HIV-associated structural brain changes as related to cognition

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Dissertation

HIV-ASSOCIATED STRUCTURAL BRAIN CHANGES AS RELATED TO COGNITION

by

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This dissertation is the culmination of over a decade’s worth of work. During that course of time, I got married, co-raised two boys that are now in elementary school, and discovered my passion for teaching secondary school science. As a result, the successful completion of this thesis would never have been possible without a number of people’s help along the way.

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HIV-ASSOCIATED STRUCTURAL BRAIN CHANGES AS RELATED TO COGNITION

MAUREEN GLESSNER COURTNEY

Boston University Graduate School of Arts and Sciences, 2016
Major Professor: Chantal E. Stern, Professor of Psychological and Brain Sciences

ABSTRACT

Nearly half of all HIV-positive individuals present with some form of HIV-associated neurocognitive disorder (HAND). The experiments described in this thesis examined the structural changes that occur in the brain as a result of HIV infection. While previous work has established that HIV targets the basal ganglia and fronto-striatal systems and impacts cortical and white matter pathways, it was unknown whether these changes occur in the absence of HAND. The studies described here focused on cognitively asymptomatic HIV+ individuals (CAHIV+) without HAND as determined by widely accepted neuropsychological performance guidelines.

Experiment 1 utilized diffusion tensor imaging (DTI) to examine HIV-associated alterations in white matter (WM) fractional anisotropy (FA) in the absence of HAND in 23 HIV+ individuals and 17 control participants (HIV-) matched for age, education, and verbal IQ. The hypothesis was that CAHIV+ participants would show lower FA values than HIV- in the corpus callosum,
fronotemporal, and parietal regions of interest (ROIs). CAHIV+ individuals demonstrated higher FA in the frontotemporal region and posterior corpus callosum, but lower FA in parietal WM relative to HIV- individuals.

Experiment 2 utilized structural MRI to compare cortical thickness in 22 CAHIV+ individuals and 19 control participants (HIV-) matched for age, education, and verbal IQ. The hypothesis was that CAHIV+ participants would have thinner frontal, temporal, and parietal regions than HIV- participants. Reduced cortical thickness measures were identified in the cingulate and superior temporal gyri, with increased cortical thickness measures in the inferior occipital gyrus, for HIV+ participants compared to HIV-.

Experiment 3 examined the relationship between the structural alterations identified in Experiments 1 and 2, neuropsychological performance on tests sensitive to HAND identification, and immunological characteristics in 30 HIV+ participants and 28 HIV- control participants. As hypothesized, regional FA values, cortical thickness, and viral load were related to neuropsychological composite scores for CAHIV+, but not HIV-.

Together, results from these three studies suggest that regional FA and cortical alterations identified in CAHIV+ patients may contribute to the cognitive deficits often seen in later stages of HIV disease.
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<td>AEF</td>
<td>Abstraction / executive function cognitive domain</td>
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<tr>
<td>ANI</td>
<td>Asymptomatic neurocognitive impairment</td>
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<td>ART</td>
<td>Anti-retroviral therapy</td>
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<td>AWM</td>
<td>Attention / working memory cognitive domain</td>
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<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
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<tr>
<td>CAHIV+</td>
<td>Cognitively asymptomatic HIV+</td>
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<tr>
<td>cART</td>
<td>Combined anti-retroviral therapies</td>
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<td>CDC</td>
<td>Centers for Disease Control</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>CVLT-II</td>
<td>California Verbal Learning Test, 2nd edition</td>
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<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<td>FA</td>
<td>Fractional anisotropy</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>gp120</td>
<td>Envelope glycoprotein GP120</td>
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<td>HAD</td>
<td>HIV-associated dementia</td>
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<td>HAND</td>
<td>HIV-associated neurocognitive disorders</td>
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<td>HAART</td>
<td>Highly active antiretroviral therapies</td>
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<td>HDS</td>
<td>HIV-dementia screening scale</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>HIVE</td>
<td>Human Immunodeficiency Virus encephalitis</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>HIV-</td>
<td>Human Immunodeficiency Virus seronegative</td>
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<td>HIV+</td>
<td>Human Immunodeficiency Virus seropositive</td>
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<tr>
<td>iEEG</td>
<td>Intracranial electroencephalography</td>
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<td>IOG</td>
<td>Inferior occipital gyrus</td>
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<td>MEG</td>
<td>Magnetoencephalography</td>
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<td>MGH</td>
<td>Massachusetts General Hospital</td>
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<td>MD</td>
<td>Mean diffusibility</td>
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<td>MND</td>
<td>Mild neurocognitive disorder</td>
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<td>MLR</td>
<td>Memory (learning and recall) cognitive domain</td>
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<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>OFA</td>
<td>Occipital face area</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>sMRI</td>
<td>Structural magnetic resonance imaging</td>
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<tr>
<td>STG</td>
<td>Superior temporal gyrus</td>
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<td>STS</td>
<td>Superior temporal sulcus</td>
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<tr>
<td>TSPO</td>
<td>18 kDa translocator protein</td>
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<td>VFL</td>
<td>Verbal fluency / language cognitive domain</td>
</tr>
<tr>
<td>WAIS-III</td>
<td>Wechsler Adult Intelligence Scale, 3rd edition</td>
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<td>WM</td>
<td>White matter</td>
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CHAPTER ONE

General Introduction

OVERVIEW

There are currently 32.1 million adults and children living with Human Immunodeficiency Virus (HIV) worldwide. These include an approximate 48,000 individuals who are newly infected here in North America each year (UNAIDS, 2013). Since the advent of highly active antiretroviral therapies (HAART), the incidence of HIV-associated dementia (HAD) has dropped dramatically (Sacktor et al., 2002). However, the prevalence of less severe HIV-associated neurocognitive impairment continues to rise as HIV+ individuals are living longer lives by taking advantage of new medicinal therapies (Cysique & Brew, 2009). Given the wide range of cognitive impairments seen in HIV-positive patients, the term HIV-associated neurocognitive disorders (HAND) was coined to encapsulate them all (McArthur, Steiner, Sacktor, & Nath, 2010).

These impairments have been related to the neurodegenerative pathology of HIV, as triggered by the influx of HIV-infected monocytes and macrophages into the Central Nervous System (CNS), a condition known as HIV encephalitis (HIVE) (Budka, 1991; Gendelman, Lipton, Tardieu, Bukrinsky, & Nottet, 1994). While combined antiretroviral therapies (cARTs) have helped to reduce the incidence HAD, they are unable to altogether eliminate HIV infection in the CNS
(Masliah, DeTeresa, Mallory, & Hansen, 2000). This is not surprising given that the majority of antiretroviral medications do not penetrate the blood-brain barrier (Letendre et al., 2008). Additionally, while cART can control replication of the virus, latent forms of HIV that become integrated into the host’s genome have been detected in the CNS (Jordan, Bisgrove, & Verdin, 2003). It appears as though the brain actually serves as a reservoir for those latent strains of the virus. In the presence of the appropriate stimulus, latently infected cells will reactivate and begin producing infectious versions of the virus (Alexaki, Liu, & Wigdahl, 2008; Redel et al., 2009). As a result, the neurodegeneration caused by HIVE continues to progress slowly over the course of many years (Cook et al., 2005; McArthur, 2004).

It has been well established that HIV targets subcortical regions with particular affinity for the basal ganglia and fronto-striatal networks (J. R. Berger & Nath, 1997). This results in the presence of HIV-infected macrophages, microglia, and multinucleate giant cells in basal ganglia nuclei (Brew, Rosenblum, Cronin, & Price, 1995), caudate atrophy (Hestad et al., 1993; Stout et al., 1998), and basal ganglia hypermetabolism (Hinkin et al., 1995; van Gorp et al., 1992). More recently, neuroimaging studies have shown that the cortex and white matter pathways are impacted as well (Masters & Ances, 2014). However, it was unknown whether these changes occur in HIV+ patients without HAND.

The purpose of this thesis was to examine the relationship between HIV-related structural brain changes in the white matter and cortex,
neuropsychological performance on tests sensitive to HAND identification, and immunological characteristics in a group of cognitively asymptomatic HIV+ (CAHIV+) patients without HAND as compared to well-matched control participants (HIV-). In three distinct, but related investigations, neuroimaging techniques and neuropsychological testing were employed to understand this relationship. The studies described here focused on CAHIV+ without HAND as determined by widely accepted neuropsychological performance guidelines from the National Institute of Mental Health and National Institute of Neurological Diseases and Stroke (Antinori et al., 2007). Studying this relationship contributes to our understanding of the neurological compromise associated with HIV-infection and highlights the brain regions most susceptible in the cognitively asymptomatic stages of the disease. Given that nearly 50% of all HIV-infected individuals suffer from some form of HAND (Griffin, Kang, Ma, & Zhang, 2015), such knowledge is clinically useful given the continuous development of and need for novel antiretroviral medications (Asahchop, Wainberg, Sloan, & Tremblay, 2012).

The results of the first two experiments, detailed in chapters two and three of this dissertation, provide evidence for structural changes that take place in the brain as a result of HIV-infection in cognitively asymptomatic HIV+ patients. The third study, found in chapter four of this thesis, examines the relationship between these structural alterations, neuropsychological performance, and HIV-specific immunological markers. Together, results from these three studies
suggest that regional white matter and cortical alterations identified in patients without HAND may contribute to the cognitive deficits seen in later stages of HIV disease.

The focus of this introductory chapter is to provide the necessary theoretical background on which the remainder of this dissertation is based. First, the cellular and molecular mechanisms of HIV as it enters the brain will be detailed. The known neurological damage caused by HIV will then be highlighted in the white matter, cortex, and cognitive domains. The specific aims for each experiment, along with their predictions, follow. Finally, the general methods are outlined.

Chapters two, three, and four encompass the background, methods, results, and discussion sections for each of the three distinct experiments. Chapter five gives a comprehensive conclusion to tie each of the previous studies together while emphasizing the significance of this work.

MOLECULAR AND CELLULAR MECHANISMS OF HIV

Shortly after initial infection, HIV enters the CNS where it primarily invades microglial cells and perivascular macrophages (Albright et al., 1999; He et al., 1997; Wiley, Schrier, Nelson, Lampert, & Oldstone, 1986). The virus latches onto CD4 immunoglobulin receptors found on the surface of those cells while additional chemokine receptors such as CXCR4 and CCR5 allow for similar entry into lymphocytes and monocytes, respectively (Deng et al., 1996; Dittmar et al.,
Once a cell is infected, the HIV structural protein complex gp160 causes the cell membrane to fuse with the membrane of the virus, thus allowing the virus to enter the cell (E. A. Berger, Murphy, & Farber, 1999). Infected lymphocytes and monocytes cross the blood-brain barrier freely and release HIV throughout the CNS (Koenig et al., 1986). Additional proposed mechanisms for spreading the infection throughout the brain include cell-free virus penetration, (N. Q. Liu et al., 2002), release by infected microvascular endothelial cells (Albright, Soldan, & González-Scarano, 2003), and disruption of blood-brain barrier integrity via astrocytes (Eugenin, Clements, Zink, & Berman, 2011).

Once infected, cells can cause synaptic injury and neuronal loss via a number of mechanisms. Some infected cells release neurotoxic chemokines and cytokines such as quinolinic acid, interleukin-1β, and tumor necrosis factor (TNF)-α which increases blood-brain barrier permeability and allows easier passage for HIV into the CNS (Matsumoto et al., 1993; Mocchetti, Bachis, & Avdoshina, 2012). These neurotoxins have a diverse range, affecting white matter and numerous CNS structures (Kaul, Garden, & Lipton, 2001). The release of HIV viral proteins from infected cells can promote toxicity via both direct and indirect neurochemical cascades as well. These viral proteins include the HIV transcription factor Tat (Nath, 2002), envelope glycoprotein GP120 (gp120), and Nef, a protein that aids in disease replication (van Marle et al., 2004). While Nef has been mainly associated with oligodendrocyte dysfunction
(Radja, Kay, Albrecht, & Jolicoeur, 2003), much more is know regarding the roles Tat and gp120 play in neurodegeneration.

When Tat is released from HIV-infected cells, it alters the blood brain barrier (Toborek et al., 2003) and interacts with other proteins to enter additional neurons (Y. Liu et al., 2000). Tat has an excitotoxic effect when it binds with $N$-methyl-$D$-aspartate (NMDA) receptors so that excessive glutamate levels are released by cell, dysregulating neighboring NMDA receptors, and leading to neural injury (Haughey, Nath, Mattson, Slevin, & Geiger, 2001). Tat has been implicated in other neurotoxic events including nitric oxide synthesis (Nath, 1999) and depolarization of mitochondrial membranes (V. Li, Brustovetsky, & Brustovetsky, 2009).

gp120 causes neurotoxicity in a variety of ways as well. Like Tat, gp120 evokes apoptosis via the release of cytokines and glutamate (Kaul & Lipton, 1999). However, it has also been shown to bind directly to chemokine receptors CXCR4 and CCR5 in neurons and astrocytes, respectively, resulting in apoptosis (Hesselgesser et al., 1998; Zheng et al., 1999). While increasing the production of destructive cytokines and glutamate, gp120 has additionally been implicated in the reduction of brain derived neurotrophic factor (BDNF), which is vital for neuronal survival (Nosheny, Bachis, Acquas, & Mocchetti, 2004).

Tat and gp120 have shown selective toxicity to striatal neurons (Brew et al., 1995; Kure, Lyman, Weidenheim, & Dickson, 1990). Additionally, the basal ganglia and hippocampus are rich in NMDA receptors (Monaghan & Cotman,
and dependent upon BDNF for normal functioning (Xie, Hayden, & Xu, 2010; Xu et al., 2000), both of which are targets of HIV proteins. Given the chemical mechanisms of these viral proteins, it is unsurprising that while some general damage to the CNS occurs with HIV infection, the disease does have reliable regional specificity.

HIV PATHOLOGY

Imaging white matter in HIV

In patients with HIV, it has been determined that HIV-infected macrophages and multinucleated giant cells target cerebral white matter (WM) with regional specificity for the corpus callosum and internal capsule (Gosztonyi, Artigas, Lamperth, & Webster, 1994). This leads to diffuse WM pallor that becomes more prominent as the disease advances (Price et al., 1988). A number of radiological studies have demonstrated overall WM volume loss in HIV (Aylward et al., 1993; J. R. Berger & Nath, 1997; Du et al., 2012; Jernigan et al., 1993; Ragin, Storey, Cohen, Epstein, & Edelman, 2004; Stout et al., 1998). Using more nuanced magnetic resonance imaging techniques, HIV-associated changes have been particularly well-documented in the corpus callosum (Chang et al., 2008; Filippi, Ulug, Ryan, Ferrando, & van Gorp, 2001; Hoare et al., 2011, 2012; Leite et al., 2013; Müller-Oehring, Schulte, Rosenbloom, Pfefferbaum, & Sullivan, 2010; Nir et al., 2014; Pfefferbaum et al., 2009; Pfefferbaum, Rosenbloom, Adalsteinsson, & Sullivan, 2007; Schulte, Müller-Oehring, Javitz,
Pfefferbaum, & Sullivan, 2008; Tate et al., 2011; Thurnher et al., 2005; Wright, Heaps, Shimony, Thomas, & Ances, 2012; Wu et al., 2006) and to a lesser extent in the corona radiate or internal capsule/centrum semiovale (Hoare et al., 2012; Leite et al., 2013; Nir et al., 2014; Pomara, Crandall, Choi, Johnson, & Lim, 2001; Wright et al., 2012).

For HIV positive participants, altered white matter, as measured by fractional anisotropy (FA) values obtained via Diffusion Tensor Imaging (DTI), has been reported across callosal regions (Hoare et al., 2011, 2012; Leite et al., 2013; X. J. Li et al., 2015; Müller-Oehring et al., 2010; Nir et al., 2014) and specifically in the genu (Corrêa et al., 2015; Filippi et al., 2001; Schulte et al., 2008; V. M. Tang et al., 2015; Thurnher et al., 2005) and splenium (Filippi et al., 2001; Wu et al., 2006). Recently, reduced FA in the corpus callosum demonstrates a significant relationship with duration of infection (Wright et al., 2015), and has been found as early as one hundred days of infection (Ragin et al., 2015).

