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Mapping the brain in younger and older asymptomatic HIV-1 men: Frontal volume changes in the absence of other cortical or diffusion tensor abnormalities

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ABSTRACT

Introduction: Over the past decade the developments made in treating people with human immune deficiency virus (HIV) have greatly improved quality of life and life expectancy. However, the nature of asymptomatic HIV-associated minor neurocognitive disorder (HAND) remains unclear. In this study we explored the occurrence of neuropsychological and neuroimaging changes in medically and psychiatrically stable HIV-1 infected patients on highly active antiretroviral treatment (HAART) from two separate age groups.

Methods: Participants included 20 HIV-1 infected younger (aged 20–40) and 20 HIV-1 older patients (aged 50–75). Comparisons were made with 20 age- and education-matched younger and 22 matched older healthy seronegative males. Participants were stable on treatment and asymptomatic at study onset with undetectable HIV-1 viral loads, and free of medical or psychiatric co-morbidity, alcohol or substance misuse. A detailed neuropsychological assessment was used and volumetric-magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) performed to assess grey and white-matter integrity.

Results: We found significant effects of ageing on memory, grey and white matter measures. Comparison of the HIV-positive and HIV-negative groups did not show significant differences on the neuropsychological tests after Bonferroni correction, and there were no significant age by HIV status interactions. However, we did find reduced grey matter volume on MRI in our HIV-positive participants within the medial and superior frontal gyri. We also found significant ageing effects in fronto-temporal grey and white matter, independent of the effect of HIV.

Conclusions: The results from this study suggest that HIV-1 disease by itself does not significantly impair cognitive function when patients are otherwise asymptomatic.

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Nevertheless, the imaging techniques were sensitive enough to detect subtle grey matter changes not normally evident until much later in the disease. If confirmed in a longitudinal study this frontal grey matter change could represent an important biomarker for trials in HIV disease.

1. Introduction

The introduction of highly active antiretroviral therapy (HAART) in 1996 for the treatment of human immune deficiency virus (HIV) has been accompanied by a dramatic decrease in HIV-associated mortality and morbidity (Detels et al., 2001; Dore et al., 2003; Hammer et al., 1997; Palella et al., 1998). In particular, a progressive decline in the incidence of HIV-associated dementia has recently been reported (Bhaskaran et al., 2008). Despite this evidence for the positive impact of HAART, its effects upon the more subtle sequelae of HIV such as neurocognitive impairment remains unclear.

Neurocognitive symptoms can result directly from HIV-infection or indirectly via toxins released from infected cells which, in turn, increase vulnerability to opportunistic infections and to HIV-related malignancies (Ances et al., 2006; Varatharajan and Thomas, 2009). Since the introduction of HAART, the reported prevalence of cognitive impairment has been estimated to fall to between 10% and 30% (Cohen et al., 2001; Deutsch et al., 2001; Ferrando et al., 1998; Letendre et al., 2004; Marra et al., 2003; Robertson et al., 2004, 2007; Suarez et al., 2001; Tozzi et al., 1999, 2001; Woods et al., 2009), and is argued to be lower than the rates in the pre-HAART era (Woods et al., 2009), although not all studies agree that the rate of prevalence has fallen (Carvalhal et al., 2006; Chang et al., 2003; Cysique et al., 2004; Giancola et al., 2006; Sacktor et al., 2002; Tozzi et al., 2005). A report by Cysique et al. (2006) showed that cognitive performance was stabilised in the majority of HAART-treated individuals over time, but with considerable variability in the individual patterns of change. This led many researchers to conclude that HAART's effectiveness is variable, some participants improving and others deteriorating, which in turn may give the overall impression that there is no apparent net change.

Despite long-term asymptomatic HIV-1 representing approximately 40% of the total HIV-infected population (Cole et al., 2007), the vast majority of the post-HAART studies have been conducted with symptomatic participants. Whilst the pre-HAART results were inconsistent (Bornstein et al., 1993; Clifford et al., 1990; Grant et al., 1987; Harrison et al., 1998; Hart et al., 1990; Heaton et al., 1995; McAllister et al., 1992; McArthur et al., 1989; Valcour et al., 2004), two post-HAART studies have concluded that there is no significant difference in the rate of cognitive impairment in asymptomatic HIV participants when compared with healthy controls (Cole et al., 2007; Stern et al., 1998), although data from the CHARTER study suggested rates of cognitive impairment in asymptomatic participants were as high as 36% (Heaton et al., 2010). As such it still remains unclear whether chronic HIV-infection can cause progressive neurocognitive dysfunction in medically stable asymptomatic HIV-1 (Cohen and Navia, 2007).

Given that older age is associated with higher HIV-1 viral loads and with immunosenescence, it has been suggested that there may be an additive or synergistic effect between HIV-1 infection and ageing, with a consequent increased incidence of cognitive impairment (Goodkin et al., 2001). Studies in older adults are therefore particularly important, especially given the increased likelihood of survival into older age, and the occurrence and increasing prevalence of newly infected HIV-1 cases in older adults. Results to date are mixed: some studies do not find a significant impact of ageing on cognitive function in HIV (Wilkie et al., 2003) whilst others have reported higher rates of cognitive impairment (Becker et al., 2007; Valcour et al., 2004, 2008). Interpretation is complicated by the fact that cognitive impairment in older adults is often associated with an increased prevalence of co-morbid risk factors, such as motor and sensory deficits, depression, substance and alcohol abuse (Valcour et al., 2004), along with cardiovascular and cerebrovascular disease (Becker et al., 2004).

