common origin and a standardized anatomical orientation, the anterior commissure (AC) and posterior commissure (PC) were manually identified on the native 2-mm-thick SPGR images and rotated into a common orientation. The parameters required to accomplish this transformation for each scan session were applied to all of the structural, FA, and diffusion images. All of the datasets were re-sliced to isotropic 1 mm\(^3\) voxels and the FOV set to 20 cm for each axis. This image volume was large enough to encompass the entire brain for all subjects in the study. Each subject’s SPGR data were then aligned to a grand average laboratory SPGR template with a 12-parameter affine model, followed by creation of a new grand average used as a template for a higher-order (3rd–5th polynomial) non-linear warp. The use of non-linear warping allowed the registration of all brains to a common space despite age- and disease-related differences in morphology. The early echo, dual-echo FSE data for each subject were also aligned to the SPGR grand average with AIRS2.5 using the standard-deviation-of-ratio-image cost function, followed by higher-order (3rd to 5th polynomial) non-linear warp (Woods et al., 1998a, b). The \( b = 0 \) images were warped to the native late echo, dual-echo FSE images in 3D, first with a 12-parameter affine, followed by stepwise 2nd and 3rd order polynomial functions. Finally, for each subject all the registration transformation matrices were then combined into one function so they could be applied only once to native data. This process allowed for anatomical identification of the corpus callosum in a common space for each subject from structural or FA images and the DTI data to be accessed either in native or common space.

Identification of the corpus callosum and its sectors
The corpus callosum was identified on the midsagittal slice extracted from the aligned FA data with a semi-automated edge identification procedure with high interrater reliability. Regional callosal areas were defined geometrically, as follows. The corpus callosum silhouette was rotated to a plane parallel to the inferior extremes of the rostrum anteriorly and splenium posteriorly. The midpoint along this plane between the anterior extreme of the genu and posterior extreme of the splenium was used as the centre of a circle, and radii were projected at +30° and +150° angles relative to
For both genu and splenium targets, the number of fibres and the transformed back to common coordinates for display (Fig. 2). After fibre detection the fibre locations were determined. MD of each voxel comprising each fibre, for all fibres, were used to identify targets for fibre tracking as described below.

To examine the possibility that observed results were due to partial-voluming, FA and MD were also measured in 3 volumes of interest placed in the middle of the genu and splenium, with no limit on the number of fibres. The mean FA and MD were also measured in 3 midsagittal slices for each of the three callosal sectors (Fig. 1). The genu and splenium sectors were also used to identify targets for fibre tracking as described below.

To identify targets for fibre tracking, each target also had a companion source, defined as a parallel plane perpendicular to the corpus callosum and the target locations manually placed directly in the middle of the genu and splenium (Fig. 2). All manual interaction with images was performed by A.P. or E.V.S, who were blind to subject age, sex and diagnosis.

Fibre tracking
Target and source regions were defined on the aligned FA data, as described above. For fibre tracking, each target also had a companion source, defined as a parallel plane perpendicular to the midsagittal plane and located 10 mm anterior to the genu target and 10 mm posterior to the splenium target (Fig. 3). The target and source were then transformed into each subject’s native coordinates using the inverse of the original warping transform (Woods et al., 1998a, b). The fibre tracking was performed on native, unwarped DTI data for each of the callosal regions of interest separately, using software distributed by Gerig et al. (2005) based on the method of Mori and colleagues (Xue et al., 1999; Mori and van Zijl, 2002; Xu et al., 2002). Tracking parameters included white matter extraction threshold (minimum FA) of 0.17, fibre tracking threshold of 0.125, and maximum voxel-to-voxel coherence minimum transition smoothness threshold of 0.80 (~37° maximum deviation between voxels), with no limit on the number of fibres. The mean FA and MD of each voxel comprising each fibre, for all fibres, were determined. After fibre detection the fibre locations were transformed back to common coordinates for display (Fig. 2). For both genu and splenium targets, the number of fibres and the mean FA and MD of all fibres were determined. We refer hereafter to the group of fibres coursing through each target region as genu or splenium ‘fibre bundles’ (Fig. 3).