*Imaging the cortex in HIV*

Early imaging studies utilized qualitative, clinical definitions of atrophy via visual examination of MRI films by neuroradiologists to examine sulcal width in different stages of HIV infection across the cortex. However, there was little uniformity among the results. Some studies reported a greater proportion of participants with an Acquired Immunodeficiency Syndrome (AIDS) diagnosis as
having enlarged sulci when compared to HIV- or pre-AIDS HIV+ participants (Harrison et al., 1998; Poutiainen et al., 1993). Additional work evaluating a large group of HIV+ participants with mild cognitive impairment failed to find any cortical atrophy (Dooneief et al., 1992). It was also reported that the most pronounced cortical atrophy in both neurosymptomatic and symptom-free HIV+ patients was localized within the temporal lobes, though the majority of patients did not have atrophy at all (Elovaara et al., 1990).

Overall cortical atrophy associated with HIVE has been documented using quantitative neuroimaging methods (Chrysikopoulos, Press, Grafe, Hesselink, & Wiley, 1990; Post et al., 1988; Wiley et al., 1991) and pathological assessment (Miller et al., 1997) as well. One postmortem study demonstrated that deceased HIV+ participants possessed increased levels of cerebral spinal fluid and a decreased cortical volume (Archibald et al., 2004). Additionally, medically symptomatic HIV+ men demonstrated a more rapid loss of cortical tissue than asymptomatic HIV+ or seronegative individuals (Stout et al., 1998). Studies examining regional volume loss have reported significant reduction of posterior gray matter in HIV+ participants suffering from dementia as compared to HIV+ patients without HAD and HIV- individuals (Aylward et al., 1995) and local cortical thinning in primary sensory, motor, and premotor cortex in AIDS patients with cognitive impairment was found to be related to low CD4 count (Thompson et al., 2005).

More recent studies have assessed cortical thickness in HIV+ patients and
identified atrophy in the anterior cingulate and temporal cortices (Küper et al., 2011), posterior and inferior temporal lobes, parietal lobes, and in the cerebellum (Becker et al., 2012). However, another group found no significant differences in cortical thickness between HIV+ patients and control participants (Corrêa et al., 2016). Finally, a reduction in cortical thickness in the bilateral insula, orbitofrontal and temporal cortices and right superior frontal cortex in patients using HAART, with detectable HIV-deoxyribonucleic acid (DNA) in peripheral blood, compared with HIV patients using HAART without HIV-DNA detected in peripheral blood has been documented as well (Kallianpur et al., 2012).

COGNITIVE IMPAIRMENT IN HIV

There is an extensive body of literature suggesting that fronto-striatal dysfunction is associated with HIV (Chang et al., 2001; Ernst, Chang, & Arnold, 2003; Hinkin et al., 2002; Llorente et al., 1998; Martin et al., 2001; Sahakian et al., 1995; Stout et al., 1995; Villa et al., 1996). A number of studies have reported cognitive deficits in the asymptomatic adult HIV+ population defined as infection with HIV prior to receiving either a diagnosis of AIDS, medically symptomatic HIV status, or cognitively symptomatic HIV status. The characteristic neuropsychological symptomatology includes working memory dysfunction (Chang et al., 2001; Ernst, Chang, Jovicich, Ames, & Arnold, 2002; Hinkin et al., 2002; Martin et al., 2001; Stout et al., 1995), higher order attentional disturbances with a lack mental flexibility (Sahakian et al., 1995), general motor
and psychomotor speed decreases (Llorente et al., 1998; Villa et al., 1996), and cognitive slowing culminating as a decrease in reaction time (Karlsen, Reinvang, & Frøland, 1992; Law et al., 1995; Mellgren et al., 2000) especially in tasks requiring response inhibition (Hinkin, Castellon, Hardy, Granholm, & Siegle, 1999; Llorente et al., 1998). More recently, visuospatial difficulties have been identified as well (Bogdanova, Neargarder, & Cronin-Golomb, 2008; Olesen, Schendan, Amick, & Cronin-Golomb, 2007).

In an effort to standardize the criteria used in the classification of HAND for HIV+ patients, testing the following set of neuropsychological domains has been recommended by the National Institute of Mental Health and National Institute of Neurological Diseases and Stroke: Verbal/language, attention/working memory, abstraction/executive function, memory (learning and recall), speed of information processing, and motor skills (Antinori et al., 2007). The same group proposed a z-score based algorithm to assist in standardized diagnostic classification of HAND and clarified three different levels of impairment: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-1-associated dementia (HAD).

**SPECIFIC AIMS OF THIS THESIS**

The occurrence and onset of damage to cortical regions and white matter pathways in cognitively asymptomatic HIV+ individuals has yet to be fully defined. The main objective of this thesis is to examine HIV-related structural
brain changes in a group of cognitively asymptomatic HIV patients as compared to well-matched control participants using structural magnetic resonance imaging (MRI) techniques. This was achieved by utilizing neuroimaging and neuropsychological methods in three independent but interrelated experiments.

In Experiment #1, DTI was employed to examine white matter alterations in cognitively asymptomatic individuals with HIV. In Experiment #2, cortical thickness measurements calculated from Magnetic Resonance Imaging scans were used to examine structural changes in the cerebral cortex in cognitively asymptomatic individuals with HIV. In Experiment #3, neuropsychological measures were used to characterize the cognitive profile of each participant in order to examine the relationship between the imaging measures collected in Experiments #1 and #2, cognitive performance on a series of neuropsychological tests, and immunological characteristics.

Experiment 1: Examination of white matter alterations in cognitively asymptomatic individuals with HIV using Diffusion Tensor Imaging (Chapter 2)

RATIONALE. Diffuse WM pallor is one of the most prominent features of advanced HIV disease (Price et al., 1988) and its severity has been associated with HAD (Gray et al., 1996). However, few studies have used DTI to examine WM in cognitively asymptomatic patients living with HIV. Previous DTI studies concluded that the method is sensitive enough to detect subtle WM changes
associated with HIV. Whole brain differences (Ragin et al., 2004) and regional 
alterations in the corpus callosum (Chang et al., 2008; Filippi et al., 2001; Hoare 
et al., 2011, 2012; Leite et al., 2013; Müller-Oehring et al., 2010; Nir et al., 2014; 
Pfefferbaum et al., 2009, 2007; Schulte et al., 2008; Tate et al., 2011; Thurnher 
et al., 2005; Wright et al., 2012; Wu et al., 2006), frontal WM (Pomara et al., 
2001; Thurnher et al., 2005; Wu et al., 2006), internal capsule (Pomara et al., 
2001), and hippocampus (Thurnher et al., 2005) were noted in the HIV groups as 
well. Many of these previous investigations did not carry out a full 
neuropsychological assessment using the updated criteria for HAND to exclude 
HIV+ participants with ANI, MND, or HAD (Filippi et al., 2001; Hoare et al., 2012; 
Leite et al., 2013; Müller-Oehring et al., 2010; Pfefferbaum et al., 2009, 2007; 
Ragin et al., 2004; Schulte et al., 2008; Tate et al., 2010; Thurnher et al., 2005; 
Wright et al., 2012). Other studies actively recruited subjects with major cognitive 
deficits or dementia as part of their study (Pomara et al., 2001; Ragin et al., 
2004; Wu et al., 2006) or focused on a specific subset of the HIV+ population 
including the elderly (Nir et al., 2014), children with vertical transmission (Hoare 
et al., 2015), patients with HIV subtype C (Hoare et al., 2011), or newly infected 
patients (Ragin et al., 2015). In the studies that specifically included cognitively 
asymptomatic HIV+ individuals, one only reported the relationship with a global 
cognitive deficit score as opposed to individual domains (Chang et al., 2008). 
Another group used tract-based special statistics (TBSS) to calculate DTI 
measures across whole brain white matter skeletons for HIV-infected participants
with and without cognitive impairment as compared to healthy control participants (Zhu et al., 2013).

This study screened for deficits indicative of dementia using the HIV Dementia Screening Scale (Power et al., 1995) in conjunction with a series of neuropsychological tests containing measures sensitive in detecting early HIV-related cognitive impairments (Heaton, et al. 1995). Following the updated nosology from The National Institute of Mental Health and The National Institute of Neurological Diseases and Stroke (Antinori et al., 2007), HIV+ patients were categorized as cognitively asymptomatic (HIV+CA), having asymptomatic neurocognitive impairment (ANI), having mild neurocognitive impairment (MND), or having HIV-associated dementia (HAD). Only the DTI measures from HIV+CA individuals were used in the comparative analysis with match HIV- control participants. Experiment 1 includes a thorough regional analysis while focusing on WM regions previously associated with HIV-infection including the left, right, and central posterior corpus callosum, central anterior callosum, left and right internal capsule, left and right parietal WM, left and right parietoccipital WM, frontotemporal WM, and left and right temporal WM.

**HYPOTHESIS.** As it is well documented that WM pallor is a prominent feature of advanced HIV (Price et al., 1988) with evidence that its severity is associated with HAD (Gray et al., 1996), it was hypothesized that regional WM tissue alterations occur in patients without HAND. Based on previous imaging studies in HIV, white matter changes were predicted to occur regionally in the
corpus callosum (Chang et al., 2008; Filippi et al., 2001; Hoare et al., 2011, 2012; Leite et al., 2013; Müller-Oehring et al., 2010; Nir et al., 2014; Pfefferbaum et al., 2009, 2007; Schulte et al., 2008; Tate et al., 2011; Thurnher et al., 2005; Wright et al., 2012; Wu et al., 2006), frontotemporal WM (Pomara et al., 2001; Thompson et al., 2005; Thurnher et al., 2005; Wu et al., 2006), internal capsule (Pomara et al., 2001), and parietal WM (Becker et al., 2012; Thompson et al., 2005) of CAHIV+ participants as compared to matched HIV- control participants. This study used voxel-based t-tests to show that these changes occur in patients without HAND.

**EXPERIMENT #1.** Whole-head, high-resolution DTI scans (Siemens, Erlangen Germany, 1.5 T Sonata System; TR=14.4s, TE=81ms, slice thickness = 2mm isotropic, 60 slices total, acquisition matrix 128mm x 128mm (FOV=256mm x 256mm), 5/8 partial Fourier, 6 averages, 6 non-collinear directions with b-value = 700s/mm², and 1 image, the T2 weighted ‘lowb’ image, with b-value = 0s/mm2) were obtained for each participant. Custom tools were used in conjunction with software available as part of the Freesurfer (Martinos Center for Biomedical Imaging, Charlestown, Mass) and FSL (FMRIB, Oxford, UK) processing streams. Fractional anisotropy (FA) maps were acquired from the calculated diffusion tensor (Pierpaoli & Basser 1996).

**Experiment 2: Examination of structural changes in the cerebral cortex in cognitively asymptomatic individuals with HIV using cortical thickness**
measurements calculated from Magnetic Resonance Imaging scans

(Chapter 3)

RATIONALE. The occurrence and onset of HIV-associated damage to cortical regions and the specific regional patterns of cortical alteration has not yet been fully defined for patients free from HAND. While other authors have previously evaluated cortical thickness of HIV-positive patients and have found diffuse brain atrophy, few studies have use sMRI to calculate cortical thickness in cognitively asymptomatic patients living with HIV. The majority of these previous investigations did not carry out a full neuropsychological assessment using the updated criteria for HAND to exclude HIV+ participants with ANI, MND, or HAD (Becker et al., 2012; Küper et al., 2011; Thompson et al., 2005). Other authors included dementia patients as part of their study (Aylward et al., 1993; Patel et al., 2002), only recruited subjects over 40 (Kallianpur et al., 2012) or 50 (Becker et al., 2012) years old, or focused on patients with executive function deficits (Corrêa et al., 2016).

As in Experiment 1, this study screened for deficits indicative of dementia by using the HIV Dementia Screening Scale (Power, Selnès, Grim, & McArthur, 1995) in conjunction with a series of neuropsychological tests containing measures sensitive in detecting early HIV-related cognitive impairments (Heaton et al., 1995) that followed the recommendations from The National Institute of Mental Health and The National Institute of Neurological Diseases and Stroke for HAND classification (Antinori et al., 2007). In experiment 2 cortical thickness
measures were compared between CAHIV+ and HIV- participants to determine whether cortical atrophy is present when HAND is not. Using automated surface reconstruction procedures to measure the thickness of the cerebral cortex from high-resolution magnetic resonance (MR) images (Fischl and Dale, 2000) allowed for the identification of regional cortical changes in CAHIV+ individuals by providing regionally specified measurements of atrophy across the cortical mantle in regions previously associated with HIV-induced damage.

**HYPOTHESIS.** Based on the findings from previous investigations of cortical thickness studies in the HIV+ population, it was hypothesized that patterns of reduced cortical thickness will be apparent in CAHIV+ participants in comparison with HIV- control participants. Specifically, this reduced thickness was predicted to be regionally apparent in posterior areas (Aylward et al., 1995), cortex surrounding the central sulcus (Kallianpur et al., 2012; Thompson et al., 2005), the temporal (Becker et al., 2012; Elovaara et al., 1990; Kallianpur et al., 2012; Küper et al., 2011) and parietal lobes (Becker et al., 2012). This pattern of cortical degeneration was predicted to be evident in patients free from HAND.

**EXPERIMENT #2.** High-resolution T1-weighted MPRAGE scans were obtained on a 1.5 Tesla Siemens Sonata scanner (Imaging parameters: resolution 1.3 x 1.3 x 1 mm, echo time (T_E) = 3.2 or 3.0 ms, repetition time (T_R) = 2730 ms, inversion time (T_I) = 1000 ms, flip angle = 7°, field of view (FOV) = 256x256 mm, matrix = 192x256). Two scans were collected, motion corrected,
and averaged to create a single high-signal, high-contrast volume for each participant.

The averaged volume was then used to create representations of the gray matter/white matter boundary for each participant (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993). Cortical thickness measurements were then calculated by measuring the distance between gray matter/white matter and gray matter/cerebrospinal fluid surfaces at each surface vertex across the cortical mantle before being mapped onto the inflated surface of each participant (Dale & Sereno, 1993; Fischl, Sereno, & Dale, 1999).

**Experiment 3: Regional white and gray matter alterations are related to performance measures of HIV-associated neurocognitive disorder (Chapter 4)**

*RATIONALE.* Cognitive change in HIV has previously been associated with cortical atrophy, white matter alterations, and immunological markers of HIV infection. Cortical atrophy in HIV+ participants has been associated with slowed information processing (Harrison et al., 1998) and poor memory test performance (Harrison et al., 1998; Poutiainen et al., 1993). Whole brain DTI measurements (Ragin et al., 2004) and regional DTI measures (Wu et al., 2006; Zhu et al., 2013) correlate with cognitive deficits in HIV patients as well. Low current and nadir CD4 counts have been associated with cortical alterations in HIV+ patients (Jernigan et al., 2011; Thompson et al., 2005), while higher CD4 counts have
been related to higher FA values in the parietal lobe (Gongvatana et al., 2011).

Relationships between cortical thickness, WM FA, and immunological characteristics had not yet been examined together in CAHIV+ patients. Therefore, the overall goal of this study was to yield valuable information regarding the extent to which structural imaging variables predict cognitive performance in patients free from HAND.

**HYPOTHESIS.** It was hypothesized that regional FA values, cortical thickness, and viral load would be related to neuropsychological composite scores for CAHIV+ individuals, but not HIV- control participants. The HIV-associated alterations in cortical ROIs and DTI FA values are predicted to correlate significantly with one another in CAHIV+ participants as well. Even with no evidence of cognitive decline in HIV+CA participants, regression analyses are predicted to reveal relations between structural measurements and neuropsychological performance in CAHIV+ participants when compared with the HIV- control participants.

**EXPERIMENT #3.** For all participants, volumes of the cortical ROIs and DTI ROI FA values were correlated with neuropsychological test performance within SPSS Statistics, version 23.0. In the HIV+ patients, current CD4 count, duration of illness, and viral load were correlated with the other variables as well. Finally, to identify whether cortical thickness, WM integrity, and immunological
markers of HIV-infection affect neuropsychological performance, stepwise multivariate linear regressions were carried out within SPSS as follows:

\[
\text{CD4 + viral load + duration of illness + Cortical ROI thickness + DTI ROI FA values } \rightarrow \text{Neuropsychological Performance}
\]

Cortical ROIs and DTI ROI FA values were calculated Experiments 2 and 1, respectively. Neuropsychological Performance was represented by six cognitive composite scores that are recommended for categorization of HAND (Antinori et al., 2007) and were computed for this analysis: Verbal Fluency/Language, Attention/Working Memory, Abstraction/Executive Function, Memory (Learning and Recall), Speed of Information Processing, and Motor Skills. The derivation of these scores has been described in detail in a previous report (Castelo et al., 2007).