Post-HAART studies of the association between neuroimaging findings and cognitive performance are limited in number and scope, but suggest reduced tissue volume in certain subcortical structures and white matter pathways (Paul et al., 2002). Where such structural brain alterations arise, it suggests that HAART may not provide complete protection for the central nervous system (CNS) against HIV-1 associated injury (Thompson et al., 2006; Thurnher and Donovan-Post, 2008). To date, very few studies have investigated asymptomatic HIV-1 participants (Paul et al., 2002), although a recent study found no major neuroimaging deterioration across a seven year follow-up of participants in the early stages of HIV-infection (Samuelsson et al., 2006). It remains unclear whether documented brain alterations (Castelo et al., 2006; Hall et al., 1996; Harrison et al., 1998; Paul et al., 2008; Ragin et al., 2004; Samuelsson et al., 2006; Stout et al., 1998; Thompson et al., 2005) occur early in the disease process (Paul et al., 2002), and whether chronic HIV-infection can cause progressive neurocognitive dysfunction in medically stable asymptomatic HIV-1 (Cohen and Navia, 2007).

Thus, it remains important to determine the impact of HIV-1 on age-related cognitive decline (Larussa et al., 2006) and on any underlying neuroimaging change. In the present study, we had three main objectives:

- The first was to investigate the occurrence and pattern of neuropsychological deficits in medically and psychiatrically stable HIV-1 infected individuals without the confounding effects of other risk factors such as a history of alcohol or substance misuse/dependence, or significant previous neurological (including cognitive) or psychiatric disorder. For simplicity, we refer to such participants as 'asymptomatic' throughout the rest of this manuscript. To further

protect against potential confounds that may not be fully revealed on interview or through assessment, we restricted our sample to a select sub-group of the HIV-1 population – namely white/Caucasian men who have sex with men. Our study did not aim to investigate the pattern of neuropsychological deficits in symptomatic individuals, as this group has already been extensively investigated.

- The second objective was to examine grey and white matter biomarkers of cognitive change in this same, well characterised, group, using volumetric-magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI).
- The third objective was to examine for any interaction between the ageing process and HIV-1 infection; and therefore we recruited participants from two different age groups (i.e., 20–40 and 50–75).

2. Methods

2.1. *Participant population*

95 participants who described themselves as 'men who have sex with men' were recruited between October 2005 and December 2008. They were selected from 4 groups: HIV-1 infected patients aged 20–40 years or 50–75 years, and age- and education-matched younger (20–40) and older (50–75) healthy seronegative males. The age of 50 years was selected as the entry point into the older groups in keeping with Cherner et al. (2004). To participate in the study, HIV-1 infected participants were required to have been stable on HAART with a viral load <50 copies/ml and CD4 cell count >200 cells/μl for at least six months, as established by medical file review. HAART regimens had been prescribed as clinically determined by the primary HIV physician, and all HIV-infected participants were on HAART which included at least one CNS penetrating antiretroviral drug. All participants were of white/Caucasian ethnicity and proficient in the English language.

Participants were excluded if they met criteria for any previous or current CNS-AIDS-defining-conditions, presence of Hepatitis B or C, confounding neurological disorder, a history of head trauma with loss of consciousness for more than 10 min, or had a history of harmful alcohol or substance usage. Harmful alcohol usage was defined as more than 25 units of alcohol per week. Harmful substance usage was defined as any use of marijuana within the two weeks before the research evaluation or regular use more than once a month, use of other substances such as ecstasy or cocaine more than 3 times a year, or any use of heroin. Other exclusion criteria were chronic medical illness that might affect cognition such as cardiac, liver or renal disease, moderate to severe psychiatric disorder, and/or current use of psychotropic medication. If an MRI could not be conducted, or showed any incidental finding likely to represent non-HIV-1 related pathology, the volunteer was also excluded.

Following the initial screening, 13 participants (8 HIV-1 infected, 5 HIV-negative) were excluded from the study: 6 because of MRI contraindications, 3 as a result of previous harmful use of substances, 3 because of significant past medical, neurological or psychiatric histories, and 1 participant withdrew from the study. This left 82 participants who were enrolled into the study. Table 1 shows that there were no significant differences between the younger HIV-1 infected participants and the matched HIV-seronegative males, or between the older HIV group and the matched HIV-seronegative males, in terms of age, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), National Adult Reading Test (NART) Full Scale Intelligence Quotient (FSIQ) or estimated Wechsler Adult Intelligence Scale (WAIS) – FSIQ, Verbal IQ (VIQ) or Performance IQ (PIQ) standardised scores. Furthermore, no significant differences were noted on current CD4 and nadir CD4 cell counts between the HIV-1 infected younger group (20–40-years old) and HIV-1 infected older group (50–75-years old). However, Table 1 also indicates that there was a significant difference between the two HIV-1 infected groups for years since diagnosis (Mann–Whitney $U = 109$, $p = .014$) and years since commencing HIV treatment ($t = 3.114$, $p = .004$).

Table 1 – Means (SD) for baseline characteristics of study participants.