Motor tests
The fine finger movement test required subjects to turn a knurled pin with their forefinger and thumb, unimanually and then bimanually (Corkin et al., 1986). Three, 30-s trials for each condition were administered. The score was the number of rotations made. The grooved pegboard test required subjects to insert grooved pegs into a 5 × 5 pegboard (Matthews and Kløve, 1964). Time to fill in all pegs with each hand separately and number of pegs dropped were scored. The two-choice task (Cahn et al., 1998) consisted of a box displaying three buttons. A warning tone preceded a light that illuminated above the left or right button. Subjects then pressed the button under the light as quickly as possible. Each trial started with the subjects pressing down the middle button (start position). Reaction time (RT; time to remove finger from the start position) and movement time (MT; time to press the left or right button – RT) were scored. The walk-a-line ataxia battery (Fregly et al., 1972) assessed gait and balance and consisted of three parts, each performed first with eyes open and then eyes closed: (i) stand heel-to-toe with arms folded across the chest for 60-s trials; (ii) stand on one foot at a time for 30-s trials; and (iii) walk heel-to-toe for 10 steps. Each condition was tested twice, unless a perfect performance was demonstrated on the initial trial. For each measure of all tests, scores were expressed as the mean of left and right hand or leg scores. Analogous to the DTI metrics as described above, these motor performance measures were then transformed into age-corrected Z-scores, based on the performance of the controls (Sullivan et al., 1994), who had an expected mean ± SD = 0 ± 1 for each measure.

Statistical analysis
Group differences were tested with one-factor and group-by-brain region repeated measures analysis of variance (ANOVA); pair-wise comparisons were made with hypothesis-driven t-tests. Relations between variables were tested with Pearson product-moment correlations.
correlations (r) and, where appropriate, Spearman rank order (rho) correlations.

Results
Effects of age on normal variation in regional callosal FA and MD
In the 120 controls, lower FA and higher MD were correlated with older age, and most of the correlations were significant. Figure 4 displays the scatterplots of the regional callosal FA and MD values by age in these controls. In the control group, FA in the callosal sectors was on average 0.45 (range = 0.32–0.57) in the genu, 0.47 (range = 0.37–0.59) in the body, and 0.55 (range = 0.43–0.66) in the splenium. These values were even higher in the rarefied focal samples, being 0.64 (range = 0.44–0.82) in the genu and 0.80 (range = 0.60–0.93) in the splenium. In all comparisons, genu FA was significantly lower than splenium FA (for all tests P = 0.0001).

Because no consistent differences in relations between regional DTI metrics and age were observed as a function of sex (cf. Sullivan et al., 2001; Pfefferbaum et al., 2006a), control men and women were combined in subsequent analyses and were based on age-corrected Z-scores. A repeated measures ANOVA comparing FA in the four subject groups over the three divisions of the corpus callosum revealed diagnosis [F(3,265) = 12.332, P = 0.0001] and region [F(2,530) = 6.529, P = 0.0016] effects but no interaction. Overall, the two alcoholic groups had significantly lower FA than controls and FA in the HIV+ALC group was lower than FA in the HIV only group (Fig. 5).

Group differences in FA and MD of the corpus callosum: sector analysis
ANOVAs examining the effects of diagnosis and sex on regional callosal FA and MD yielded no significant diagnosis-by-sex interactions. Consequently, data from men and women were combined in subsequent analyses and were based on age-corrected Z-scores. A repeated measures ANOVA comparing FA in the four subject groups over the three divisions of the corpus callosum revealed diagnosis [F(3,265) = 12.332, P = 0.0001] and region [F(2,530) = 6.529, P = 0.0016] effects but no interaction. Overall, the two alcoholic groups had significantly lower FA than controls and FA in the HIV+ALC group was lower than FA in the HIV only group (Fig. 5).
genu and body than splenium of the ALC group and in the callosal body than genu or splenium of the HIV+ALC group (Fig. 5).