**SIGNIFICANCE**

As HIV+ positive individuals are living longer lives thanks to improvements in cART, the prevalence of HIV-associated neurocognitive disorders is on the rise. Cognitive impairments including working memory dysfunction (Chang et al., 2001; Ernst et al., 2003; Hinkin et al., 2002; Martin et al., 2001; Stout et al., 1995), difficulty with set-shifting (Sahakian et al., 1995), decreased motor and psychomotor speed (Llorente et al., 1998; Villa et al., 1996), increased reaction time (Karlsen et al., 1992; Law et al., 1995; Mellgren et al., 2000), and poor response inhibition (Hinkin et al., 1999; Llorente et al., 1998) challenge
individuals that suffer from HAND. As these types of impairments are associated with damage to the fronto-striatal networks (J. R. Berger & Nath, 1997) and HIV is known to target the basal ganglia soon after infection (Brew et al., 1995), it has been hypothesized that HIV-related cognitive dysfunction is related to degeneration that begins prior to the onset of cognitive decline. The included studies focus on cognitively asymptomatic HIV+ individuals as characterized by a series of neuropsychological tests containing measures sensitive in detecting early HIV-related cognitive impairments (Heaton et al., 1995) in conjunction with the HIV Dementia Screening Scale (Power et al., 1995) to identify and exclude patients with deficits indicative of cognitive impairment (Antinori et al., 2007).

This research is a valuable extension to the existing body of literature on structural changes in HIV as it would be the first to examine all facets of the cognitively asymptomatic HIV+ brain – the cortex, WM, and cognitive performance together. The experiments additionally investigate the relationship between the structural changes that occur in the absence of HAND and the already well researched cognitive alterations associated with HIV infection. Knowing if and how the changes in the cortex and WM are related is key to understanding how HIV compromises the healthy brain. Studying this relationship contributes to our understanding of the neurological compromise associated with HIV-infection and highlights the brain regions most susceptible in the cognitively asymptomatic stages of the disease. Given that nearly 50% of all HIV-infected individuals suffer form some form of HAND (Griffin et al., 2015), such knowledge
is additionally clinically useful given the continuous development of and need for novel antiretroviral medications (Asahchop et al., 2012).

**GENERAL METHODS**

Detailed methods for experiments 1, 2, and 3 are provided in chapters two, three, and four, respectively. General methods that pertain to all of the experiments are reviewed in the following section.

**Participant Recruitment and Screening**

All participants were recruited from various community health centers, adult education centers, local universities, and advertisements in neighborhood newspapers. HIV+ patients and control participants were matched for gender, age, education, verbal IQ, and handedness. All participants were between the ages of 22 and 60 years as previous experiments in our laboratory have found that age range to be optimal for study of cognitively asymptomatic HIV. Potential participants were given an extensive health questionnaire and screened over the telephone. They were invited to take part in the study if all initial inclusion criteria as described below were met. HIV+ patients provided consent for their doctors to release medical records of their current CD4 count and plasma viral load. Subjects received $50 for approximately one half hour of scanning and $25 for approximately two hours of neuropsychological testing. In addition, HIV+
patients were provided with radiological films resulting from fifteen additional minutes of clinical scans, if requested.

Exclusion criteria on the health screening questionnaire included a history of intravenous drug use, alcohol or drug dependence, history of fungal infection of the neurological system, history of psychiatric illness, head injury with loss of consciousness exceeding 30 minutes, history of learning disability, history of psychosis, a history of neurological illness, or any abnormalities seen on a previous MRI or CT of the head. Due to the MRI scanning procedure, participants with metal in the body, claustrophobia, or Ménière’s disease were also excluded. HIV+ patients were excluded if they had previous neurological complications associated with HIV infection including cryptococcal meningitis or toxoplasmosis. HIV+ participants disclosed diagnosis of HIV infection via self-report based on a blood serum assay given by their physician. Medicinal regimen was also collected via self-report and confirmed with the patient’s physician when possible. All HIV+ participants were given the HIV Dementia Screening Scale (Power et al., 1995) in conjunction with a series of neuropsychological tests containing measures sensitive in detecting early HIV-related cognitive impairments (Heaton et al., 1995) to identify and exclude patients with deficits indicative of neurocognitive disorder.

Participants gave consent to participate in the study with approval from the Boston University and Partners Human Research Committees upon arrival at Boston University. All participants were provided written informed consent prior to
the neuropsychological testing that took place at the Cognitive Neuroimaging Laboratory at Boston University. If HIV+ participants were unable to bring current immunological information from their physician with them to the testing, they were asked to fill out an additional consent form so the information could be obtained from their doctor. MRI scanning took place at the Athinoula A. Martinos Center for Biomedical Imaging in Charlestown, MA within one month of the neuropsychological testing if the participant met all cognitive inclusionary criteria. The structural MRI scans necessary for obtaining cortical thickness measurements were collected in the same session as the DTI scan.

**Neuropsychological Testing**

All participants underwent a series of neuropsychological tests containing measures sensitive in detecting early HIV-related cognitive impairments (Heaton et al., 1995). Presence of cognitive impairment, Verbal Fluency/Language, Attention/Working Memory, Abstraction/Executive Function, Memory (Learning and Recall), Speed of Information Processing, and Motor Skills were examined along with estimated pre-morbid verbal intelligence as determined by performance on the National Adult Reading Test (Blair & Spreen, 1989).

*Cognitive Impairment Assessment.* HIV-positive participants were evaluated for HIV-associated neurocognitive disorders (HAND) based on the revised research criteria outlined in Antinori et al., 2007. HIV-associated asymptomatic neurocognitive impairment (ANI) was documented by performance
of at least 1.0 standard deviation (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores. HIV-1-associated mild neurocognitive disorder (MND) was documented by performance of at least 1.0 standard deviations (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores along with patient self-report of reduced mental acuity. HIV-1-associated dementia (HAD) was documented by performance of at least 2.0 standard deviations (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores or greater than 2.5 SD below normative data on 1 domain and greater than 1 SD below on another. Additionally, all HIV+ participants were administered the HIV Dementia Screening Scale (Power et al., 1995) and excluded from the study if they received a score below 10, which is indicative of dementia.

**Verbal Fluency and Language.** Participants were administered the Boston Naming Test (Kaplan, Goodglass, & Weintraub, 1983) and Category Fluency Test (Animals, Spreen and Strauss, 1998) to assess verbal fluency and language.

**Attention/ Working Memory.** Participants were administered the third edition of the Wechsler Adult Intelligence Scale (WAIS-III). The WAIS-III Letter-Number Sequencing Subtest, WAIS-III Arithmetic Subtest, and WAIS-III Digit Span Subtest were used to assess attention and working memory (Wechsler, 1997).
Abstraction/Executive Function. In order to assess abstraction and executive function, participants were administered the Controlled Oral Word Association Test (FAS) (Benton & Hamsher, 1983; Gladsjo et al., 1999), the WAIS-III Matrix Reasoning Subtest (Wechsler, 1997), and Trails B of the Trailmaking Test (Army Test Battery, 1944; Reitan, 1992).

Memory (Learning and Recall). The second edition of the California Verbal Learning Test (CVLT-II) was administered to participants as it has been shown to be sensitive in assessing HIV-related cognitive decline (Peavy et al., 1994) through single-item supraspan list learning. CVLT-II Learning trials 1-5 and CVLT-II immediate and delayed recall tasks were used to assess memory (Delis, Kramer, Kaplan, & Ober, 2000).

Speed of Information Processing. In order to assess information processing speed, participants were administered Trails A of the Trailmaking (Army Test Battery, 1944; Reitan, 1992) and the WAIS-III Digit Symbol and Symbol Search Subtests, (Wechsler, 1997).

Motor Speed. Previous studies have documented that the Grooved Pegboard is sensitive to HIV-related motor slowing (Davis, Skolasky, Selines, Burgess, & McArthur, 2002) while the Finger Tapping Test from the Halstead-Reitan Battery is sensitive to HIV-related reaction time increases even in early stages of cognitive impairment (Hardy & Hinkin, 2002). Both tests were administered to all participants.
Depression and Anxiety Assessment. All participants were administered the Beck Depression Inventory-II and the Beck Anxiety Inventory (Beck, Brown, Berchick, Stewart, & Steer, 1990; Beck, Guth, Steer, & Ball, 1997) to screen for mood changes associated with HIV.

General Imaging Methods

All participants who met the cognitive criteria after neuropsychological testing were scanned on a 1.5 Tesla Siemens Sonata System at the Martinos Center for Biomedical Imaging in Charlestown, MA. Participants were in the scanner for approximately one half hour for T1 and DTI scanning.
CHAPTER TWO

Experiment 1: Examination of white matter alterations in cognitively asymptomatic individuals with HIV using Diffusion Tensor Imaging

INTRODUCTION

The human immunodeficiency virus (HIV) enters the CNS and can be detected in the brain within two weeks of infection (Krebs, Ross, McAllister, & Wigdahl, 2000). Early studies focused on the impact of the HIV virus on subcortical regions (Budka, 1991; Heindel et al., 1994; Navia, 1997), identifying the basal ganglia as a particular target (Aylward et al., 1993; J. R. Berger & Arendt, 2000; J. R. Berger & Nath, 1997). There is now considerable evidence that the other end of the fronto-striatal network is affected as demonstrated by volumetric loss within frontal lobe regions (Cohen et al., 2010; Pfefferbaum et al., 2014; Thompson et al., 2005; Towgood et al., 2012). Additionally, temporal and parietal cortical areas are compromised (Becker et al., 2012; Cohen et al., 2010; Küper et al., 2011; Pfefferbaum et al., 2014; Thompson et al., 2005; Towgood et al., 2012).

In patients with HIV encephalitis, it has been determined that HIV-infected macrophages and multinucleated giant cells target cerebral white matter (WM) with regional specificity for the corpus callosum and internal capsule (Gosztonyi et al., 1994). This leads to diffuse WM pallor that becomes more prominent as
the disease advances (Price et al., 1988). A number of radiological studies have demonstrated overall WM volume loss in HIV (Aylward et al., 1993; J. R. Berger & Nath, 1997; Du et al., 2012; Jernigan et al., 1993; Ragin et al., 2004; Stout et al., 1998). Using more nuanced magnetic resonance imaging techniques, HIV-associated changes have been particularly well-documented in the corpus callosum (Chang et al., 2008; Filippi et al., 2001; Hoare et al., 2011, 2012; Leite et al., 2013; Müller-Oehring et al., 2010; Nir et al., 2014; Pfefferbaum et al., 2009, 2007; Schulte et al., 2008; Tate et al., 2011; Thurnher et al., 2005; Wright et al., 2012; Wu et al., 2006) and to a lesser extent in the corona radiate or internal capsule/centrum semiovale (Hoare et al., 2012; Leite et al., 2013; Nir et al., 2014; Pomara et al., 2001; Wright et al., 2012).

Diffusion tensor imaging (DTI) allows for excellent distinction among white matter structures and provides a means of collecting non-invasive regional measures of tissue microstructure. DTI measures the three-dimensional magnitude and direction of tissue water mobility, known as self-diffusion. Fractional anisotropy (FA), a scalar metric describing the WM microstructure, is a measurement calculated from DTI data. In brain WM, the diffusion signal is thought to represent diffusion restriction (anisotropy) by the myelinated axonal projections due to hydrophobic lipid-based myelin interacting with water. Lower fiber density or decreased myelination results in less tightly-packed fiber bundles. In this case, water diffusion is less restricted, lowering FA values (Basser, 1995). For HIV positive participants, decreased FA values have been reported across
callosal regions (Hoare et al., 2011, 2012; Leite et al., 2013; X. J. Li et al., 2015; Müller-Oehring et al., 2010; Nir et al., 2014) and specifically in the genu (Corrêa et al., 2015; Filippi et al., 2001; Schulte et al., 2008; V. M. Tang et al., 2015; Thurnher et al., 2005) and splenium (Filippi et al., 2001; Wu et al., 2006). Recently, reduced FA in the corpus callosum has been found as early as the first 100 days of infection (Ragin et al., 2015), while demonstrating a significant relationship with duration of infection (Wright et al., 2015).

Given HIV’s strong affinity for the basal ganglia (J. R. Berger & Arendt, 2000; Chang et al., 2008; Ragin et al., 2005), it is not surprising that FA abnormalities in HIV positive participants have been reported in the fronto-striatal network, including the frontal lobes (J. Li et al., 2015; Pomara et al., 2001; Smith et al., 2008; Stebbins et al., 2007; Thurnher et al., 2005; Zhu et al., 2013). There is mounting evidence that parietal and temporal cortices are affected by HIV-infection as well (Cohen et al., 2010; Küper et al., 2011; Thompson et al., 2005). Altered diffusion measures (Chen et al., 2009) and FA values (Chang et al., 2008; Gongvatana et al., 2011; J. Li et al., 2015; Stebbins et al., 2007) have been reported in the parietal lobes of HIV positive individuals while temporal lobe FA increases have been correlated with use of antiretroviral therapies (Gongvatana et al., 2011). A recent longitudinal study of the Simian immunodeficiency virus (SIV) also found that decreased FA in inferotemporal regions correlated significantly with elevated blood CD4+/CD8+ ratios in primates (Z. Tang et al., 2015).
The purpose of the present study was to investigate white matter alterations in cognitively asymptomatic HIV-positive individuals as compared to HIV- control participants. Using a hypothesis driven approach, ROI analysis was limited to those WM regions previously shown to be most impacted by HIV infection. All participants underwent a neuropsychological battery that followed the revised research criteria for the identification of HAND (Antinori et al., 2007). The assessment examined the following cognitive domains: verbal fluency/language, attention/working memory, abstraction/executive function, memory (learning and recall), speed of information processing, and motor skills. Based on previous studies, we measured FA within the corpus collosum, internal capsule, parietal lobe, parieto-occipital, temporal lobe, and fronto-temporal WM. We hypothesized that there would be evidence of WM injury in all of these regions for the cognitively asymptomatic HIV-positive group in the form of decreased FA measures.

METHODS

Participants

Seventeen cognitively asymptomatic HIV+ individuals without HAND as determined by widely accepted neuropsychological performance guidelines (CAHIV+), seventeen HIV-negative control participants matched for age, education, and verbal IQ, (HIV-) and six HIV-positive individuals with measurable cognitive impairment (HAND) for secondary analyses were analyzed in this
study. All participants provided informed consent, following procedures that were approved by the Partners Human Research Committee, which oversees human research at the Athinoula A. Martinos Center for Biological Imaging (site of DTI imaging) and Boston University (site of neuropsychological testing and data analysis) institutional review board (IRB). Participants were recruited via advertisements in local newspapers and flyers posted at community health centers. Women were not excluded from the study, however due to the demographics of the local HIV population, all participants that met our inclusion criteria were men. Participants represented Caucasian, African American, Asian, and Hispanic ethnicities. Exclusion criteria for all participants included a history of intravenous drug use, alcohol or drug dependence, history of fungal infection of the neurological system, history of psychiatric illness other than major depression, head injury with loss of consciousness exceeding 30 minutes, history of learning disability, history of psychosis, a history of neurological illness, or any abnormalities seen on a previous MRI or CT of the head. Participants with metal in the body, claustrophobia, or Meniere's disease were also excluded. Additional exclusionary criteria for HIV-positive participants included any previous HIV-related Central Nervous System infections secondary to immunodeficiency. Each participant underwent neuropsychological testing (described below), followed by the MRI protocol (described below) within one month. The cognitively asymptomatic HIV+ and HIV- groups were matched for age, education, and pre-morbid verbal intelligence as determined by performance on the National Adult
Reading Test (Blair & Spreen, 1989) as summarized in Table 2.1. The smaller cognitively symptomatic HIV+ HAND group was subsequently matched on these same measures with a subset of the cognitively asymptomatic HIV+ population as summarized in Table 2.2.