	HIV-1 infected		HIV-negative	
	Older group (N = 20)	Younger group (N = 20)	Older group (N = 22)	Younger group (N = 20)
Age	58.70 (6.62)	34.25 (4.39)	57.64 (7.61)	31.75 (5.91)
IQ				
NART	119.45 (6.71)	112.05 (9.02)	117.86 (8.46)	115.25 (7.49)
WAIS-III FSIQ	117.35 (12.41)	115.55 (11.33)	117.77 (11.04)	118.20 (14.14)
WAIS-III VIQ	117.05 (12.76)	112.95 (11.67)	116.36 (14.41)	116.05 (14.34)
WAIS-III PIQ	113.95 (13.92)	115.05 (15.26)	115.50 (9.47)	116.75 (14.61)
Mood				
Depression Task	5.17 (4.20)	4.50 (5.07)	3.40 (3.00)	3.90 (5.60)
Anxiety Task	6.37 (6.57)	3.45 (4.32)	4.30 (5.60)	3.40 (3.80)
HIV variables				
CD4 cells/mm^3	735.5 (343.53)	596.45 (191.40)		
Nadir CD4 cells/mm^3	170.7 (91.99)	175.4 (101.72)		
Yrs since diagnosis[a]	13.00 (7.00)	8.10 (4.40)		
Yrs since HAART treatment[a]	8.85 (4.26)	5.00 (2.32)		

a Significant difference between groups found.

2.2. Procedures

2.2.1. Neuropsychological assessment

A range of neuropsychological tests was administered by trained psychologists (AF, KT), according to standardised procedures. Tests were broadly based on the Heaton et al. (1995) HIV battery, although equivalent tests developed and standardised in the United Kingdom were substituted where available. The neuropsychological battery took approximately 2 h to administer, and the order of testing was the same for all participants. The battery consisted of:

- the revised NART (NART-R;Nelson and Willison, 1991) as a test of general premorbid ability;
- the Vocabulary, Digit-Symbol Coding, Similarities, Block Design, Matrix Reasoning, Digit Span, Digit Symbol and Letter-Number Sequencing subtests from the WAIS (Third Edition) (WAIS-III; Wechsler, 1997) as tests of General intelligence;
- the McKenna Graded Naming Test (McKenna and Warrington, 1980) and the Controlled Oral Word Association Test (COWAT; Benton, 1968) to assess language function;
- the Logical Memory I & II and Visual Reproduction I & II from the Wechsler Adult Memory Scale (Revised) (WMS-R; Wechsler, 1987), plus the Rey Auditory Verbal Learning Test (RAVLT; Spreen and Strauss, 1998), to assess anterograde verbal and visual memory. In addition, an experimental word-pair and single-word recognition test (Castel and Craik, 2003) was also included as a potentially more sensitive measure of ageing effects;
- the Trail Making Test (Reitan, 1958) and Paced Serial Addition Test (PASAT; Spreen and Strauss, 1998) to assess processing speed;
- the Grooved Pegboard Test (Lafayette Instruments, 2002) to assess motor speed;
- the Modified Card Sorting Test (Nelson, 1976) to assess executive function.

2.2.2. Medical and psychiatric evaluation

Participants underwent a medical and brief neurological examination performed by an HIV physician. Blood samples were obtained to verify HIV status, CD4 cell count and plasma HIV-RNA, and syphilis and hepatitis B and C serology, as well as a full blood count, renal and liver function, bone and lipid profile, vitamin B12, folate and glucose, thyroid stimulating hormone (TSH) and C-reactive protein. All participants were also evaluated for psychiatric disorder or a history of alcohol and substance usage using a structured clinical interview administered by a consultant psychiatrist to establish a diagnosis according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria (American Psychiatric Association, 1994). In addition, depression and anxiety symptoms were assessed using the BDI and the BAI (Beck, 1987; Beck and Steer, 1990).

2.3. Data analysis

2.3.1. Neuropsychological and neuropsychiatric data

Normality and heterogeneity of variance were checked using the Shapiro–Wilks and Levene's tests respectively. If these criteria were fulfilled, two-way analyses of variance were conducted to examine the interaction between HIV-1 status (infected, negative) and age (younger, older). If these criteria were not fulfilled, Mann–Whitney U tests were used. Results were also Bonferroni corrected for multiple comparisons. An initial alpha level of .05 was set and then corrected for the 35 neuropsychological test scores that were analysed. Correlations between each of the individual neuropsychological and neuropsychiatric variables and HIV disease status (i.e., current and nadir CD4 count) were also investigated within the HIV groups. With the exception of the standardised NART and WAIS-III IQ scores, all neuropsychological data analysis was conducted on the raw scores. With regard to the neuropsychological measures, previous findings (Tozzi et al., 2001) suggested that comparison of 40 HIV and 40 non-HIV participants in total would give a power of 85% on verbal memory tests at alpha = .05.

2.3.2. MRI data acquisition

Scans were acquired on a General Electric SIGNA HDx 3.0T MR scanner (General Electric, Milwaukee, WI, USA) at the Centre for Neuroimaging Sciences, Institute of Psychiatry, King's College London, using the body coil for transmission, and an 8 channel head coil for signal reception. For radiological purposes, multi-slice, whole brain, T_2-weighted Fast Spin Echo (FSE) and Fluid Attenuated Inversion Recovery (FLAIR) scans were collected. Volumetric data were acquired using a 3D inversion recovery prepared fast spoiled gradient echo (IR-SPGR) sequence ($T_R = 7.1$ msec, $T_E = 2.8$ msec, $T_I = 450$ msec, excitation flip angle = 20°) giving isotropic 1.1 mm voxels. The DTI protocol, based on that described by Jones et al. (2002), used a multi-slice peripherally gated echo-planar imaging (EPI) acquisition with a parallel imaging (ASSET) speed up factor of two and an effective repetition time that varied between subjects in the range 12 and 20 RR intervals. Each volume was acquired using a doubly refocused spin echo EPI sequence that gave isotropic 2.4 mm voxels over 60 contiguous near-axial slice locations. The maximum diffusion weighting was 1300 sec mm^{-2}, and at each slice location 4 images were acquired with no diffusion gradients applied, together with 32 diffusion-weighted images in which gradient directions were uniformly distributed in space.