Presence versus absence of AIDS or alcoholism in modulating group differences in FA and MD in callosal sectors

Each HIV-infected patient was classified as having AIDS or not, where AIDS was determined by either AIDS-defining event or CD4+ count under 200 at study entry. The resulting groups comprised 31 HIV without AIDS, 11 HIV with AIDS, 37 HIV+ALC without AIDS and 15 HIV+ALC with AIDS. These four groups did not differ in age, viral load, estimated age of HIV onset, or body mass index. Irrespective of alcoholism, those with AIDS had lower Karnofsky scores than those without AIDS \[F(1,90) = 11.735, P = 0.0009\], and irrespective of AIDS, HIV+ALC had lower GAF scores than HIV only \[F(1,88) = 28.204, P = 0.0001\]. Lifetime alcohol consumption was not statistically different between AIDS

**Fig. 4** Correlations of FA (top) and MD (bottom) of each callosal region with age for 120 controls. The decrease in FA and increase in MD is more prominent in the genu and body than splenium of the corpus callosum. The MD-age correlations in the genu and body were best fitted with a quadratic model.

**Fig. 5** Mean ± SEM of age-corrected Z-scores of each regional callosal measure of FA and MD in each of the four subject groups.
and non-AIDS patients within the HIV only group or within the HIV+ALC group.

Group differences in DTI measures were re-examined with a series of ANOVAs (HIV versus HIV+ALC and no AIDS versus AIDS) and yielded a series of HIV group by AIDS group interactions: total callosal FA $[F(1,90) = 4.282, P = 0.0414]$, total MD $[F(1,90) = 3.728, P = 0.0567]$, genu FA $[F(1,90) = 5.738, P = 0.0187]$, and genu MD $[F(1,90) = 6.296, P = 0.0139]$. In general, the greatest FA and MD abnormalities occurred in the two HIV+ALC subgroups, but patients with AIDS had by far the lowest FA and highest MD, which was especially prominent in the genu (Fig. 6).

**Group differences and modulating factors in FA and MD of callosal fibre bundles**

This set of analyses focused on FA and MD measured in the fibre bundles coursing through the genu and splenium. A diagnosis-by-region ANOVA for FA yielded significant effects of diagnostic group $[F(3,265) = 11.198, P = 0.0001]$ and callosal region $[F(1,265) = 9.028, P = 0.0029]$ and a group-by-region interaction $[F(3,265) = 2.999, P = 0.0311]$. The two alcoholic groups had significantly lower FA than controls in both fibre bundles and lower FA than the HIV only group in the genu bundles (Fig. 7). Follow-up within-group repeated measures ANOVAs indicated that the genu bundles were disproportionately affected in the two alcoholic groups, but that the effect was significant in the ALC group $[F(1,173) = 14.927, P = 0.0002]$ and showed a trend in the HIV+ALC group $[F(1,103) = 3.584, P = 0.064]$. An analogous set of ANOVAs for MD revealed a significant effect of diagnostic group $[F(3,265) = 6.245, P = 0.0004]$ but neither a region effect $[F(1,265) = 2.682, P = 0.1027]$ nor an interaction $[F(3,265) = 1.918, P = 0.127]$. Only the ALC group showed a significant regional effect $[F(1,173) = 9.223, P = 0.0032]$, where MD was higher in the genu and splenium fibre bundles (Fig. 7).

We next considered the influence of presence versus absence of AIDS in the HIV groups, with versus without alcoholism, using separate ANOVAs for fibre bundle FA and MD. For FA, the alcoholism-by-AIDS interactions were significant and indicated that HIV patients with AIDS were disproportionately adversely affected if they were comorbid for alcoholism (genu $[F(1,90) = 5.116, P = 0.0261]$; splenium $[F(1,90) = 5.0175, P = 0.0276]$). A similar pattern held for MD $[genu F(1,90) = 5.070, P = 0.0268]$; splenium $F(1,90) = 3.908, P = 0.0511]$ (Fig. 8).