**Immunological information**

CD4 cell count (cells/mL), plasma viral load (log RNA/mL), and medication status were obtained, with consent, from participant medical records. Medication status was characterized as HAART (two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor, or two NRTIs and a nonnucleoside reverse transcriptase inhibitor (NNRTI), non-HAART, or none. CD4 counts were unavailable for 4 participants and plasma viral load was unavailable for 5 participants. CD4 counts, plasma viral loads, and percentage of patients in each medication class is summarized in Table 1 for cognitively asymptomatic HIV-positive subjects and in Table 2 for HIV-positive subject with HAND.

**Neuropsychological evaluation**

Within one month of the MRI, all subjects underwent a neuropsychological battery that followed the revised research criteria for the identification of HAND (Antinori et al., 2007). Verbal/language, attention/working memory, abstraction/executive function, memory (learning and recall), speed of
information processing, and motor skills were examined as well as performance on the National Adult Reading Test (Blair & Spreen, 1989) to estimate verbal intelligence. Additionally, all HIV-positive participants were administered the HIV Dementia Scale (Sacktor et al., 2005). The following six cognitive domain composite scores were computed for this analysis: verbal fluency and language (Boston Naming Test, BNT (Kaplan et al., 1983); Category Fluency Test (Animals (Spreen & Strauss, 1998)), attention/ working memory (WAIS-III Letter-Number Sequencing Subtest, WAIS-III Arithmetic Subtest, WAIS-III Digit Span Subtest (Wechsler, 1997)), abstraction/ executive function (Controlled Oral Word Association Test (FAS) (Benton & Hamsher, 1983; Gladsjo et al., 1999); WAIS-III Matrix Reasoning Subtest (Wechsler, 1997), Trailmaking Test Part B (Army Test Battery, 1944; Reitan, 1992)), memory learning and recall (California Verbal Learning Test I learning trials 1-5, immediate and delayed recall, (Delis et al., 2000), speed of information processing (Trailmaking Test Part A, (Army Test Battery, 1944; Reitan, 1992)) and WAIS-III Digit Symbol and Symbol Search Subtests, (Wechsler, 1997)), and motor speed (Grooved Pegboard ((Davis et al., 2002)) and Finger Tapping Tests dominant and nondominant (Hardy & Hinkin, 2002). Cognitive composite scores were computed using previously published methods from our group (Castelo, Courtney, Melrose, & Stern, 2007). Briefly, all raw scores were converted to z scores based on published normative data. The z scores for the individual tests from each cognitive domain were then averaged to compute a single composite score per person.
HIV-positive participants were evaluated for HIV-associated neurocognitive disorders (HAND) based on the revised research criteria outlined in Antinori et al., 2007. HIV-associated asymptomatic neurocognitive impairment (ANI, n=2) was documented by performance of at least 1.0 standard deviations (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores. HIV-1-associated mild neurocognitive disorder (MND, n=1) was documented by performance of at least 1.0 standard deviations (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores along with patient self-report of reduced mental acuity. HIV-1-associated dementia (HAD, n=3) was documented by performance of at least 2.0 standard deviations (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores or greater than 2.5 SD below normative data on 1 domain and greater than 1 SD below on another. Collectively these 6 subjects formed an exploratory analysis group, HAND, for ROI comparison with the remainder of HIV-positive subjects classified as cognitively asymptomatic (CAHIV+).

**MRI scan protocol**

Whole-head, high-resolution DTI scans (Siemens, Erlangen Germany, 1.5 T Sonata System; TR=14.4s, TE=81ms, slice thickness = 2mm isotropic, 60 slices total, acquisition matrix 128mm x 128mm (FOV=256mm x 256mm), 5/8 partial Fourier, 6 averages, 6 non-collinear directions with $b$-value = 700s/mm$^2$,
and 1 image, the T2 weighted ‘lowb’ image, with $b$-value = 0s/mm²) were obtained for each participant. DTI acquisition utilized an eddy-current suppressed echo pulse sequence to significantly reduce distortions (Reese, Heid, Weisskoff, & Wedeen, 2003).

**DT Image analysis**

Custom diffusion tools developed at the Martinos Center for Biomedical Imaging were used in conjunction with software available as part of the Freesurfer (http://surfer.nmr.mgh.harvard.edu) and FSL (http://fmrrib.ox.ac.uk/fsl) processing streams for image processing and statistical analysis. A multistep pre-processing procedure was performed on the DTI data for each participant to correct for motion and distortion, calculate FA maps, and spatially transform T2-weighed lowb volumes to a lowb template in normalized space based on MNI/Talairach space developed at the Martinos Center using similar diffusion sets. FA maps were then resampled using the newly created lowb transform.

T2-weighed lowb structural volumes were collected using the same sequence parameters as the directional volumes. This allowed each directional volume from the diffusion dataset to be resampled to the lowb image to correct for eddy current distortion or participant movement in the scanner. This was achieved using the FMRIB’s Linear Image Registration Tool (FLIRT) as a part of the FSL analysis stream. The diffusion tensor was then calculated for each voxel in the volume using a least-squares fit to the diffusion signal (Basser, Mattiello, &
LeBihan, 1994). FA maps were then acquired from the calculated diffusion tensor (Pierpaoli & Basser, 1996). This analysis stream replicated methods previously used to study WM alterations associated with age (Salat, Tuch, Greve, et al., 2005; D. H. Salat, Tuch, Hevelone, et al., 2005), Huntington’s Disease (Rosas et al., 2006), and cerebral amyloid angiopathy (Salat et al., 2006).

Whole Brain Maps

Exploratory voxel based analyses were performed to examine the regional distribution of HIV-related FA alterations and to guide additional ROI analyses. Spatially normalized FA maps were smoothed using a 3D spatial smoothing filter with a full width half maximum of 4 mm and compared by voxel based $t$-test between the combined HIV+ (asymptomatic and HAND) and HIV- groups. As there is a potential for misalignment in voxel-based volume registration, particularly in patient populations with atrophy, we excluded all voxels outside of WM thresholds set by the trace volume and visually examined each transformed volume carefully as explained in detail elsewhere (Salat, Tuch, Greve, et al., 2005; Salat, Tuch, Hevelone, et al., 2005). Since misregistrations could potentially alter the results, these maps should be considered exploratory and are secondary to the ROI analyses described below.
Regional Analysis

We measured FA in seven ROIs: left, right, and central posterior corpus callosum, central anterior callosum, left and right internal capsule, left and right parietal lobe white matter (WM), left and right parieto-occipital WM, frontotemporal WM, and left and right temporal lobe WM. Uniform ROIs (squares, 9 voxels in size) were manually inserted on the spatially normalized lowb volume for each participant using the Tkmedit function in the Freesurfer package (Illustrated in Figure 2.3). Spatial normalization was achieved by creating a lowb template. First, FSL registration tools were used to perform a mutual information cost function based registration between each participant’s lowb volume and the T2 template provided by the Montreal Neurological Institute (MNI; Montreal, Canada). Next, the normalized volumes were averaged. Each ROI was placed in a region previously associated with HIV-related neurodegeneration using a standardized placement procedure. This uniform square avoided arbitrary sizes of ROIs across participants. ROI analyses were performed blind to participant HIV-status. By studying the FA and tensor maps for each participant, anatomical boundaries were defined for ROI placement in an effort to stay within homogeneous regions of WM fibers and to avoid sampling voxels that included gray matter or cerebral spinal fluid. Left and right posterior corpus callosum ROIs were defined on the axial plane by placing the square where the splenium meets the forceps major in each hemisphere. A central posterior callosum ROI was centered in the splenium on the axial plane. A central anterior callosum ROI
was placed in the same axial slice in the center of the genu. Left and right internal capsule ROIs were defined on the axial plane, lateral to the center of the thalamus. Left and right parietal ROIs were defined in deep WM (at least 3mm from the gray matter/white matter border) in the center of the axial plane just above the corpus callosum. Left and right parieto-occipital ROIs were defined in deep WM in the axial plane of the posterior corpus callosum. Frontotemporal ROIs were defined in the sagittal plane centered and just superior to the corpus callosum. Left and right temporal ROIs were placed in the deep WM in the coronal plane just lateral to the inferior horn of the lateral ventricle.

**Statistical Analysis**

The HIV- (n=17) and cognitively asymptomatic HIV+ (n=17) groups were matched with respect to age, education, estimated verbal I.Q., and handedness (see Table 1). For additional analyses, the cognitively symptomatic HIV+ group (HAND, n=6) was matched to a subset of the asymptomatic HIV+ participants (n=8) with respect to age, education, estimated verbal I.Q., and CD4 count, viral load, and HIV Dementia Scale score (see Table 2). We conducted independent-sample t tests to examine between-group differences in FA for the HIV- and cognitively asymptomatic HIV+ groups as well as the cognitively asymptomatic HIV+ subset and HAND groups.
RESULTS

Visualization of exploratory global FA maps indicated increased FA in a combined group of all HIV positive participants (HIV+ and HAND) across several regions including the posterior callosum and temporal lobes as well as regional FA decreases in the parietal lobe (see Figure 2.1) when compared to the HIV-group. Apparent FA increases on global exploratory maps for all HIV+ in the PCC can be viewed as both group and individual maps in Figure 2.2. Regional FA differences were highly significant in the frontotemporal ROI (p=.00008) where FA was increased for the CAHIV+ group as compared to HIV-. The left, right, and total posterior callosum ROIs were also significant (p=.04, .04, and .02, respectively) for increased FA in the CAHIV+ group as compared with HIV-. The left and right parietal ROIs showed a significant decrease in FA (p=.04, .03, respectively) for CAHIV+ compared to HIV-. All regional FA differences are illustrated in Figure 2.3. No significant FA differences were identified between the HAND group and matched subset of CAHIV+ individuals. Individual FA values for the highly significant frontotemporal ROI are illustrated in Figure 2.4.

DISCUSSION

The current study used DTI methods to detect subtle WM changes associated with HIV infection in a group of patients with well-characterized cognitive status. The cognitively asymptomatic HIV-positive individuals demonstrated higher FA values in the frontotemporal region and posterior callosum.
callosum, but lower FA values in parietal WM when compared with the control group. When combining participants from both HIV-positive asymptomatic and HAND groups, we detected negative associations between left posterior callosum FA and two domains of cognition.

The most significant finding for this study was increased FA in the frontotemporal region for HIV positive participants without HAND when compared to HIV negative individuals. This finding runs contrary to our initial hypothesis and was somewhat unexpected given the nature of anisotropic diffusion. In brain WM, the diffusion signal is thought to represent diffusion restriction (anisotropy) by the myelinated axonal projections due to hydrophobic lipid-based myelin interacting with water. Lower fiber density or decreased myelination would result in less tightly packed fiber bundles, and thus, water diffusion would be less restricted, lowering FA values (Basser, 1995). While this is not the first study to report increased FA in an HIV positive population (Gongvatana et al., 2011; Hoare et al., 2011, 2012; Pomara et al., 2001; Smith et al., 2008; Stebbins et al., 2007; Stubbe-Drger et al., 2012; Thurnher et al., 2005), it is generally assumed that FA will decrease with HIV-associated WM damage. One interpretation is that the FA increase reflects a loss of regional white matter complexity (Stebbins et al., 2007) from damaged crossing and nonparallel WM fibers. With parallel fibers left preserved, increased FA would occur. In a combined gross anatomical and morphometric study of the corpus callosum in HIV-infected brains, swelling of axons and myelin sheaths was seen along with diminished myelin thickness in
other regions (Wohlschlaeger, Wenger, Mehraein, & Weis, 2009). Diminished myelin thickness would likely result in less tightly packed fiber bundles while swelling could potentially spread out WM tracts, decreasing the complexity and resulting in higher FA values for our CAHIV+ group. Other studies have reported elevated gray matter volumes for HIV+ individuals (Castelo et al., 2007; Jernigan et al., 2011) giving the possible explanation of tissue swelling resulting from immune reactivation. It is also important to note a potential role of antiretroviral medications as a cause of increased FA in this region. Gongvatana and colleagues (2011) found that HIV patients undergoing antiretroviral treatment exhibited higher anisotropy specifically in the temporal lobe when compared to patients not on a medicinal regimen.

While frontostriatal compromise has been well documented in HIV (J. R. Berger & Nath, 1997), recent evidence suggests that temporal cortices are affected both functionally and structurally by HIV-infection as well. An fMRI investigation of HIV-positive patients during an episodic encoding task demonstrated reduced signal intensity in the right medial temporal lobe when compared to an HIV- control group (Castelo, Sherman, Courtney, Melrose, & Stern, 2006). Structurally, one study documented loss of gray matter in the anterior cingulate and temporal cortices for HIV positive individuals with neurocognitive impairment (Küper et al., 2011). Another showed an association between gray matter loss in the anterior temporal lobe and neuropsychological performance (Becker et al., 2012). It is unsurprising that underlying temporal lobe
white matter would be altered with HIV-infection in the absence of cognitive impairment. Increased temporal lobe FA has previously been documented in two other DTI studies of HIV, for HIV positive individuals with significant depressive symptoms (Smith et al., 2008) and those undergoing antiretroviral treatment (Gongvatana et al., 2011).

Additional significant FA changes were observed in the posterior corpus callosum and parietal lobe WM for our cognitively asymptomatic HIV+ group as compared to HIV- participants. Several imaging studies have reported WM compromise in the corpus callosum of HIV patients (Chang et al., 2008; Corrêa et al., 2015; Filippi et al., 2001; Hoare et al., 2011, 2012, 2015; Leite et al., 2013; J. Li et al., 2015; Müller-Oehring et al., 2010; Nir et al., 2014; Pfefferbaum et al., 2009, 2007; Ragin et al., 2015; Schulte et al., 2008; V. M. Tang et al., 2015; Tate et al., 2011; Thurnher et al., 2005; Wright et al., 2015, 2012; Wu et al., 2006). Additionally, correlations between corpus callosum alterations and measures of cognitive health have been reported (Hoare et al., 2012; Nir et al., 2014; Schulte et al., 2008; V. M. Tang et al., 2015; Wu et al., 2006; Zhu et al., 2013). The current study demonstrated similar findings with the left, right, and total posterior corpus callosum ROIs showing significant increased FA in the HIV+ group as compared with HIV-. As with the increased FA seen in the frontotemporal ROI, this may be attributed to a loss of regional white matter complexity.

While FA increases were noted in the posterior callosum and frontotemporal WM, left and right parietal lobe ROIs showed a significant
decrease in FA for the cognitively asymptomatic HIV+ group compared to HIV-.

Previous studies have reported altered diffusion measures (Chen et al., 2009) and both reduced (Chang et al., 2008; X. J. Li et al., 2015) and increased (Gongvatana et al., 2011; Stebbins et al., 2007) FA values in the parietal lobes of HIV positive individuals. In a study examining patients at baseline and a one-year follow up, cognitive change in HIV patients as measured by a global deficit score correlated with parietal lobe FA values (Chang et al., 2008). Additionally, neuropsychological evaluations have shown HIV-related impairment in a parietal-dependent mental rotation tasks (Olesen et al., 2007; Weber, Woods, Cameron, Gibson, & Grant, 2010) while an fMRI investigation demonstrated posterior parietal hyperactivity for HIV+ individuals during an episodic encoding task (Castelo et al., 2006).