2.3.3. Image processing and analysis

The MRI datasets were first assessed qualitatively by an experienced neuroradiologist (NS) blind to participant group status, to check for evidence of radiological abnormalities outside of the normal range. Participants with such abnormalities were excluded from the study, as discussed above.

2.3.4. Voxel-based morphometry (VBM)

VBM was used to compare MRI based measures of grey and white matter between groups (Ashburner and Friston, 2001). In the current study, segmentation (i.e., determination of the distribution of these tissue types within the brain) was performed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neurosciences, University College London, UK). After completion of the unified segmentation process (Ashburner and Friston, 2005), which included a 'modulation' step yielding grey matter volumes, the resulting tissue maps were smoothed with a $10 \times 10 \times 10$ mm Gaussian kernel to reduce noise and allow for the effects of small residual mis-registrations. We chose

this kernel width based on a review of existing studies in HIV and ageing (Carasig et al., 2003; Tisserand et al., 2002, 2004).

A two-way (HIV-1 status by age group) analysis of covariance (ANCOVA) model, co-varying for total intracranial grey and white matter brain volumes, was fitted at each intracerebral voxel in standard space. As structural brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics) are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al., 1999). We therefore first tested data on a voxelwise basis, using a deliberately lenient p-value ($p \leq .05$) to detect voxels putatively demonstrating differences between groups, and then searched for spatial clusters among the voxels highlighted. No parametric distribution is known for cluster mass, however, so it is necessary to use permutation testing to assess the statistical significance of these clusters. Both voxel- and cluster-wise tests were therefore performed in the BAMM package (Brain Activation and Morphological Mapping, a joint development of the Brain Mapping Unit, Department of Psychiatry, University of Cambridge and The Institute of Psychiatry, London, UK http://www-bmu.psychiatry.cam.ac.uk/BAMM/index.html), and we chose thresholds for the cluster mass statistic such that the number of clusters expected by chance alone (the number of false positives) would be less than one over the whole brain volume (i.e., over all comparisons made). We also conducted separate post-hoc t-test analyses to examine relevant contrasts in more detail.

2.3.5. *DTI analysis*

Following correction for image distortion due to the eddy currents, performed using in-house software based on the fMRIB software library (FSL) 'eddy correct' software (http://www.fmrib.ox.ac.uk/fsl/index.html), the diffusion tensor was determined for each voxel following the method of Basser et al. (1994), allowing calculation of fractional anisotropy (FA) and mean diffusivity (MD) measures (Basser and Pierpaoli, 1996). To allow between group comparisons, these maps were then spatially normalised using a two stage process. The mean T_2-weighted ($b = 0$) image for each subject was first registered (using SPM2, Wellcome Department of Imaging Neurosciences, University College London, UK) to the EPI template provided; the derived warping parameters were then applied to corresponding FA images, allowing these to be averaged and smoothed to create new, study-specific, template. Each subject's FA images were then re-registered to this template and the registration parameters thus determined were also applied to the corresponding MD images. The normalised FA images were also segmented to give maps of the probability of a tissue being either white or grey matter, and these segmented images were thresholded at a low (10%) probability to provide a (deliberately relatively liberal) binary mask of white matter (Kyriakopoulos et al., 2008; Shergill et al., 2007). Finally, the FA and MD images were mildly smoothed with a $5 \times 5 \times 5$ mm Gaussian kernel (to reduce noise and minimize the effects of small residual mis-registrations) before multiplying by the white matter mask. This masking procedure, along with a requirement that all subjects contribute data at a particular position, restricted the analyses to core white matter regions only, reducing the search volume (and thus the number of comparisons made) and also avoiding testing at the grey/white interfaces. As with the VBM analysis, the size of the smoothing kernel chosen at this point can affect the sensitivity of the analysis to inter-group differences of differing spatial extents (Jones et al., 2005). In the absence of a specific hypothesis about the size of the areas in which we expected to see changes, we chose to use a conservative degree of smoothing, with a kernel size of the same order of magnitude as the width of many white matter tracts.

Between-group differences in white matter FA and MD were again estimated by fitting a two-way (HIV-1 status by age group) analysis of variance (ANOVA) model at each intracerebral voxel in standard space. As before, we searched for spatial clusters among the voxels highlighted, and tested the significance of the cluster mass, in this instance using the XBAM package (developed at The Institute of Psychiatry, London, UK http://www.brainmap.co.uk/) (Bullmore et al., 1999). At the cluster level we again calculated for a range of p-values the number of clusters which would be expected by chance alone and set the statistical threshold such that the expected number of false positive clusters by chance alone would be less than one; we have reported this value for any analyses yielding significant clusters.

3. Results

Table 2 shows mean scores (±1 SD) on the neuropsychological tests in the HIV-1 infected and healthy control groups.

3.1. *Group difference analysis*

Age: There were significant main effects of age, with older adults performing worse on the RAVLT total words recalled ($F_{1,78} = 21.34$, $p = .001$), RAVLT immediate recall ($F_{1,78} = 24.31$, $p < .001$), RAVLT delayed memory recall ($F_{1,78} = 21.30$, $p < .001$) and Word-Pairs false acceptance rate ($F_{1,74} = 13.59$, $p < .001$). These findings remained statistically significant after Bonferroni correction for multiple comparisons.