**Demographic and disease factors associated with FA and MD in each patient group**

We tested the correlations between each principal demographic and disease variable listed in Table 1 and each DTI sector and fibre tracking measure in the different patient groups. Applying family-wise Bonferroni correction for 12 comparisons with predicted directions per brain measure, $P$ values $\leq 0.008$ were considered significant. The only correlation that met this criterion was between lower genu FA and older age in the alcoholic only group ($r = -0.29, P = 0.0068$). In addition, the 14 HIV+ALC patients who reported
having peripheral neuropathy had higher MD in the total callosal region \[t(50) = 3.252, P = 0.0021\], genu \[t(50) = 2.736, P = 0.0086\], and body \[t(50) = 3.365, P = 0.0015\] and lower FA in the body \[t(50) = 2.824, P = 0.0068\] than the 38 patients who did not report neuropathy. Hepatitis C, lifetime consumption of alcohol, family history of alcoholism, T-cell count and viral load were not significant correlates of sector or fibre tracking DTI measures in any group.

Motor performance associations with FA and MD in each patient group

We examined whether the DTI sector and fibre tracking measures were predictive of motor performance. Applying family-wise Bonferroni correction for seven comparisons with predicted directions per brain measure, \(P\) values \(< 0.014\) were considered significant.

In the HIV only group, three splenium measures were significant predictors of three upper motor tasks (Fig. 9): lower FA in the sector-defined splenium correlated with slower choice reaction time \((r = −0.41, P = 0.0085)\), lower FA in the splenium fibre bundles correlated with fewer fine finger movement rotations \((r = 0.38, P = 0.0131)\), and higher MD in the splenium fibre bundles correlated with longer type to complete the grooved pegboard task \((r = 0.50, P = 0.0009)\). Multiple regression analyses entering genu and splenium FA or MD measures, depending on the pairing observed in the simple correlations, identified the splenium measure as contributing significantly to the motor prediction over and above that for the genu measure.

In the HIV+ALC group, FA or MD in a number of callosal sector and fibre bundle metrics correlated with performance on grooved pegboard, fine finger movement (Fig. 10), or ataxia tests. Significant predictors of grooved pegboard were FA in the sectors of the genu \((r = −0.38, P = 0.0051)\) and body \((r = −0.43, P = 0.0013)\), FA in the genu fibre bundles \((r = −0.38, P = 0.0059)\), and MD in the body sector \((r = 0.34, P = 0.0137)\). Fine finger movements were correlated with FA in the splenial fibre bundles \((r = 0.40, P = 0.0037)\). The ataxia measure of standing on one foot with eyes closed was correlated with FA \((r = 0.48, P = 0.0006)\) in the total corpus callosum and specifically in the body \((r = 0.48, P = 0.0007)\) and splenium \((r = 0.48, P = 0.0006)\). In addition, lower FA in the genu fibre bundles correlated with poorer scores on this balance test \((r = 0.37, P = 0.0095)\).

None of the correlations was significant in the ALC group.

Focal callosal samples: controlling for partial-voluming effects

This set of analyses was based on FA and MD from manually identified, focal samples \((3 \times 3 \times 3 \text{mm}^3)\) of the corpus callosum that minimized the effect of partial voluming, that is, the effect of including non-white matter signal in callosal tissue.

Separate repeated measures ANOVA based on age-corrected FA and MD in the genu and splenium across the four subject groups revealed the same pattern of abnormalities observed with the anatomically-defined regions (Fig. 11). Specifically, the genu sample had lower FA and tended to have higher MD than the splenium sample \[FA interaction: \(F(3,265) = 4.499, P = 0.0042\); MD interaction: \(F(3,265) = 2.09, P = 0.0874\)\]. Within-group ANOVAs identified these regional differences as significant for FA in the ALC \([F(87,173) = 15.484, P = 0.0002]\) and HIV+ALC
[F(52,103) = 19.833, \( P = 0.0001 \)] groups and for MD in the ALC group [F(87,173) = 10.967, \( P = 0.0014 \)], but none was significant in the HIV only group. Thus, the anatomically-defined and focal sample approaches yielded similar findings especially in the genu, suggesting that group differences identified with the larger regions of interest were likely not due to the unintentional inclusion of non-white matter signal in the callosal regions examined. Table 2 presents the uncorrected FA values for the sectors and focal samples.

Analyses considering the HIV groups divided by presence versus absence of AIDS and alcoholism revealed significant interactions for the regional genu FA [F(1,90) = 4.914, \( P = 0.0292 \)] and MD [F(1,90) = 3.972, \( P = 0.0493 \)], indicating severely decreased FA and increased MD in the HIV+ALC with AIDS. The interactions were not significant for the regional splenium measures.