It is important to note the limitations of this study. First, due to the small sample size and resultant limited power, the results have not been corrected for multiple comparisons. This limitation is offset by restricting our analysis to a priori regions previously associated with HIV-related cognitive decline. It is also worth noting that our results are consistent with prior DTI studies investigating regional HIV-associated alterations (Gongvatana et al., 2011; Hoare et al., 2012; Nir et al., 2014; Schulte et al., 2008; Smith et al., 2008; V. M. Tang et al., 2015; Wu et al., 2006; Zhu et al., 2013). An additional limitation is that while exclusion criteria for all participants included a history of intravenous drug use, alcohol or drug dependence, some subjects included in our study had a history of recreational
illicit drug use, and it has been documented that co-morbid substance abuse (Chang, Ernst, Speck, & Grob, 2005; Jernigan et al., 2005) and alcoholism (Pfefferbaum et al., 2007) may influence HIV-associated brain alterations. Finally, in terms of methodological considerations, we utilized a balanced echo sequence to reduce eddy current distortion and post-processing was used to reduce residual distortion and motion. However, echo-planar scans are susceptible to additional distortions such as reduced signal (Jezzard & Clare, 1999). In an attempt to limit such distortions, regions were only sampled within deep WM. Despite these limitations, our data suggest that increases in frontotemporal and posterior corpus callosum FA and decreases in parietal lobe FA occur in HIV patients, even those that are cognitively asymptomatic.
Table 2.1. Subject demographics for cognitively asymptomatic HIV-positive participants (CAHIV+) and HIV- control participants (HIV-) presented as means with standard deviations. P-values reflect results of independent sample t-tests. NART: National Adult Reading Test.
<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>CAHIV+</th>
<th>(p)-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
<td>N/A</td>
</tr>
<tr>
<td>Age</td>
<td>42.35 ± 9.18 (31-54)</td>
<td>38.17 ± 10.74 (22-59)</td>
<td>0.50</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.06 ± 2.01 (12-18)</td>
<td>15.33 ± 1.97 (12-18)</td>
<td>0.48</td>
</tr>
<tr>
<td>NART Estimated Verbal IQ</td>
<td>119.5 ± 4.27 (107.34-126.03)</td>
<td>117.5 ± 7.3 (106.45-126.92)</td>
<td>0.13</td>
</tr>
<tr>
<td>Handedness</td>
<td>94% Right</td>
<td>94% Right</td>
<td>N/A</td>
</tr>
<tr>
<td>†CD4 count at testing</td>
<td>N/A</td>
<td>688.6 ± 309.9 (249-1433)</td>
<td>N/A</td>
</tr>
<tr>
<td>†Viral load at testing (copies/µl)</td>
<td>N/A</td>
<td>6.27 ± 11.09 (&lt;.05-43.8)</td>
<td>N/A</td>
</tr>
<tr>
<td>HIV Dementia Screening Score</td>
<td>N/A</td>
<td>15.2 ± 1.17 (13-16)</td>
<td>N/A</td>
</tr>
<tr>
<td>HIV Medications</td>
<td>N/A</td>
<td>9 HAART, 1 non-HAART, 7 none</td>
<td>N/A</td>
</tr>
</tbody>
</table>

† Information not available for two participants.
Table 2.2. Subject demographics for HIV-positive participants with HIV-associated neurocognitive disorders (HAND) as compared to a subset of cognitively asymptomatic HIV+ participants (CAHIV+) presented as means with standard deviations. P-values reflect results of independent sample t-tests. NART: National Adult Reading Test, ANI: asymptomatic neurocognitive impairment, MND: mild neurocognitive impairment, HAD: HIV-associated dementia.
<table>
<thead>
<tr>
<th></th>
<th>HAND</th>
<th>CAHIV+</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td>Age</td>
<td>$43 \pm 7.72$ (31-51)</td>
<td>$41.4 \pm 7.63$ (34-54)</td>
<td>0.66</td>
</tr>
<tr>
<td>Education (years)</td>
<td>$13.5 \pm 1.51$ (12-16)</td>
<td>$14 \pm 2$ (12-16)</td>
<td>0.27</td>
</tr>
<tr>
<td>NART Estimated Verbal IQ</td>
<td>$100.96 \pm 5.38$ (93.99-108.23)</td>
<td>$104.85 \pm 10.37$ (86.87-110.9)</td>
<td>0.21</td>
</tr>
<tr>
<td>Handedness</td>
<td>100% Right</td>
<td>75% Right</td>
<td>N/A</td>
</tr>
<tr>
<td>†CD4 count at testing</td>
<td>$646.5 \pm 464.23$ (132-1240)</td>
<td>$621.22 \pm 290.63$ (249-1064)</td>
<td>0.92</td>
</tr>
<tr>
<td>§Viral load at testing (copies/µl)</td>
<td>$248.23 \pm 425.89$ (&lt;.075-740)</td>
<td>$2.87 \pm 4.46$ (&lt;.075-6.99)</td>
<td>0.17</td>
</tr>
<tr>
<td>HIV Dementia Scale Score</td>
<td>$14.58 \pm 1.5$ (12-16)</td>
<td>$15.4 \pm 1.34$ (13-16)</td>
<td>0.54</td>
</tr>
<tr>
<td>HIV Medications</td>
<td>3 HAART, 1 non-HAART, 2 none</td>
<td>4 HAART, 1 non-HAART, 3 none</td>
<td>N/A</td>
</tr>
<tr>
<td>HAND Classification</td>
<td>2 ANI, 1 MND, 3 HAD</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

† Information not available for two HAND, 2 HIV+.

§ Information not available for three HAND, 2 HIV+. 
Figure 2.1. Global fractional anisotropy maps for all HIV+ and HIV- subjects. Yellow indicates areas of increased FA for the HIV+ as compared to HIV-. Blue indicates areas of decreased FA for HIV+ as compared to HIV-.
Fig. 2.2. Illustration of increased fractional anisotropy in the posterior corpus callosum for all HIV+ subjects. The images on the left are averages of all HIV- (top) and HIV+ (bottom) participants. Those on the right are FA maps for individual subjects.
Fig. 2.3. Comparison between cognitively asymptomatic HIV+ participants (CAHIV+) and seronegative control participants (HIV-) in *a priori* regions of interest previously associated with HIV-related neurodegenerative disease. (A) Localization of regions of interest. (B) Bar graphs show mean white matter fractional anisotropy and standard error within each ROI.
* denotes a p-value <.05. ** denotes a p-value of 0.00008.
**Fig. 2.4.** Scatterplot illustrating the individual frontotemporal region white matter fractional anisotropy values for HIV- participants, cognitively asymptomatic HIV-positive individuals (CAHIV+), and HIV-positive participants with HIV-associated cognitive disorder (HAND).
Individual Frontotemporal ROI FA Values

FA Measurements

HIV- (n=17)  CAHIV+ (n=17)  HAND (n=8)
CHAPTER THREE

Experiment 2: Examination of structural changes in the cerebral cortex in cognitively asymptomatic individuals with HIV using cortical thickness measurements calculated from Magnetic Resonance Imaging scans

INTRODUCTION

Previous studies have demonstrated that the human immunodeficiency virus (HIV) predominantly affects subcortical regions of the brain with particular affinity for the basal ganglia and white matter pathways (J. R. Berger & Nath, 1997; Budka, 1991; Navia, 1997). However, the occurrence and onset of damage to cortical regions and the specific regional patterns of cortical alteration is still being defined.

The earliest imaging studies utilized qualitative clinical definitions of atrophy as defined by radiologists in which sulcal width was examined in different stages of HIV infection across the cortex. These studies reported a greater proportion of participants with an AIDS diagnosis as having enlarged sulci when compared to HIV- or pre-AIDS HIV+ participants (Harrison et al., 1998; Poutiainen et al., 1993), overall cortical atrophy being associated with HIV encephalitis (Chrysikopoulos et al., 1990; Post et al., 1988; Wiley et al., 1991), and greater gray matter volume loss related to more advanced stages of HIV illness as defined by the Centers for Disease Control (CDC) (Di Sclafani et al.,
1997). One investigation reported pronounced atrophy localized within the
temporal lobes of HIV+ participants (Elovaara et al., 1990). However, no cortical
atrophy was reported for evaluation including comparatively large group of 233
HIV+ participants with mild cognitive impairment (Dooneief et al., 1992).

While MR imaging techniques and quantitative data analysis tools have
emerged, questions still remain regarding the regional pattern and onset of HIV-
related alterations in the cortex. One such volumetric report demonstrated that
deceased HIV+ participants had decreased cortical volumes as compared to a
HIV- control group (Archibald et al., 2004). Studies examining regional volume
loss have reported significant reduction of posterior gray matter in HIV+
participants with dementia as compared to HIV+ and HIV- individuals without
dementia (Aylward et al., 1995). A more rapid loss of cortical tissue was
additionally documented in both medically asymptomatic and symptomatic HIV+
men compared to a seronegative group (Stout et al., 1998). More recently, local
cortical thinning has been reported in the frontal and temporal lobes of HIV+
individuals and associated with low CD4 count (Thompson et al., 2005) and high
HIV DNA levels in peripheral blood (Kallianpur et al., 2012) while brain volume
abnormalities have been associated with HIV-related medical factors such as
nadir CD4 count, history of an AIDS-defining event, age at infection, and clinical
staging (Pfefferbaum et al., 2012). Another investigation found no differences in
cortical thickness between HIV-infected individuals with and without cognitive
deficits and matched control participants.
There is an extensive body of literature suggesting that fronto-striatal dysfunction is associated with HIV (Chang et al., 2001; Ernst et al., 2003; Hinkin et al., 2002; Llorente et al., 1998; Martin et al., 2001; Sahakian et al., 1995; Stout et al., 1995; Villa et al., 1996). Poorer performance on neuropsychological measures has correlated both with reduced brain regional brain volumes in HIV+ individuals (Becker et al., 2012; Küper et al., 2011; Paul et al., 2008; Thompson et al., 2005) and enlarged subcortical structures (Castelo et al., 2007). However, it is unknown whether this degeneration occurs in the absence of HAND.

The purpose of the present study was to investigate cortical thickness in cognitively asymptomatic HIV-positive individuals as compared to HIV- control participants. Using a hypothesis driven approach, ROI analysis was limited to those cortical regions previously shown to be most impacted by HIV infection via exploratory analyses. All participants underwent a neuropsychological battery that followed the revised research criteria for the identification of HAND (Antinori et al., 2007). The assessment examined the following cognitive domains: verbal fluency/language, attention/working memory, abstraction/executive function, memory (learning and recall), speed of information processing, and motor skills. We used automated surface reconstruction procedures to measure the thickness of the cerebral cortex from high-resolution magnetic resonance (MR) images (Fischl and Dale, 2000). This quantitative method allowed us to identify regional cortical changes, providing regionally specified measurements of atrophy across the entire mantle. Based on previous research and exploratory whole brain
maps, we measured cortical thickness within the cingulate gyrus, superior temporal gyri, and middle occipital gyrus. We hypothesized that there would be reduced regional cortical thickness within all of these regions for the HIV-positive group without HAND when compared to the HIV- group.

**METHODS**

*Participants.*

Nineteen HIV-negative (HIV-) participants and 29 HIV-positive participants were analyzed in this study. For the HIV+ participants, 22 were cognitively asymptomatic (CAHIV+), and 7 participants were cognitively symptomatic using the criteria for HIV-associated neurocognitive disorders (HAND). All participants provided informed consent, following procedures that were approved by the Partners Human Research Committee, which oversees human research at the Athinoula A. Martinos Center for Biological Imaging (site of DTI imaging) and Boston University (site of neuropsychological testing and data analysis) institutional review board (IRB). Participants were recruited via advertisements in local newspapers and flyers posted at community health centers. Women were not excluded from the study, however due to the demographics of the local HIV population, all participants that met our inclusion criteria were men. Participants represented Caucasian, African American, Asian, and Hispanic ethnicities. Exclusion criteria for all participants included a history of intravenous drug use, alcohol or drug dependence, history of fungal infection of the neurological
system, history of psychiatric illness other than major depression, head injury with loss of consciousness exceeding 30 minutes, history of learning disability, history of psychosis, a history of neurological illness, or any abnormalities seen on a previous MRI or CT of the head. Participants with metal in the body, claustrophobia, or Meniere’s disease were also excluded. Additional exclusionary criteria for HIV-positive participants included any previous HIV-related Central Nervous System infections secondary to immunodeficiency. Each participant underwent neuropsychological testing (described below), followed by the MRI protocol (described below) within one month. The cognitively asymptomatic HIV+ and HIV- groups were matched for age, education, and pre-morbid verbal intelligence as determined by performance on the National Adult Reading Test (Blair & Spreen, 1989) as summarized in Table 3.1. The smaller cognitively symptomatic HIV+ HAND group was subsequently matched on these same measures with a subset of the cognitively asymptomatic HIV+ population as summarized in Table 3.2.

Immunological Information.

CD4 cell count (cells/mL), plasma viral load (log RNA/mL), and medication status were obtained, with consent, from participant medical records. Medication status was characterized as HAART (two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor, or two NRTIs and a nonnucleoside reverse transcriptase inhibitor (NNRTI), non-HAART, or none. CD4 counts and
plasma viral loads were unavailable for 2 CAHIV+ participants. CD4 counts, plasma viral loads, and percentage of patients in each medication class is summarized in Table 3.1 for cognitively asymptomatic HIV-positive subjects and in Table 3.2 for HIV-positive subject with HAND.

**Magnetic Resonance Imaging.**

High-resolution T1-weighted MPRAGE scans were obtained on a 1.5 Tesla Siemens Sonata scanner (Imaging parameters: resolution 1.3 x 1.3 x 1 mm, echo time (T_E) = 3.2 or 3.0 ms, repetition time (T_R) = 2730 ms, inversion time (T_I) = 1000 ms, flip angle = 7°, field of view (FOV) = 256x256 mm, matrix = 192x256). These parameters were empirically optimized for high gray matter/white matter and gray matter/cerebral spinal fluid contrast. Two scans were collected, motion corrected, and averaged to create a single high-signal, high-contrast volume for each participant. Head motion was minimized using padding.

**Image Analysis.**

The averaged volume was used to create representations of the gray matter/white matter boundary for each participant (Dale et al., 1999; Dale & Sereno, 1993). This surface was then deformed outward to locate the gray matter/cerebrospinal fluid border to produce measures of cortical thickness with sub-millimeter accuracy (Fischl & Dale, 2000). This method uses both intensity
and continuity information in the creation of surface boundaries. Additionally, both sets of surfaces were automatically topologically corrected (Fischl, Liu, & Dale, 2001). Cortical thickness measurements were calculated by measuring the distance between gray matter/white matter and gray matter/cerebrospinal fluid surfaces at each surface vertex across the cortical mantle and then mapped onto the inflated surface of each participant (Dale & Sereno, 1993; Fischl, Sereno, & Dale, 1999). This allowed for visualization of the data across the entire cortical surface (i.e. sulci and gyri) without being obscured by folds in the cortex. Each participant’s reconstructed brain was registered to an average spherical surface representation that optimally aligned sulcal and gyral patterns across participants for interparticipant registration (Fischl, Sereno, & Dale, 1999; Fischl, Sereno, Tootell, & Dale, 1999). The maps were then smoothed using a circularly symmetric Gaussian kernel across the surface with a standard deviation of 7 mm. This technique has been validated by direct comparison with measurements made on postmortem brains (Rosas et al., 2002) and provides comparable data to manually drawn regions of interest (Kuperberg et al., 2003; Salat et al., 2004).

**Exploratory whole brain analyses.**

We first created exploratory whole brain surface maps of HIV-associated cortical thickness alterations in a subset of 20 participants (10 HIV+, 10 HIV-). These analyses were considered exploratory because prior studies have noted the potential for misalignment in voxel-based volume registration, particularly for
images where atrophy is expected (Jernigan, Gamst, Fennema-Notestine, & Ostergaard, 2003). While each image went through the detailed registration process described above and was carefully inspected visually, it is still possible that misregistrations can contribute to the presented results. Therefore, these maps are considered secondary to the ROI analyses.

Regional analyses.

All ROIs were created using an automated parcellation technique based on a manually labeled training set using cortical folds for regional boundaries (Fischl et al., 2004). Our preliminary whole brain maps (described above) suggested that regions spanning the temporal and occipital lobes along with smaller areas in the parietal and frontal lobes were most thinned in the group of HIV+ individuals. We obtained measures from these same regions in an independent sample of 39 participants (20 HIV+, 19 HIV-) to determine whether the effect was replicable. The results were similar across the two independent analyses. Additionally, with the exception of the occipital cortex, these same regions have been previously associated with HIV-associated atrophy (Becker et al., 2012; Thompson et al., 2005). Therefore, thickness was analyzed in anatomical parcellations encompassing these regions for the final reported analysis of CAHIV+ as compared to HIV- individuals. Anatomical parcellations of theses ROIs are shown in Figure 3.2. We conducted independent-sample t tests to examine between-group differences in cortical ROIs.
RESULTS

*Exploratory whole brain analyses.*

The preliminary whole brain surface map of HIV-associated cortical thickness alterations for 49 participants (30 HIV+, including both cognitively asymptomatic and symptomatic and 29 HIV-) highlighted regionally variable reduced thickness with strongest statistical effects in the temporal and occipital lobes (Figure 3.1).