HIV status: Only on immediate recall in the Visual Reproduction task was there a significant effect of HIV status. Because of the heterogeneity of variance on this task, the Mann–Whitney U test was employed, and it showed that the HIV group performed worse than seronegative group ($U = 530.0$, $p = .004$). However, this did not survive a Bonferroni correction. The trend was in the same direction for delayed recall on this test (Mann–Whitney $U = 636.0$, $p = .058$).

Age by HIV interaction: There were no significant interaction effects between age and HIV status at the .05 level on any of the neuropsychological tests.

3.2. *Neuroimaging findings*

Age: Significant effects of general ageing consistent with the normal ageing process were detected, with the older individuals having significantly less grey matter than younger participants in a number of brain regions encompassing the frontal lobes, insula and temporal lobes (cluster test significance $p = .01$; see Fig. 1a). We also detected significantly increased grey matter in a region encompassing the thalamus

Table 2 – Means (SD) on all neuropsychological variables.

	HIV-1 infected (N = 40)[c]	HIV-1 negative (N = 42)[d]	Sig.	Cohen's d
WAIS-III				
Vocabulary	54.85 (8.42)	54.93 (8.85)	.967[a]	−.009
Similarities	25.87 (3.88)	26.02 (4.84)	.879[a]	−.034
Block Design	48.00 (12.27)	47.00 (10.84)	.696[a]	−.086
Matrix Reasoning	17.85 (4.79)	18.93 (3.73)	.258[a]	−.252
Digit Span	19.31 (4.47)	18.55 (4.20)	.649[b]	.175
Digit Symbol	71.18 (18.21)	75.50 (13.73)	.227[a]	−.268
Symbol Search	36.03 (7.14)	34.81 (8.27)	.486[a]	.158
Letter-Number Sequencing	12.24 (1.88)	11.62 (2.63)	.265[a]	.271
WMS-R				
Logical Memory I	29.03 (6.14)	31.19 (6.05)	.111[a]	−.354
Logical Memory II	25.38 (6.93)	27.52 (6.62)	.155[a]	−.316
Visual Reproduction I	36.65 (3.17)	37.76 (3.94)	.004[b]	−.310
Visual Reproduction II	32.67 (6.41)	34.50 (7.45)	.058[b]	−.131
Graded Naming Test	23.67 (3.96)	23.21 (3.57)	.591[a]	.122
PASAT				
2.4 sec Adjusted	3.76 (1.37)	3.39 (1.02)	.451[b]	.306
1.6 sec Adjusted	3.89 (1.94)	3.29 (1.05)	.217[b]	.385
Errors sum	7.05 (4.93)	6.29 (3.42)	.436[a]	.179
Percentage errors	.11 (.10)	.08 (.05)	.195[b]	.379
Verbal fluency	46.62 (13.11)	46.62 (12.89)	.999[a]	0
Trail Making Test				
Trails A	28.09 (10.04)	28.74 (8.03)	.458[b]	−.072
Trails B	64.99 (24.31)	60.44 (17.75)	.241[a]	.214
Grooved Pegboard				
Dom. Hand Time	70.95 (16.02)	70.09 (11.35)	.810[b]	.062
Dom. Hand Drops	.38 (.54)	.48 (.71)	.731[b]	−.159
Non-Dom. Hand Time	81.40 (18.49)	76.15 (12.94)	.371[b]	.329
Non-Dom. Hand Drops	.41 (.72)	.52 (.80)	.452[b]	−.145
Modified Card Sorting Test				
Categories completed	5.89 (1.58)	6.18 (1.23)	.688[b]	−.205
Total errors	7.51 (6.50)	5.57 (4.82)	.250[b]	.339
Preservative errors	.26 (.20)	.23 (.21)	.617[b]	.146
RAVLT				
List 1–5 total[e]	51.70 (9.28)	53.38 (8.97)	.407[a]	−.184
Immediate recall[e]	10.60 (2.64)	11.26 (2.91)	.284[a]	−.238
Delayed recall[e]	9.98 (2.72)	10.98 (3.14)	.142[b]	−.340
Recognition	12.93 (1.65)	13.31 (1.87)	.160[b]	−.215
Single-word recognition				
Item hit rate	.56 (.14)	.53 (.16)	.341[a]	.200
Item false accept rate	.16 (.13)	.17 (.14)	.879[b]	−.074
Word-pair recognition				
Pairs hit rate	.62 (.20)	.59 (.21)	.508[a]	.146
Pairs false accept rate[e]	.28 (.20)	.29 (.15)	.452[b]	.057

a Independent t-test (2-tailed).
b Mann–Whitney U.
c A limited number of participants did not complete the full battery of tests, as such the sample size for each individual tests ranges from N = 34 to N = 40.
d A limited number of participants did not complete the full battery of tests, as such the sample size for each individual tests ranges from N = 37 to N = 42.
e Significant main effect of age.

(cluster test significance $p = .01$; see Fig. 1b). From the DTI analysis, we detected a number of areas of reduced FA and increased MD, including frontal and temporal white matter tracts, all consistent with the ageing process (see Fig. 2).