**Discussion**

Using *in vivo* DTI anisotropy and diffusivity measures, we examined the microstructural integrity of regions of the corpus callosum and their frontal and parietal-occipital
Table 2 FA values for the corpus callosum

<table>
<thead>
<tr>
<th>Callosal sectors</th>
<th>Control</th>
<th>Alcohol</th>
<th>HIV</th>
<th>HIV+ALC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu FA (mean ± SD)</td>
<td>45.5 ± 4.7</td>
<td>42.0 ± 5.1</td>
<td>44.4 ± 4.5</td>
<td>41.1 ± 5.1</td>
</tr>
<tr>
<td>Splenium FA (mean ± SD)</td>
<td>56.3 ± 4.2</td>
<td>53.7 ± 4.8</td>
<td>55.5 ± 4.3</td>
<td>53.5 ± 4.5</td>
</tr>
<tr>
<td>Callosal focal samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genu FA (mean ± SD)</td>
<td>64.2 ± 7.2</td>
<td>58.8 ± 8.4</td>
<td>56.4 ± 9.3</td>
<td>62.4 ± 6.5</td>
</tr>
<tr>
<td>Splenium FA (mean ± SD)</td>
<td>80.4 ± 6.2</td>
<td>79.1 ± 6.4</td>
<td>78.6 ± 5.2</td>
<td>80.4 ± 6.9</td>
</tr>
</tbody>
</table>

extents with quantitative fibre tracking in individuals with HIV infection, alcoholism, and those comorbid for both conditions. Compared with controls, all patient groups had lower FA and higher MD in callosal regions and fibre bundles than would be expected for their age. In general, however, these effects were only significant in the two groups with alcoholism, which on average exhibited 0.65–1.2 SD deficits in FA and similar effect sizes in regional microstructural MD. The callosal regions were differentially affected by alcoholism, with the anterior regions more affected than the splenium. The genu-splenium gradient in the alcoholics was even more pronounced in the fibre bundles. When the HIV-infected groups were singled out for comparison in terms of HIV disease progression, defined as an AIDS-defining event or low CD4+ counts (<200), and alcoholism comorbidity, the effect of HIV disease progression was further exacerbated by alcoholism comorbidity. In particular, the HIV-infected subgroup with AIDS and alcoholism exhibited ~2 SD FA and MD abnormalities in the callosal sectors, abnormalities that were more than twice the effect sizes observed in the other three HIV-infected subgroups and pervasive from the anterior-to-posterior extent of the corpus callosum. These differences were also present in the callosal fibre bundles and in the rarefied focal samples from the genu, which by the method of their identification have a reduced likelihood of finding age or disease effects.

Study selection criteria specified that volunteers had to have achieved a Karnofsky rating of at least 70 out of 100, which is indicative of ability to live independently, and absence of clinically detectable cognitive impairment that could be diagnosed psychometrically. In fact, all subjects received Karnofsky ratings of ≥80, indicating that they were ‘able to carry on normal activity and to work; no special care needed;’ 27% of subjects scored 90 and 66% scored 100. Despite the relatively intact functions of the HIV patients, a subset had AIDS-defining events or had a T-cell nadir <200. Clearly, the presence of AIDS is not a surrogate marker for neurological disease per se but when combined with alcoholism enhanced the risk of having neuroradiological evidence for CNS abnormalities (cf. Pfefferbaum et al., 2006c; Thompson et al., 2006).

The pattern of callosal macrostructural abnormality and factors influencing this effect comports well with our recent study of callosal macrostructure and ventricular volume (Pfefferbaum et al., 2006c) conducted in the patients of the present study. Consistent with the anterior-to-posterior gradient observed in the callosal microstructure, the prior report showed that the frontal ventricular and callosal macrostructures were more adversely affected than the posterior loci. Similarly, Thompson et al. (2006) reported that frontal horn ventricular enlargement distinguished AIDS patients from controls better than did measures of occipital or temporal horn.