*Global Thickness.*

There were no significant global or hemispheric thickness differences between the CAHIV+ participants and matched HIV- individuals (demographics listed in Table 3.1) or between HAND patients and the subset of CAHIV+ participants (demographics listed in Table 3.2).

*Regions of Interest.*

Mean cortical thickness measurements (in mm) for the ROIs showing statistically significant between-group thickness differences are presented in Table 3.3a. Two regions showed significant statistical differences between the CAHIV+ and HIV- groups – the superior temporal gyrus (STG) and the inferior occipital gyrus. A mean reduction of 6.0% thickness was calculated in the planum polare region of the STG while a mean increase of 16.7% thickness was calculated in the inferior occipital gyrus.
Additional comparisons between HAND patients and the matched subset of the CAHIV+ participants, yielded significant reduced thickness measures in the STG, cingulate sulcus, and additional parietal ROIs. These results are presented in Table 3.3b.

DISCUSSION

The goal of this study was to examine HIV-related cortical thickness alterations using a quantitative, automated computational approach that estimates thickness across the entire cortical mantle using high-resolution MR images (Fischl & Dale, 2000). The results demonstrate that regional HIV-associated cortical alterations occur in the cingulate sulcus, temporal, occipital, and parietal lobes. Importantly, these data suggest that degenerative processes occur in the cortex in the absence of HAND and that these processes are regionally specific.

Our findings of reduced regional thickness in the temporal lobe for CAHIV+ participants and temporal, parietal, and cingulate cortices for HAND participants confirm and build on prior work that examined cortical atrophy in HIV+ participants. Aylward and colleagues (1995) reported their HIV+ group with dementia as having a significantly smaller posterior gray matter percentage than non-demented HIV+ and HIV- control participants. No group differences were found for measurements of anterior gray matter in that study. Wiley and colleagues (1991) reported atrophy of the cortical ribbon that was most evident in
the parietal and temporal regions of neocortex in patients with HIV encephalitis. Finally, in a study carried out by Elovaara and colleagues (1990) using qualitative neuroradiological evaluation, the most pronounced degeneration of HIV+ participants who exhibited cortical atrophy was found in the temporal lobes. More recent imaging studies have reported cortical thinning in anterior cingulate and temporal cortices (Küper et al., 2011), primary sensory and motor cortices, parietal association cortex, medial frontal and premotor cortex (Thompson et al., 2005), posterior and inferior temporal lobes, parietal lobes, and cerebellum (Becker et al., 2012). While we did not hypothesize that regional thickness in the occipital lobe would be increased in the CAHIV+ individuals when compared to control participants, elevated volume estimates for subcortical gray matter (Jernigan et al., 2011) and putamen volumes (Castelo et al., 2007) have been previously reported for the HIV+ population.

The characteristic neuropsychological symptomatology in HIV+ participants includes working memory dysfunction, lack of mental flexibility (Sahakian et al., 1995), decreases in motor and psychomotor speed ((Llorente et al., 1998; Villa et al., 1996), and increases in simple reaction time (Karlsen et al., 1992; Law et al., 1995; Mellgren et al., 2000) and reaction time during response inhibition (Hinkin et al., 1999; Llorente et al., 1998). Taking into account these findings, which are suggestive of fronto-striatal dysfunction in participants during varying stages of HIV illness, it was surprising to find no significant evidence of reduced thickness in the prefrontal cortex for our participants. One possibility is
that the cognitive deficits attributed to changes in the fronto-striatal system are due to white matter damage that spares prefrontal cortical regions. A recent DTI investigation found increased mean diffusivity (MD) in the white matter of the posterior frontal lobe for neurologically asymptomatic HIV+ patients when compared to a control group (Zhu et al., 2013).

Our demonstration of reduced cortical thickness measures in the STG in patients free from HAND is interesting given the hypothesized functions of this region. With primary auditory cortex housed within the STG and abutting Wernike’s area, it is unsurprising that intracranial electroencephalography (iEEG) investigations have linked the STG to speech perception (Chan et al., 2014; E. F. Chang et al., 2010). A recent magnetoencephalography (MEG) study revealed the necessity of the STG in attending to speech in a “multitalker” situation with noise distractors (Vander Ghinst et al., 2016). A number of studies have documented hearing difficulties in HIV+ individuals (Zuniga, 1999). One such report stated that HIV+ individuals on a steady regimen of antiretroviral therapy (ART) had significantly greater difficulties understanding speech in noise, and were significantly more likely to report that they had difficulty understanding speech than the patients not taking medication (Maro et al., 2014). This finding is line with the hypothesis that antiretroviral medications have an ototoxic effect (Thein, Kalinec, Park, & Kalinec, 2014).

Additional research has suggested that the STG is damaged in patients with spatial neglect (Karnath, Rennig, Johannsen, & Rorden, 2011; Kenzie et al.,
2015; Rousseaux, Allart, Bernati, & Saj, 2015), and that it is necessary for spatial processing (Mayer, Dorflinger, Rao, & Seidenberg, 2004), and feature-based serial exploratory search tasks (Ellison, Schindler, Pattison, & Milner, 2004; Gharabaghi, Fruhmann Berger, Tatagiba, & Karnath, 2006). HIV+ patients have deficits in related spatial and attentional tasks. For example, one study reported that HIV+ participants perform significantly worse than control participants on a spatial variant of the N-back working memory task (Hinkin et al., 2002) while another found HIV-related deficiencies on a visuospatial temporal ordering task (Woods et al., 2013). Results of a study assessing automobile driving performance in a group of HIV+ patients as compared to control participants showed that the HIV+ participants who were also neuropsychologically impaired performed poorly on a spatial navigation task that required mental rotation (Marcotte et al., 2004).

A potential explanation for the smaller thickness measurement of the STG in CAHV+ individuals when compared to the HIV- control group comes from anatomical research showing that the STG has direct connections with the caudate nucleus and putamen in monkeys (Yeterian & Pandya, 1998). Specifically, the rostral and middle parts of the STG project to ventral portions of the head, body, and tail of the caudate nucleus while the caudal STG project to the dorsal head and body of the caudate. The rostral and middle STG are connected to the rostroventral and caudoventral putamen and the caudal STG projects to the caudodorsal putamen (Yeterian & Pandya, 1998). There is a
wealth of evidence supporting basal ganglia dysfunction associated with HIV infection (for review see Berger and Arendt, 2000). The results presented here suggest that HIV infection damage may include the STG in addition to the basal ganglia.

The other statistically significant difference found in the CAHIV+ group was increased thickness of the inferior occipital gyrus (IOG) as compared to the HIV- control group. While we hypothesized that reduced cortical measures would be observed for the CAHIV+, other studies have reported putamen hypertrophy (Castelo et al. 2007) and brain volume increases in the HIV+ population (Jernigan et al., 2011) where immune activation was thought to lead to mild swelling of tissues. A similar hypothesis has been made regarding increases in cortical volumes associated with substance dependence (Jacobsen, Giedd, Gottschalk, Kosten, & Krystal, 2001; Jernigan et al., 2005). Another possible explanation is that underlying white matter damage would be expected to result in increased thickness of the cerebral cortex in HIV.

Located within the IOG is an area that has been dubbed the occipital face area or OFA (Gauthier et al., 2000). A variety of imaging studies have associated OFA activation with accurate face perception, suggesting that it constructs an initial representation of a face (see Pitcher et al., 2011 for a review). Impairment on a facial emotion recognition task has been associated with reduced subcortical volumes in HIV+ individuals (Clark et al., 2015). Disruption in this area may account for some of the other visuospatial (Bogdanova et al., 2008;
Hinkin et al., 2002; Marcotte et al., 2004; Olesen et al., 2007) and attentional (Chang et al., 2004) difficulties documented in the HIV population.

We have demonstrated both reduced thickness measures of the cerebral cortex within the temporal lobe and increased thickness measures of the occipital cortices in HIV+ patients without HAND using an automated computational approach that estimated thickness across the entire cortical mantle from high-resolution MR images (Fischl & Dale, 2000). Although these methods utilized high quality data sets and have been validated using histological methods (Rosas et al., 2002) as well as manual methods (Kuperberg et al., 2003; Salat et al., 2004), it is possible that increases in white matter signal intensities associated with HIV infection could alter the placement of the gray matter/white matter boundary (Thurnher et al., 2000). Motion correcting, averaging of two scans per participant, and visual inspection of each surface for accuracy reduces this potential confound. Additionally, having similar findings in both the preliminary group of 20 participants and an independent sample of 39 participants suggests that our results are reliable. However, it is also important to consider further the variability within the participant group itself. While all HIV+ participants were well characterized in terms of neuropsychological assessment according to the guidelines recommended by the National Institutes of Health (Antinori et al., 2007), they were fairly diverse in terms of CD4 counts, plasma viral loads, years since diagnosis, and medication status. We compared thickness in the STG and inferior occipital gyrus across CAHIV+ groups based on
the type of medicinal regimen they are on given that other reports have postulated that taking HAART may have an influence on gray matter volume in HIV+ individuals (Corrêa et al., 2016; Kallianpur et al., 2012). There was no difference among groups, suggesting that medication does not contribute to the observed effects (Figures 3.3). The results of this study suggest that selective alteration of the cerebral cortex begins during the cognitively asymptomatic stages of HIV-infection within the temporal and occipital cortices.
Table 3.1. Subject demographics for cognitively asymptomatic HIV-positive participants (CAHIV+) and HIV- control participants (HIV-) presented as means with standard deviations. P-values reflect results of independent sample t-tests. NART: National Adult Reading Test.
<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>CAHIV+</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>22</td>
<td>N/A</td>
</tr>
<tr>
<td>Age</td>
<td>41.3 ± 9.64</td>
<td>41.14 ± 9.23</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>(26-61)</td>
<td>(22-59)</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.1 ± 1.96</td>
<td>15.4 ± 1.81</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(12-19)</td>
<td>(12-18)</td>
<td></td>
</tr>
<tr>
<td>NART Estimated Verbal IQ</td>
<td>119.7 ± 4.04</td>
<td>115.1 ± 9.93</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(107.3-124.3)</td>
<td>(86.9–125.1)</td>
<td></td>
</tr>
<tr>
<td>Handedness</td>
<td>89% Right</td>
<td>86% Right</td>
<td>0.77</td>
</tr>
<tr>
<td>†CD4 count at testing</td>
<td>N/A</td>
<td>649.7 ± 344.2</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(249-1433)</td>
<td></td>
</tr>
<tr>
<td>†Viral load at testing (copies/µl)</td>
<td>N/A</td>
<td>6.8 ± 11.4</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Undetectable – 43.8)</td>
<td></td>
</tr>
<tr>
<td>†Years since HIV+ diagnosis</td>
<td>N/A</td>
<td>8.7 ± 5.3</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1-20)</td>
<td></td>
</tr>
<tr>
<td>Antiretroviral medications</td>
<td>N/A</td>
<td>10 HAART</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 non-HAART</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 None</td>
<td></td>
</tr>
<tr>
<td>HIV Dementia Screening Score</td>
<td>N/A</td>
<td>15.3 ± 1.2</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12-16)</td>
<td></td>
</tr>
</tbody>
</table>

† Information not available for two CAHIV+ participants.
Table 3.2. Subject demographics for a subset of the cognitively asymptomatic HIV-positive participants (CAHIV+) as compared to patients with HIV-associated neurocognitive disorder (HAND) presented as means with standard deviations. P-values reflect results of independent sample t-tests. NART: National Adult Reading Test.
<table>
<thead>
<tr>
<th></th>
<th>HAND</th>
<th>CAHIV+</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>7</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td>Age</td>
<td>40.3 ± 7.83</td>
<td>43.13 ± 10.9</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>(31-51)</td>
<td>(22-54)</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>13 ± 1.53</td>
<td>14.5 ± 1.69</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(12-16)</td>
<td>(12-16)</td>
<td></td>
</tr>
<tr>
<td>NART Estimated Verbal IQ</td>
<td>103.0 ± 7.07</td>
<td>107.8 ± 11.0</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>(94.0-108.2)</td>
<td>(86.9–118.9)</td>
<td></td>
</tr>
<tr>
<td>Handedness</td>
<td>100% Right</td>
<td>87.5% Right</td>
<td>0.37</td>
</tr>
<tr>
<td>†CD4 count at testing</td>
<td>556 ± 324.1</td>
<td>689.5 ± 406.7</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(492-878)</td>
<td>(267-1240)</td>
<td></td>
</tr>
<tr>
<td>¢Viral load at testing</td>
<td>3.73 ± 5.3</td>
<td>9.8 ± 17.1</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>(0.075-7.4)</td>
<td>(0.05 – 43.8)</td>
<td></td>
</tr>
<tr>
<td>§Years of diagnosis</td>
<td>14 (10-20)</td>
<td>9.2 ± 5.4 (3-20)</td>
<td>0.32</td>
</tr>
<tr>
<td>§Antiretroviral medications</td>
<td>3 HAART</td>
<td>3 HAART</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 non-HAART</td>
<td>1 non-HAART</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 None</td>
<td>4 None</td>
<td></td>
</tr>
<tr>
<td>HIV Dementia Screening Score</td>
<td>14.1 ± 1.9</td>
<td>14.3 ± 1.5</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>(11-16)</td>
<td>(12-16)</td>
<td></td>
</tr>
</tbody>
</table>

† Information not available for 3 HAND and 2 CAHIV+ participants.
¢ Information not available for 5 HAND and 2 CAHIV+ participants.
§ Information not available for 4 HAND one 1 CAHIV+ participant.
Table 3.3. Regions of interest thickness measures (in mm) presented as mean, standard error of the mean, and range for CAHIV+ as compared to HIV- participants (A) and HAND patients as compared to a subset of the CAHIV+ group.
### A.

<table>
<thead>
<tr>
<th>Region Name</th>
<th>ROI # from Fig. 3.2</th>
<th>HIV- Mean ± SE (range)</th>
<th>CAHIV+ Mean ± SE (range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Superior Temporal Gyrus (Planum Polare)</td>
<td>35</td>
<td>3.28 ± 0.80 (2.95-3.79)</td>
<td>3.08 ± 0.78 (2.66-3.57)</td>
<td>0.014</td>
</tr>
<tr>
<td>Right Inferior Occipital Gyrus</td>
<td>2</td>
<td>1.57 ± 0.38 (1.24-2.23)</td>
<td>1.89 ± 0.51 (1.33-2.41)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### B.