HIV status: Although the HIV-infected and control participants did not differ in terms of total grey, total white matter, and total combined (grey/white) brain volumes (Table 3), there were significant regional grey matter differences. On the VBM analysis, the HIV-1 infected individuals had significantly less grey matter than the seronegative group in a region, encompassing the medial and superior frontal-gyrus (cluster test significance $p = .01$; see Fig. 3). Post-hoc analyses showed that this held good within the individual younger and older groups alone. There is clear overlap between the findings in older

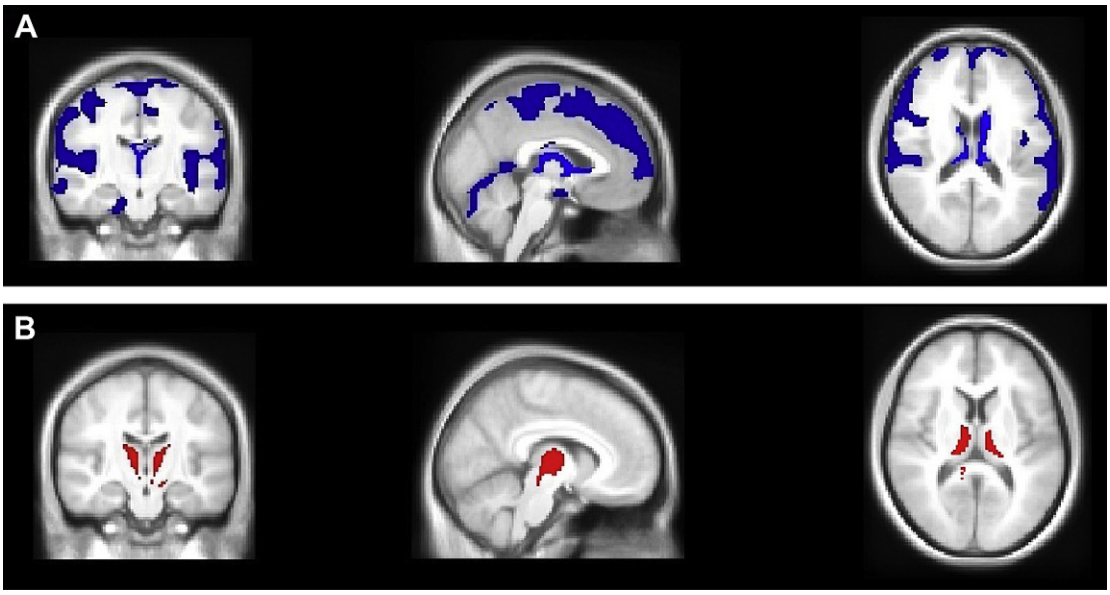

Fig. 1 – Areas of change in older participants when compared to younger participants ($p = .01$) as represented by (A) reduced grey matter in a cluster predominantly encompassing the frontal lobes, insula and temporal lobes and (B) increased grey matter in a cluster incorporating the thalamus.

(Fig. 4a) and younger groups (Fig. 4b), although there are also noticeable differences; neither these differences nor the specific regions highlighted in either age group should be over-interpreted, however, due to the small sample size and post-hoc nature of this sub-group analysis. In addition, no significant correlations were detected between neuropsychological and grey matter measures within these regions. There were no significant differences on measures of FA or MD from the DTI analysis.

Age by HIV interaction: No significant interactions were detected between age and HIV status on the grey matter, FA, or MD measures.

3.3. *Correlations between HIV, neuropsychological, neuropsychiatric and neuroimaging variables*

No significant correlations were found between current CD4 count and any of the neuropsychological variables in the total

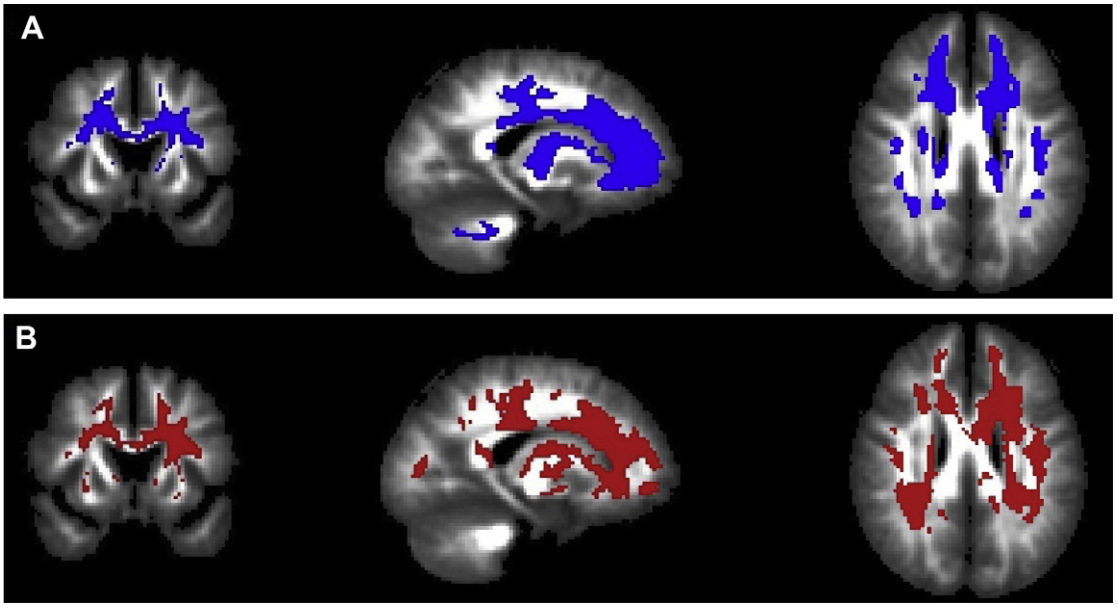

Fig. 2 – Areas of change in the frontal and temporal lobes older participants when compared to younger participants ($p = .01$) as represented by (A) reduced FA and (B) increased MD.

Table 3 – Global brain volumes.

	HIV-infected (N = 37)	HIV-negative (N = 41)	*p*-Value (t test)
Total grey matter	694.5	712.3	.274
Total white matter	480.0	484.5	.679
Total brain (grey + white)	1174.5	1196.8	.374

Note: all measurements in millilitres.