Focal samples of rarefied white matter FA and MD used in this analysis minimized partial-voluming effects. Whether the observed water motility is more abundant in intracellular than extracellular spaces cannot currently be definitively discerned in vivo. It may, however, reflect disease-related breakdown of myelin sheathing, trapping of fluid between thin or lysed sheathes and between fibres and bulbous swelling of oligodendrocytes as occurs in normal ageing (Peters et al., 2001; Peters and Sethares, 2002, 2003). Axonal damage is associated with high viral loads in post-mortem study (Mankowski et al., 2002). Previously, we noted relations between greater viral load burden and more extensive WMHIs in both HIV only and HIV+ALC (Pfefferbaum et al., 2006c), demonstrating the functional relevance of these signs of white matter degradation to indexing HIV severity, noted in early MRI studies of AIDS (Olsen et al., 1988). Hyperintense regions of white matter may reflect myelin pallor and rarefaction associated with presence of multinucleated giant cells, located primarily in white matter, and considered the neuropathological hallmark of HIV encephalitis (Navia et al., 1986; Budka, 1997; Navia, 1997; Langford et al., 2002), and may be heralded by increasing diffusivity in fully-volumed tissue.

Mechanisms of alcohol exposure in accelerating the progress of HIV infection include immune suppression (Wang and Watson, 1995; Wang et al., 2002), blockage of pharmacological effectiveness (Miguez et al., 2003), and potentiation of the neurotoxicity of retroviral proteins shed by the HIV virus during glial infection (Self et al., 2004; Chen et al., 2005). Slice culture studies of rat brain have shown a locus of ethanol’s action on the HIV glycoprotein 120 (gp120), which activates glia to cause neurotoxicity through the NMDA receptor (Collins et al., 2000). Further, some in vitro studies indicate a neuroprotective effect of moderate (i.e. 20–30 μM) ethanol pretreatment (Collins et al., 2000; Belmadani et al., 2001) and a potentiation at
higher ethanol levels (100 μM) (Belmadani et al., 2003). By contrast, another study, using an NT2.N human neuron model (Wu et al., 1996), found that both low (16.7 μM) and moderate (50 μM) ethanol concentrations potentiated gp120-induced neural apoptosis (Chen et al., 2005). While some in vitro studies suggest a neuroprotective effect of alcohol in HIV infection, our in vivo human studies demonstrate a clear synergistic deleterious effect, where HIV-infected individuals who also are alcohol abusers or dependents have substantially higher risk of brain insult than do those with alcoholism or HIV infection alone. Indeed, even non-alcoholic HIV infected individuals with a history of an AIDS-defining event have evidence for far less brain macrostructural (Pfefferbaum et al., 2006c) and microstructural abnormalities (present study) than do those with alcoholism comorbidity.

The adverse effect of alcoholism on the HIV-infected brain is not a simple sequela of substance abuse; rather, it is different from that observed in HIV-methamphetamine comorbidity. Volumes of the basal ganglia and parietal cortex were significantly larger in individuals with HIV-methamphetamine comorbidity but smaller in those with HIV alone; further, the extent of the volume abnormality was predictive of cognitive impairment in each group (Jernigan et al., 2005). DTI evidence of the present study combined with our prior volumetric study of the ventricles and corpus callosum (Pfefferbaum et al., 2006c) in the subjects of the present study indicate the typical direction of abnormality, with enlargement in CSF-filled spaces and shrinkage in tissue that was profound in HIV/alcoholism comorbidity. Comparing these studies suggests different types of neurotoxic mechanism of brain tissue disruption arising from methamphetamine and alcohol (for review, see Koob and Le Moal, 2005).

Correlations between DTI metrics and motor performance in the HIV-infected groups is evidence for the functional meaningfulness of these brain measures and indicate potential mechanisms for motor slowing often reported in HIV infection (Heaton et al., 1995; Sacktor et al., 1996; Llorente et al., 1998; Lopez et al., 1998; Rothlind et al., 2005) and exacerbation by alcoholism comorbidity (Fein et al., 1995; Rothlind et al., 2005). Indeed, an ERP study by Fein et al. (1995) observed prolongation of P300 latency in HIV-infected patients with alcoholism, suggesting compromised neuronal transmission possibly attributable to impaired white matter connectivity. Quantitative fibre tracking measures of FA and MD in fibre bundles coursing through the splenium selectively predicted speed of fine finger movements and grooved pegboard performance in the HIV-infected group. The HIV-infected group with alcoholism showed correlations between callosal FA and grooved pegboard performance, like their HIV-infected only counterparts, and also showed relations between FA in the body of the corpus callosum and ataxia scores and likely reflects a contribution from alcoholism (cf. Pfefferbaum et al., 2000a; Sullivan et al., 2006b).