<table>
<thead>
<tr>
<th>Region Name</th>
<th>ROI # from Fig. 3.2</th>
<th>CAHIV+ Mean ± SE (range)</th>
<th>HAND Mean ± SE (range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Superior Temporal Gyrus (Transverse)</td>
<td>74</td>
<td>2.06 ± 0.43 (1.88-2.22)</td>
<td>1.96 ± 0.43 (1.91-2.01)</td>
<td>0.054</td>
</tr>
<tr>
<td>Right Cingulate Sulcus</td>
<td>7</td>
<td>2.36 ± 0.60 (2.31-2.44)</td>
<td>2.21 ± 0.58 (2.08-2.46)</td>
<td>0.013</td>
</tr>
<tr>
<td>Left Intraparietal Sulcus</td>
<td>56</td>
<td>1.73 ± 0.38 (1.50-2.0)</td>
<td>1.54 ± 0.34 (1.42-1.66)</td>
<td>0.020</td>
</tr>
<tr>
<td>Right Intraparietal Sulcus</td>
<td>56</td>
<td>1.71 ± 0.36 (1.47-1.91)</td>
<td>1.51 ± 0.39 (1.41-1.59)</td>
<td>0.003</td>
</tr>
<tr>
<td>Right Postcentral Sulcus</td>
<td>67</td>
<td>1.65 ± 0.44 (1.49-1.79)</td>
<td>1.51 ± 0.41 (1.41-1.79)</td>
<td>0.020</td>
</tr>
<tr>
<td>Left Angular Gyrus</td>
<td>25</td>
<td>2.33 ± 0.12 (2.19-1.54)</td>
<td>2.15 ± 0.13 (1.91-2.2)</td>
<td>0.024</td>
</tr>
</tbody>
</table>
**Figure 3.1.** The preliminary average statistical map of 30 participants with HIV (including CAHIV+, HAND, and unclassified patients) as compared with the average map of 29 HIV- participants. Maps are presented on the semi-inflated cortical surface of an average brain with darker gray areas corresponding to sulci and lighter gray areas corresponding to gyri. Non-neocortical regions and regions that are not part of the cortical mantle (i.e. the corpus callosum) have been excluded from the analysis. The colorscale represents the significance of the thickness change with yellow indicating regions with the greatest between group reduction in thickness measures.
Figure 3.2. Anatomical parcellations corresponding to the numbered regions of interest (ROIs) reported in Table 3.3 as previously published (Destrieux et al., 2010). These were created using an automated parcellation technique based on a manually labeled training set using cortical folds for regional boundaries (Bruce Fischl et al., 2004).
Figure 3.3. The average superior temporal gyrus (STG) and inferior occipital gyrus (IOG) thickness (in mm) for 19 HIV- control participants, 22 CAHIV+ participants sorted into a group taking HAART (n=11), and a CAHIV+ group not on a HAART regimen (n=11). This graph demonstrates that there is not a strong effect of medication on STG or IOG thickness in the CAHIV+ group.
**Superior Temporal Gyrus**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAHIV+ on HAART</td>
<td>3</td>
</tr>
<tr>
<td>CAHIV+ w/o HAART</td>
<td>3</td>
</tr>
<tr>
<td>HIV-</td>
<td>3</td>
</tr>
</tbody>
</table>

**Inferior Occipital Gyrus**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAHIV+ on HAART</td>
<td>2</td>
</tr>
<tr>
<td>CAHIV+ w/o HAART</td>
<td>2</td>
</tr>
<tr>
<td>HIV-</td>
<td>1</td>
</tr>
</tbody>
</table>
CHAPTER FOUR

Experiment 3: Regional white and gray matter alterations are related to performance measures of HIV-associated neurocognitive disorder

INTRODUCTION

Previous studies have demonstrated an association between WM volume loss and cognitive decline in HIV (Aylward et al., 1993; Stout et al., 1998). Investigations using MR imaging to focus on WM tracts have shown that whole-brain DTI measurements correlate with cognitive deficits in HIV positive patients (Ragin et al., 2004; Tate et al., 2010). Specifically, regional DTI measurements acquired in the corpus callosum (Hoare et al., 2012; Nir et al., 2014; Schulte et al., 2008; V. M. Tang et al., 2015; Wu et al., 2006; Zhu et al., 2013), corona radiate (Hoare et al., 2012; Nir et al., 2014; Zhu et al., 2013), uncinate fasciculus (Schulte, Muller-Oehringer, Sullivan, & Pfefferbaum, 2012), frontal WM (Chang et al., 2008; Wu et al., 2006), parietal WM (Chang et al., 2008), sagittal stratum (Hoare et al., 2011), subcortical structures (Chang et al., 2008; Ragin et al., 2005), anterior commissure, and internal capsule (V. M. Tang et al., 2015) correlate with a host of different cognitive deficits in HIV positive patients. Additionally, DTI fiber-tracking measures in the splenium (Pfefferbaum et al., 2007) and cerebellum (Sullivan et al., 2011) have predicted slowed psychomotor performance in HIV-infected groups.
Cortical atrophy in HIV+ participants has been associated with slowed information processing (Harrison et al., 1998) and poor memory test performance (Harrison et al., 1998; Poutiainen et al., 1993) as well. While poorer performance on neuropsychological measures has correlated with reduced regional brain volumes in HIV+ individuals (Becker et al., 2012; Castelo et al., 2007; Corrêa et al., 2016; Küper et al., 2011; Paul et al., 2008; Thompson et al., 2005) in more recent imaging studies as well. Other disease-related factors of HIV-illness have been associated with cortical degeneration as well. HIV+ patients who had experienced an AIDS-defining event have shown thinner primary sensory, motor, and premotor cortices (Thompson et al., 2005), smaller cortical volumes with larger ventricles (Cohen et al., 2010), and smaller volumes in the corpus callosum (Pfefferbaum et al., 2006; Thompson et al., 2006). Brain volume abnormalities have been associated with the lowest CD4 lymphocyte count measured after HIV infection, referred to as the nadir CD count, (Clark et al., 2015; Jernigan et al., 2011; Pfefferbaum et al., 2012) and plasma viral load as well (Cardenas et al., 2009; Jernigan et al., 2011).

Other studies have investigated the association between HIV-associated structural changes and a series of cognitive domains recommended for HAND classification by The National Institute of Mental Health and The National Institute of Neurological Diseases and Stroke (Antinori et al., 2007). One report looked at the changes in a global cognitive deficit score, where all individual domains were averaged, one year after baseline measurements were recorded for in HIV+ patients.
participants. They found that overall cognitive deficits correlated with FA alterations in parietal and frontal WM and putamen (Chang et al., 2008). Another investigation utilized tract-based special statistics (TBSS) to calculate DTI measures throughout all white matter tracts for HIV-infected participants with and without cognitive impairment as compared to healthy control participants (Zhu et al., 2013). The study found that two cognitive domains – speed of information processing and verbal fluency – significantly correlated with reduced FA and increased mean diffusivity values across multiple WM structures. However, to our knowledge, no prior work has investigated the relationship between cortical and WM regions previously associated with HIV-infection and cognitive domains known to be sensitive to the detection of HIV-associated neurocognitive disorders (HAND).

Thus, the purpose of the present study was to identify correlations between cortical volumes, DTI FA values, neuropsychological test performance, and immunological characteristics of HIV-infection. Using stepwise multivariate linear regressions, we sought to identify whether cortical thickness, WM integrity, and immunological markers of HIV-infection correlate with neuropsychological performance. We hypothesized that regional FA values, cortical thickness, and viral load would be related to neuropsychological composite scores for CAHIV+ individuals, but not HIV-control participants.
METHODS

Participants.

Twenty-eight HIV-negative (HIV-) participants and 30 HIV-positive participants were analyzed in this study. For the HIV+ participants, 23 were cognitively asymptomatic (CAHIV+), and 7 participants were cognitively symptomatic using the criteria for HIV-associated neurocognitive disorders (HAND). All participants were recruited for Experiments 1 and 2, following the same exclusionary criteria, neuropsychological assessment, and immunological data collection outlined in those Methods sections of chapters 2 and 3.

Correlational Analyses.

Five structural ROIs were examined for correlation with neuropsychological test performance: frontotemporal WM, posterior corpus callosum WM, parietal WM, STG thickness, and IOG thickness. Bivariate correlation analyses were only implemented to examine relationships for regions with FA and cortical thickness differences between the cognitively asymptomatic HIV+ and HIV- groups as reported in Experiments 1 and 2. The relationship between FA values, cortical thickness, and immunological health characteristics (CD4 counts and plasma viral loads) was explored for both the cognitively asymptomatic HIV+ and HAND groups. A set of secondary post-hoc analyses were carried out in which we combined the asymptomatic HIV+ and HAND groups to explore correlations between FA values and cognitive performance for
our entire HIV positive population. Statistical analyses were performed using SPSS, version 23.0 statistical analysis software for Macintosh (SPSS Inc, Chicago, Illinois).

**Regression Analyses.**

Stepwise multivariate linear regressions were implemented to examine the extent to which structural alterations in the WM and cortex, identified in Experiments 1 and 2, affected neuropsychological performance. Six cognitive composite scores (attention/working memory, abstraction/executive function, speed of information processing, memory (learning and recall), motor speed, and verbal fluency and language) were computed in the following manner. First, all raw scores were computed to z scores based on published normative data. The z scores for the tests in a given cognitive category were then averaged to compute 1 composite score per person for each cognitive domain. Finally, the z scores from each cognitive domain were averaged to create a single global functioning score. The global functioning composite score was entered as the dependent variable while CD4 lymphocyte count, viral load, duration of illness, and each of the ROIs, and were submitted as independent variables in the stepwise linear regression model. Statistical analyses were performed using SPSS, version 23.0 statistical analysis software for Macintosh (SPSS Inc, Chicago, Illinois).
RESULTS

**WM FA and Neuropsychological/Immunological Characteristic Correlations.**

The association between FA and neuropsychological test performance was calculated separately for each participant group. The relationship with cognitive performance was only explored for regions showing between-group differences in FA measures collected in Experiment 1 (frontotemporal, posterior corpus callosum, parietal lobe). HIV negative subjects had no significant associations with cognitive domain scores. For the cognitively asymptomatic HIV+ group, the negative correlation between viral load and right parietal FA was significant ($r=-.660$, $p=.010$) where viral load increases with lower FA values. The cognitively asymptomatic group also had a significant negative correlation between left posterior corpus callosum FA and one measure of the verbal fluency/language abilities domain (Boston Naming Test score, $r=-.518$, $p=.040$) such that lower FA is correlated with better test performance. The HAND group showed a negative correlation between frontotemporal FA and the Attention/Working Memory cognitive domain ($r=-.950$, $p=.004$) so that FA increases as performance decreases. In the combined HIV positive group (asymptomatic HIV+ and HAND), correlations were identified where FA increased as test performance decreased. These include left posterior callosum FA and the Attention/Working Memory Domain ($r=-.446$, $p=.038$), and the Verbal Fluency Domain ($r=-.478$, $p=.029$). Increased viral load correlated with increased FA in the left posterior callosum as well ($r=.506$, $p=.032$). These results are outlined in Table 4.1A.
Cortical Thickness and Neuropsychological/Immunological Characteristic Correlations.

The association between cortical thickness and neuropsychological test performance was calculated separately for each participant group. Relationship with cognitive performance was only explored for regions showing between-group differences in cortical thickness measures in Experiment 2 (superior temporal gyrus, inferior occipital gyrus, cingulate sulcus, intraparietal sulcus, postcentral sulcus and angular gyrus) as shown in Table 3.3. For participants with HAND, correlations were identified between the Attention/Working Memory domain score and the right superior temporal sulcus ($r=.906$, $p=.013$) and right cingulate gyrus ($r=.902$, $p=.014$), the Abstraction/Executive Function domain score and the right intraparietal sulcus ($r=.818$, $p=.047$), and the global functioning score and right superior temporal sulcus ($r=.906$, $p=.005$) where cortical thickness decreases as test performance decreases.

While the CAHIV+ participants had no significant associations with cognitive domain scores on their own, a number of additional correlations were significant once the HIV+ groups (CAHIV+ and HAND) were combined. These included the Attention/Working Memory domain score with the transverse aspect of the left STG ($r=.419$, $p=.03$) and the right superior temporal sulcus ($r=.486$, $p=.01$), the Memory (Learning and Recall) domain score with the right superior temporal sulcus ($r=.386$, $p=.042$), the right intraparietal sulcus ($r=.458$, $p=.014$), and the right postcentral sulcus ($r=.483$, $p=.009$), the Verbal Fluency/ Language
domain score with the left temporal pole \((r=.428, p=.037)\), and the global functioning score with the right intraparietal sulcus \((r=.374, p=0.046)\) and the right postcentral sulcus \((r=.492, p=.007)\). All associations were such that thickness decreased as test performance decreased. An additional significant correlation included viral load with the Verbal Fluency/ Language Domain, \((r=-0.636, p=.003)\) where viral load increased as test performance decreased. For the HIV- group, a correlation between thickness in the planum polare of the STG and the Memory (Learning and Recall) domain score was significant \((r=.561, p=.003)\) where STG thickness decreases with lower test score. These results are outlined in Table 4.1A.

**WM FA and Cortical Thickness Correlations.**

The association between white matter fractional anisotropy and cortical thickness was calculated separately for each participant group. The CAHIV+ individuals demonstrated significant correlations between angular gyrus thickness and both frontotemporal \((r=.623, p=0.017)\) and right posterior callosum \((r=.566, p=0.035)\) where FA increases along with angular gyrus thickness. Those same relationships between angular gyrus thickness and frontotemporal \((r=.529, p=0.017)\) and right posterior callosum \((r=.659, p=0.002)\) FA were significant for the combined HIV+ group (CAHIV+ and HAND together) as well. The HAND individuals also had a significant association between the angular gyrus and right posterior callosum FA \((r=.942, p=.017)\) (Figures 4.1 and 4.2). Temporal pole
thickness decreases correlated with left posterior callosum FA increases in both CAHIV+ ($r=-.602$, $p=.008$) and all HIV+ ($r=-.430$, $p=.032$) (Figure 4.3). HAND participants showed an additional correlation between frontotemporal FA and superior temporal sulcus thickness ($r=-.869$, $p=0.025$) where frontotemporal FA increases as superior temporal sulcus thickness decreases. The combined HIV+ group additionally had a significant association between frontotemporal FA and the cingulate gyrus ($r=-.445$, $p=0.026$), where frontotemporal FA increases as cingulate gyrus thickness decreases. For HIV- participants, the only significant correlation identified was between right inferior occipital gyrus thickness and parietal FA ($r=.602$, $p=0.014$). These results are outlined in Table 4.1B.

**Regression Analyses.**

To assess the extent to which immunological health, FA alterations, and cortical thickness affected cognitive function in the HIV+ groups, a stepwise linear regression analysis was conducted. In the model, immunological health characteristics (CD4 lymphocyte count, plasma viral load, duration of illness), the significant ROIs for CAHIV+ individuals from Experiment 1 (frontotemporal FA, posterior callosum FA, parietal lobe FA), and Experiment 2 (superior temporal gyrus and inferior occipital gyrus thickness) were submitted as independent variables, and the global functioning composite score was the dependent variable. The model was not statically significant for the CAHIV+, HAND, or combined HIV+ group.
DISCUSSION

A series of statistical analyses were calculated to further investigate the relationship between altered FA and cortical thickness measures identified in the CAHIV+ group in Experiments 1 and 2, neuropsychological scores, and immunological markers of HIV disease. The most significant ROIs identified for CAHIV+ individuals included the increased frontotemporal FA (p=.00008) in Experiment 1 and the decreased superior temporal gyrus thickness (p=.01) and the increased inferior occipital gyrus thickness (p=.001) in Experiment 2. While there were no significant correlations identified between frontotemporal FA and cognitive measures for the HIV- or cognitively asymptomatic HIV+ group, the HAND cohort showed a correlation in this region where frontotemporal FA increased as performance on the Attention/Working Memory cognitive domain decreased. These data suggest that FA alterations identified in the absence of cognitive impairment contribute to the cognitive deficits seen in more advanced stages of HIV disease. When the cognitively asymptomatic HIV+ and HAND groups were combined, this association was no longer significant. While the small sample size limits the conclusions we can draw, it is possible that there is a nonlinear association between FA in frontotemporal regions and cognition in HIV such that cognitive deficits arise after significant damage to the underlying white matter occurs. This would support previous findings where a significant association between neurocognitive domains (Speed of Information Processing and Verbal Fluency) and altered diffusion measures across multiple WM
structures in HIV+ individuals were demonstrated (Zhu et al., 2013).

While the CAHIV+ participants had no significant associations between cognitive domain scores and superior temporal gyrus or inferior occipital thickness on their own, a number of correlations involving the superior temporal region were significant once the HIV+ groups (CAHIV+ and HAND) were combined. These included the Attention/Working Memory domain score with the transverse aspect of the left STG and the right superior temporal sulcus, and the Memory (Learning and Recall) domain score with the right superior temporal sulcus. These results are similar to the cognitive associations we identified for frontotemporal FA in the HAND cohort; the results further suggest that the cortical alterations documented in the absence of cognitive impairment may contribute to the cognitive deficits seen as HAND progresses. It is also important to note that no significant interactions were identified between neuropsychological performance and these regions for the HIV- group.