(young and old) HIV-1 infected group. However, nadir CD4 cell count correlated significantly with Logical Memory II ($r = .417$, $p = .007$) and with preservative errors on the Modified Card Sorting Test ($r = -.519$, $p = .001$). The former did not survive a Bonferroni correction for multiple comparisons at an initial alpha of .05. There were no significant correlations between the neuropsychiatric variables of BDI and BAI and either current or nadir CD4 count. There were also no significant correlations between the significant neuroimaging findings and current or nadir CD4 count.

4. Discussion

Whether or not there is cognitive deterioration in medically and psychiatrically stable HIV-1 participants remains a matter of some debate. In our study we found that, in general, there was no evidence of significant neuropsychological impairment in our stable HIV-1 patient group. There was significant impairment on visual recall on the Visual Reproduction test, but this did not survive Bonferroni correction. The results from this study are in keeping with some post-HAART investigations (Clifford et al., 1990; McArthur et al., 1989) although others show impairment in less selected groups (Becker et al., 2007; Dawes et al., 2008). It suggests that stable HIV-1 asymptomatic participants with long-term suppression of viral load and CD4 counts above 200 cells/μl do not necessarily show cognitive decline. Those asymptomatic HIV-1 infected individuals, such as those we have investigated, who do show decline may be a minority, not necessarily representative of the typical course of the disease (Cole et al., 2007).

Where previous studies have found significantly increased rates of neuropsychological impairment in asymptomatic HIV-1 infected individuals, there have been inconsistencies in the cognitive domains affected and in the reported rates of neuropsychological impairment (Reger et al., 2002). The differences between our results and those studies which have shown high rates of impairment may very largely reflect such factors as rates of alcohol and substance misuse, medical and psychiatric co-morbidities, for which our study carefully controlled.

In examining for age effects, we found that overall our older participants performed worse than the younger on specific aspects of visual and verbal memory, but we did not find evidence of accelerated ageing effect in older HIV-1 adults. Some previous studies have found that older HIV-1 infected individuals are at greater risk for developing cognitive impairment than younger HIV-1 participants (Becker et al., 2004; Valcour et al., 2004, 2008), although not all investigations have found this (Cohen and Navia, 2007; Goodkin et al., 2001). Brew et al. (2008) concluded that there was no consistent relationship between age and HIV-1 associated cognitive impairment. Again, some authors (Wilkie et al., 2003) have cautioned that the presence of various co-morbid diseases and risk factors may confound reports of a positive relationship between HIV status and ageing (Becker et al., 2007). Our findings in medically and psychiatrically stable HIV participants also provide grounds for caution in interpreting comparisons of older and younger HIV participants where co-morbid risk factors have not been adequately controlled for.

Whalley et al. (2004) described cognitive ageing as the net outcome of multiple positive and negative determinants. Our patient groups had the negative determinant of HIV-1 infection, but also a number of positive factors including high premorbid IQ and good medical and psychiatric health. These may well have given them sufficient 'cerebral reserve' (Valcour et al., 2004) to protect them against the risk of neurological pathology. However, these protective factors may be less effective as HIV-1 individuals age further, and future longitudinal studies of older HIV-1 participants will be important.

In terms of neuroimaging, we found significant regional grey matter and white matter effects of ageing in our sample overall. These findings match the results of studies of normal ageing which have found reduced areas of grey matter density throughout the cortex and sub-cortex, including in the temporal lobes and frontal lobes (Raz et al., 2005). Previous studies give no clear evidence of brain changes in the asymptomatic stage of HIV-infection (Hall et al., 1996; Harrison et al., 1998; Samuelsson et al., 2006). In our study, we found evidence that both younger and older HIV-1 infected individuals had significantly less grey matter in a region encompassing the

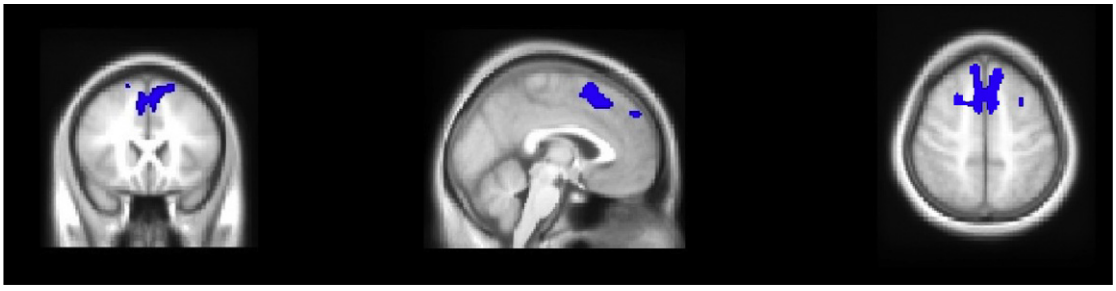

Fig. 3 – Reduced grey matter in a cluster encompassing the medial and superior frontal-gyrus in HIV-1 infected participants when compared to healthy participants ($p = .01$).