Limitations
Fundamental differences in the demographic make-up of the patient groups relative to the healthy control group impose limitations on the interpretation of the observed group effects despite their statistical significance. Within subject-group correlational analyses provided one empirical approach to examine the relations between demographic factors and brain measures, but only one correlation met statistical significance after correction for multiple comparisons. Nonetheless, the lower educational level, estimated IQ, and lower global functioning scores may be part of the disease state that contribute to a poorer condition of the brain, and some of these factors may be markers of lower premorbid status, thus precluding true matching of healthy controls to patients on such factors. Our contention is that occult comorbidity for alcoholism may be a relevant factor in other studies reporting group differences, or lack thereof, of brain pathology in HIV infection; similarly, some alcoholics in Cohort 1, who did not have serology testing, may have had occult HIV infection. Our experience from Cohort 3, wherein only one alcoholic has seroconverted over a 3-year period, suggests that the number would be small. Finally, this study focused on DTI indices of white matter pathology in alcoholism and HIV infection. Such pathology is ubiquitous in alcoholism, whereas subcortical (e.g. basal ganglia) brain pathology has been traditionally associated with HIV infection. Thus, the DTI metrics may be differentially sensitive to alcoholism compared with HIV infection, as may also be the case for the functional measures used here. Nonetheless, the strong DTI findings in HIV-alcoholism comorbidity highlight the role of white matter abnormalities when the HIV infection progresses to AIDS.

Conclusions
This DTI study of HIV infection provides indirect evidence to support the hypothesis that inflammation affecting white matter fibres and their cytoskeletal or myelin constituents is at least one mechanism by which HIV infection affects the brain. DTI’s sensitivity to detection of disruption of microstructural integrity of white matter may provide an early indication of the potential for HIV-associated dementia (Berger and Avison, 2001) and signs of insult from co-existing alcoholism.

Acknowledgements
We would like to thank our diligent research assistants (Jeffrey Eisen, Donna Murray, Marya Schulte, Andrea Spadoni, Carla Raassi, Daniel J. Pfefferbaum, Ted Sullivan, Alexander Jack, Julia Sandler, Carrie McCloskey, Shara Vinco, Marissa Huang, Shannon Muir and Suzanne Franklin), research clinicians (Julia Buss RN, Crystal Caldwell, Stephanie A. Sassoon, PhD, Anne O’Reilly, PhD, Anjali Deshmukh, PhD), and clinical collaborators (Carol A. Kemper, MD and Stanley Derensenski, MD) for their
Alcohol, HIV and white matter microstructure

invaluable work in subject recruitment, clinical evaluation, medical examination, scheduling, screening, contact maintenance, data collection and data entry. This work was supported by NIAAA grants AA12999, AA12388, AA05963, and AG17919. Funding to pay the Open Access publication charges for this article was provided by AA12999.

References


Belmadani A, Neafsey EJ, Collins MA. Human immunodeficiency virus type 1 gp120 and ethanol coexposure in rat organotypic brain slice cultures: curtailment of gp120-induced neurotoxicity and neurotoxic mediators by moderate but not high ethanol concentrations. J Neurol 2010; 257: 45–54.


Cloak CC, Chang I, Ernst T. Increased frontal white matter diffusion is associated with lateralized metabolites and psychomotor slowing in HIV. J Neuroimmunol 2004; 157: 147–52.


Fein G, Higgins CA, MacKay S. Alcohol abuse and HIV infection have additive effects on frontal cortex function as measured by auditory evoked potential P3a latency. Biol Psychiatry 1995; 37: 183–95.


Alcohol, HIV and white matter microstructure