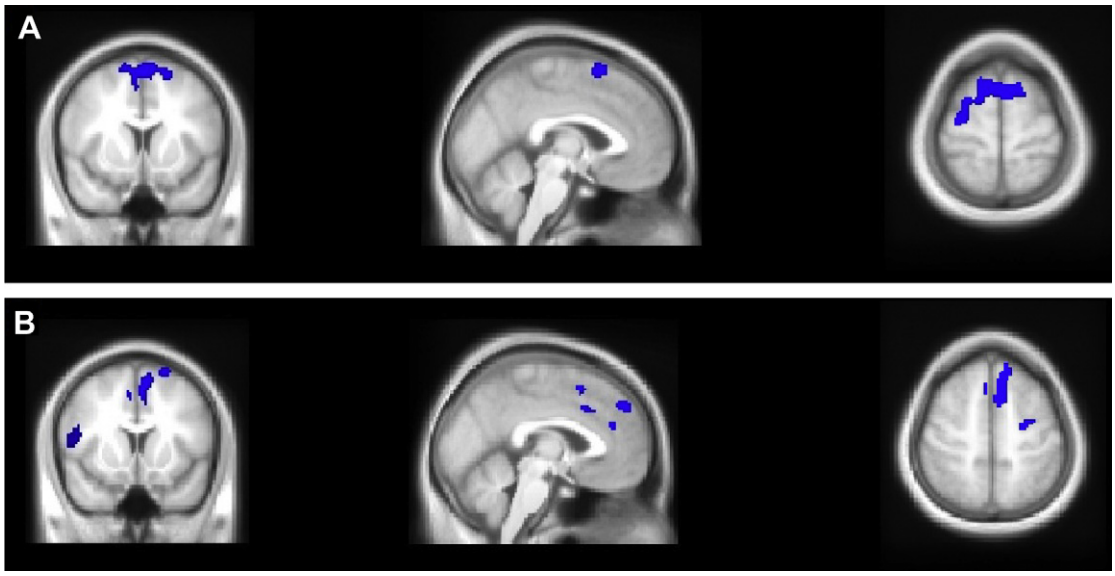

Fig. 4 – Area of reduced grey matter (A) in a cluster encompassing the superior frontal-gyrus in older participants HIV-1 infected participants when compared to older healthy participants ($p = -.01$) and (B) in a cluster encompassing the medial and superior frontal-gyrus in younger HIV-1 infected participants when compared to younger healthy participants ($p = .01$).

medial and superior frontal gyri than the matched HIV-negative healthy seronegative group. These findings are broadly consistent with observations previously obtained much later in the disease, and may reflect the sensitivity of our imaging protocol to detect subtle changes in the early disease stages. For example, Thompson et al. (2005) found that participants with AIDS had regionally specific reduced frontal grey matter volumes, though no thinning of temporal or prefrontal cortices. Not surprisingly, given the advanced stage of infection in their participant group, they found more extensive changes beyond that which we have observed with reduced grey matter volumes in the parietal lobes as well (Thompson et al., 2005). Similarly, Chiang et al. (2007) reported reductions in grey matter volume in the medial and basal frontal lobes in more advanced HIV cases, although again these were more extensive changes than those we detected in our early stage HIV-1 infected cases. There is also some evidence of histopathological loss of frontal neurons in individuals with HIV (Everall et al., 1991, 1993), and of abnormal frontal activations on functional MRI (Ernst et al., 2002).

In contrast to a number of other studies that have previously reported changes in DTI measures of FA (Chang et al., 2008; Filippi et al., 2001; Pomara et al., 2001; Ragin et al., 2004) and MD (Chang et al., 2008; Stebbins et al., 2007), we did not find any evidence of white matter integrity changes associated specifically with HIV-infection. However, we did detect significant regional white matter effects of ageing, thereby confirming that our imaging techniques were sensitive and adequately powered to detect such lifespan differences. Thompson et al. (2006) have discussed the difficulty in detecting what may be only subtle white matter alterations in HIV, suggesting that these alterations may occur later in the disease process than the grey matter alterations. Similarly, Stout et al. (1998) have reported that white matter changes in HIV may be less robust than atrophy in cortical grey matter because of apparent fluctuations in the white matter signal over time. Moreover, as discussed above, participants had been excluded from the sample where confounding factors such as hepatitis C, substance and alcohol abuse histories, or depression were present. Furthermore, we did not obtain significant correlations between our imaging findings and our neuropsychological variables, and this may also have reflected the early and asymptomatic stage at which our HIV participants were assessed.

It is important to acknowledge a number of limitations in our study. As noted previously, our patient group comprised a select group of individuals with high premorbid IQ and well managed HIV. These factors may have protected them against the negative impact of the disease, and therefore it is important to determine whether our results generalise to other sectors of the HIV population who are not so well protected. In addition, in order to reduce the potential impact of confounding co-morbid disease factors, we restricted our sample to those Caucasian men who have sex with men, and again it will be important to examine how well our results generalise to other sections of the HIV population. Finally, whilst our sample size was relatively large for an imaging study, it would be useful to replicate our findings in a still larger sample.

In conclusion, the findings from this study have indicated that asymptomatic HIV-1 participants, with long-term suppression of viral load, did not manifest significant cognitive impairment, nor any significant interaction with the ageing process, after factors such as alcohol and substance misuse, cardiovascular disease, and depression had been excluded. This, in itself, has important implications for the HIV-infected population as it may suggest that the impact of cohort effects are more important than other factors such as ageing. However, we also found evidence of reduced grey matter content in a region encompassing the medial and superior frontal gyri in our HIV participants, despite their preserved

neuropsychological performance. This finding may be partly attributable to recent advances in imaging (including higher magnetic field strengths), thereby enhancing the sensitivity of our techniques to changes not normally evident until later in the disease. The findings from this study suggest that reduced frontal grey matter may precede the appearance of cognitive impairment. This finding needs to be replicated and explored further in a longitudinal study and, if confirmed, it may provide an important biomarker for drug trials in early HIV disease.

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