Several anatomical relationships were identified for the HIV+ groups that were not evident in the HIV- participants. Namely, FA in the right posterior corpus callosum correlated with angular gyrus thickness in the CAHIV+, HAND, and combined HIV+ groups. Angular gyrus thickness also correlated positively with frontotemporal FA for the CAHIV+ and combined HIV+ groups. These results were somewhat unexpected given the direction of the association. Both posterior callosum and frontotemporal FA increased in the HIV+ groups when compared to HIV- measurements (as detailed in chapter 2), but angular gyrus thickness
decreased in the HAND group when compared to the subset of CAHIV+ individuals. As discussed in chapter 3, increased volumetric measures have been documented in the HIV+ population before, potentially due to edema of the neural tissue (Jernigan et al., 2011). These correlations suggest increased angular gyrus volumes for the HIV+ groups. This finding is interesting given fMRI evidence for HIV-associated hyperactivity in the posterior parietal lobe during both an episodic encoding task (Castelo, Sherman, Courtney, Melrose, & Stern, 2006) and mental rotation task (Schweinsburg et al., 2012). The other significant anatomical finding for both CAHIV+ and the combined HIV+ group was an inverse correlation between left posterior corpus callosum FA and the temporal pole. This finding is in agreement with the posterior callosum FA increase reported in the chapter 2 and the temporal pole decrease reported in chapter 3. There were no significant correlations identified for the HIV- individuals involving the increased FA measures in the frontotemporal or posterior corpus callosum regions. This is further evidence to suggest that these associations within the temporal lobe are unique for HIV+ participants, even those free from HAND.

Fewer associations between immunological characteristics and either structural ROIs or cognitive performance were identified than hypothesized. No signification correlations were found for CD4 lymphocyte count or years since diagnosis. This is likely due to the fact that we collected CD4 count from our patients at the time the neuropsychological battery was administered. However, nadir CD4 count has been shown to be a superior predictor of HIV-associated
cognitive impairment (Ellis et al., 2011). We found that viral load increased as test performance on the Verbal Fluency/Language Domain decreased, but we also observed a negative relationship between viral load and right parietal FA for CAHIV+, which is not entirely consistent with the between group finding in Experiment 1. As viral load increases, it would be expected that FA values drop.

While our regressional analyses failed to show that altered FA and cortical measures are able to predict global neuropsychological functioning, several unique correlations were identified for the HIV+ groups when compared to the HIV- control participants. The results of this study highlight a pattern of association between neuropsychological test performance and regional alterations found in the cortex and white matter for HIV+ individuals. This is most pronounced in the temporal lobe, suggesting that regional changes to WM and cortex that occur while HIV patients are cognitively asymptomatic may contribute to the deficits seen as HAND develops.
Table 4.1. A. Correlations between structural ROIs (calculated in Experiments 1 and 2) and cognitive domain scores. B. Correlations between regional FA values (calculated in Experiment 1) and cortical thickness measures (calculated in Experiment 2). PCC = posterior corpus callosum, STG = superior temporal gyrus, STS = superior temporal sulcus, AWM = Attention/Working Memory, VF = Verbal Fluency/Language, MLR = Memory (Learning and Recall), AEF = Attention/Executive Function, GF = Global Functioning.
### Table A.

<table>
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<th>GROUP</th>
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<th>Cognitive Domain</th>
<th>Pearson’s r</th>
<th>p-value</th>
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<td>Left PCC FA</td>
<td>VFL</td>
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<td>AWM</td>
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<td>Right STS</td>
<td>AWM</td>
<td>.486</td>
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<td>Right STS</td>
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<td>p-value</td>
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<tr>
<td>HIV-</td>
<td>Left Parietal FA</td>
<td>Right IOG</td>
<td>.602</td>
<td>0.014</td>
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</table>
Figure 4.1. Elevated FA measures in frontotemporal white matter for both CAHIV+ and all HIV+ patients are associated with increases in angular gyrus thickness.
Increased frontotemporal FA is associated with increased angular gyrus thickness for CAHIV+

\[ r^2 = .389 \]
\[ p = .017 \]

Increased frontotemporal FA is associated with increased angular gyrus thickness for all HIV+

\[ r^2 = .280 \]
\[ p = .017 \]
Figure 4.2. Elevated FA measures in posterior corpus callosum white matter for CAHIV+, HAND, and all HIV+ patients are associated with increases in angular gyrus thickness.
Increased right posterior corpus callosum FA is associated with angular gyrus thickness for CAHIV+

\[ r^2 = .320 \]
\[ p = .035 \]

Increased right posterior corpus callosum FA is associated with angular gyrus thickness for HAND

\[ r^2 = .887 \]
\[ p = .017 \]

Increased right posterior corpus callosum FA is associated with angular gyrus thickness for all HIV+

\[ r^2 = .434 \]
\[ p = .002 \]
**Figure 4.3.** Elevated FA measures in posterior corpus callosum white matter for CAHIV+ and combined the combined HIV+ patient groups are associated with decreases in temporal pole thickness.
Increased left posterior corpus callosum FA is associated with decreased temporal pole thickness for CAHIV+

\[ r^2 = .362 \]
\[ p = .008 \]

Increased left posterior corpus callosum FA is associated with decreased temporal pole thickness for all HIV+

\[ r^2 = .185 \]
\[ p = .032 \]
CHAPTER FIVE

Integrated Discussion

The goal of this thesis was to examine the associations between HIV-related structural brain changes in the white matter and cortex, neuropsychological performance on tests sensitive to HAND identification, and immunological characteristics in a group of cognitively asymptomatic HIV+ (CAHIV+) patients without HAND as compared to well-matched control participants (HIV-). The integration of diffusion tensor imaging (DTI), structural magnetic resonance imaging (sMRI), and neuropsychological testing allowed for a comprehensive investigation of these structure-function relationships in the pre-dementia stages of HIV illness. DTI was used to collect regional fractional anisotropy (FA) values reflective of white matter integrity in both cognitively asymptomatic HIV+ individuals (CAHIV+) and well-matched control participants. Additionally, sMRI was used to collect regional cortical thickness measurements in both of these groups. Neuropsychological measures were utilized in all three studies for the purpose of staging HIV+ participants regarding HAND severity so that only HIV+ participants free from any form of neurocognitive disorder (asymptomatic, mild, or dementia) were included in the CAHIV+ groups. The neuropsychological measures were also used to examine the relationship between structural measures and cognition. This discussion section will briefly
summarize the main findings of each experiment and offer integrative interpretations for the collective results.

**SUMMARY OF FINDINGS**

Results from Experiment #1 (chapter 2) documented significantly elevated FA values in frontotemporal white matter (WM) in cognitively asymptomatic HIV+ (CAHIV+) individuals, compared to well-matched control participants. Based on earlier studies, the prediction was that WM changes would be evident in the CAHIV+ group and be most prominent in the corpus callosum with alterations in the internal capsule, frontotemporal, and parietal, WM. While a subset of studies have reported increased FA values in various WM regions for the HIV+ population, the majority report reduced FA, particularly in the corpus callosum. Along with the increased FA in the frontotemporal region, we found increased FA in the posterior callosum for CAHIV+ individuals, and reduced FA in the parietal ROI.

Results from Experiment #2 (chapter 3) revealed statistically significant reduced cortical thickness measures in the superior temporal gyrus (STG) for the CAHIV+ group as compared to the HIV- control participants. A 17% increase in thickness was calculated in the inferior occipital gyrus (IOG) for CAHIV+ individuals as well. While our prediction was that CAHIV+ participants would show signs of reduced cortical thickness across frontal, temporal, and parietal when compared to HIV- control individuals, significant cortical alterations were
confined to the STG and IOG for the patients free from HIV-associated neurocognitive disorder (HAND).

Finally, the results from Experiment #3 (chapter 4) highlighted significant relationships between the same ROIs calculated in Experiment #1 and #2 (chapters 2 and 3, respectively) and measures of neuropsychological performance and immunological health. For CAHIV+ individuals, associations were revealed between both frontotemporal and posterior callosum FA with thickness of the angular gyrus and posterior callosum FA with temporal pole thickness. These relationships were not significant for the HIV- individuals. While none of the cognitive domain scores (calculated from neuropsychological tests known to be sensitive to HAND) correlated significantly with the structural ROIs for CAHIV+ participants, there was a relationship between increased FA in the posterior callosum and poorer performance the Boston Naming Test - one measure of the Verbal Fluency/Language abilities domain. CAHIV+ individuals also had a significant relationship between viral load and right parietal FA where viral load increased with lower FA values. When the CAHIV+ and HAND groups were combined, a number of significant correlations between structural ROIs and cognitive domain scores were identified. These findings suggest that regional changes to WM and cortex that occur while HIV patients are cognitively asymptomatic may contribute to the deficits seen in the later stages of HIV illness as HAND develops.
PRE-HAND STRUCTURAL BRAIN CHANGES

The findings from Experiments #1 and #2 are consistent with previous imaging studies of HIV+ patients where regional WM injury has been documented in the corpus callosum (Hoare et al., 2011, 2012; Leite et al., 2013; X. J. Li et al., 2015; Müller-Oehring et al., 2010; Nir et al., 2014), frontal (J. Li et al., 2015; Pomara et al., 2001; Smith et al., 2008; Stebbins et al., 2007; Thurnher et al., 2005; Zhu et al., 2013), temporal (Gongvatana et al., 2011), and parietal lobes and regional cortical thinning has been reported in the temporal cortices (Becker et al., 2012; Küper et al., 2011) various stages of illness. However, in Experiments #1 and #2, the HIV+ patients in the CAHIV+ group were limited to those showing no signs of cognitive impairment as determined by the neuropsychological criteria for HAND established by the National Institute of Mental Health and National Institute of Neurological Diseases and Stroke (Antinori et al., 2007).

Many of the previous imaging investigations did not carry out a full neuropsychological assessment using the updated criteria for HAND to exclude HIV+ participants with asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), or HIV-associated dementia (HAD) (Becker et al., 2012; Küper et al., 2011; Thompson et al., 2005). Other authors actively recruited subjects with major cognitive deficits or dementia as part of their study (Aylward et al., 1993; Patel et al., 2002; Pomara et al., 2001; Ragin et al., 2004;
Wu et al., 2006), focused on a specific subset of the HIV+ population including the elderly (Nir et al., 2014), patients over 40 (Kallianpur et al., 2012) or 50 years old (Becker et al., 2012), children with vertical transmission (Hoare et al., 2015), patients with HIV subtype C (Hoare et al., 2011), or patients infected with the virus within 100 days of scanning (Ragin et al., 2015). Of those using the updated nosology for HAND, patients with ANI were considered neurologically asymptomatic, despite demonstrating impairment of documented by performance of at least 1.0 standard deviation (SD) below the mean for appropriate normative data on two or more of the composite ability domain scores (Zhu et al., 2013).

**Temporal Lobe.**

In the CAHIV+ participants, free from all levels of HAND, we identified temporal lobe alterations in both the white matter and cortex. These same regions showed an inverse relationship between each one another in the combined HIV group such that frontotemporal FA increased as cortex within the STG thinned. The same was true of the HAND group where frontotemporal FA increased as the STS decreased. STG thickness also correlated with poorer performance on the Attention/ Working Memory (AWM) cognitive domain score for the combined HIV+ group. The HAND participants demonstrated a negative correlation between the same AWM domain and frontotemporal FA such that FA increases as test performance decreases.
As is well-established in previous literature, basal ganglia dysfunction is associated with HIV infection (Berger and Arendt, 2000); anatomical research has identified direct connections between the STG and the caudate nucleus and putamen in monkeys (Yeterian & Pandya, 1998). Other work suggests that the STG is necessary for spatial processing (Mayer et al., 2004), and feature-based serial exploratory search tasks (Ellison et al., 2004). Given that HIV+ patients have been documented as having deficits in related spatial and attentional tasks (Grassi et al., 1999; Hinkin et al., 2002; Marcotte et al., 2004), it is unsurprising that we identified irregular measures in both the WM and cortex of the superior temporal lobe.

**Parietal Lobe.**

There was a significant decrease in parietal lobe FA for the CAHIV+ group compared to HIV- in Experiment #1. This parietal FA decrease correlated with increased plasma viral loads for the CAHIV+ group in Experiment #3. These results were unsurprising given that previous studies have reported reduced FA values in HIV+ participants (Chang et al., 2008; X. J. Li et al., 2015). When comparing the CAHIV+ and HAND groups in Experiment #2, mean thickness of the intraparietal sulcus was significantly decreased for HAND participants. Furthermore, Experiment #3 revealed that reduced cortical thickness measures of the intraparietal sulcus correlated with poorer performance on both the Memory (Learning and Recall) domain and overall global functioning score for
the combined HIV+ group. Global deficit scores have correlated with parietal lobe FA values in a previous report (Chang et al., 2008). Associations between increased angular gyrus thickness and both frontotemporal and posterior callosum elevated FA measures were also identified. Given that HIV-related impairment has been reported during parietal-dependent mental rotation tasks (Olesen et al., 2007; Weber et al., 2010) and fMRI investigations demonstrating posterior parietal hyperactivity (Castelo et al., 2006; Schweinsburg et al., 2012), the parietal lobe is clearly impacted by HIV illness.

**Occipital Lobe.**

While we did not identify WM alterations in the occipital lobe, a 17% increase in thickness was calculated in the inferior occipital gyrus (IOG) for the CAHIV+ participants as compared to the control group – a highly significant result. A recent PET study showed significant regional increases of 18 kDa translocator protein (TSPO) in the occipital lobe for HIV+ patients (Vera et al., 2016). TSPO is a marker of inflammation and has been associated with poorer cognitive performance and white matter microstructural pathology. Immune activation leading to tissue swelling has been hypothesized in other reports of volume increases in the HIV+ population (Jernigan et al., 2011). Another possible explanation is that underlying white matter damage could result in increased thickness of the cerebral cortex in HIV. Whatever the direct cause, occipital lobe alteration may account for some of the visuospatial (Bogdanova et al., 2008;
Hinkin et al., 2002; Marcotte et al., 2004; Olesen et al., 2007) and attentional (Chang et al., 2004) difficulties previously documented in the HIV population.

_Corpus callosum._

White matter alterations in the corpus callosum have been well documented in HIV+ individuals (Hoare et al., 2011, 2012; Leite et al., 2013; X. J. Li et al., 2015; Müller-Oehring et al., 2010; Nir et al., 2014) and specifically identified in the genu (Corrêa et al., 2015; Filippi et al., 2001; Schulte et al., 2008; V. M. Tang et al., 2015; Thurnher et al., 2005) and splenium (Filippi et al., 2001; Wu et al., 2006). Recently, reduced FA in the corpus callosum has been found as early as within the first one hundred days of infection (Ragin et al., 2015). Experiment #1 highlighted increased FA in the posterior corpus callosum for the CAHIV+ individuals as compared to the HIV- group. No cognitive associations were identified for the posterior corpus callosum within the CAHIV+ group. However, in the combined HIV positive group (HIV+ and HAND), the left posterior callosum FA was inversely associated with both the Attention/Working Memory Domain and Verbal Fluency Domains, supporting the interpretation that increased FA in this sample is indicative of disease progression (as discussed above regarding the temporal and occipital lobes). Also consistent with this interpretation, increased viral load correlated with increased FA in the left posterior callosum for the combined HIV+ group.
OVERALL CONCLUSIONS

Collectively, our results conclude that temporal, occipital, and parietal lobe dysfunction occurs in cognitively asymptomatic HIV+ individuals. The work in this thesis demonstrates that both white matter integrity and the cortical ribbon are regionally altered in the absence of HAND. Within a combined group of individuals spanning HAND classification, these same regions correlate with cognitive performance in neuropsychological domains known to be sensitive to HIV-related illness. Together, the results from these three studies suggest that regional FA and cortical alterations identified in the absence of HAND may contribute to the cognitive deficits seen in later stages of HIV disease.

These findings significantly contribute to our understanding of the cognitively asymptomatic HIV-infected brain. While the lives of HIV+ individuals have been extended by highly active antiretroviral therapies, the prevalence of HAND continues to rise. HIV infection is becoming a chronic illness with few treatment options specifically designed to address the cognitive aspects. Understanding the path of HIV-infection in the central nervous system, when patients are free from cognitive illness, is crucial for the development of new treatments.
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http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2711895&tool=pm


CURRICULUM VITAE

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RESEARCH
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TEACHING
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EDUCATION
Sept 2003 - May 2016 Boston University, Graduate School of Arts and Sciences, Boston, MA
Doctoral Candidate in Brain, Behavior, and Cognition Program
• Dissertation: “HIV-associated structural brain changes as related to cognition”
• Advisor and First Reader: Chantal E Stern, D.Phil.

May 2003 - May 2000 Boston University, Graduate School of Arts and Sciences, Boston, MA
M.A. in Psychology

FELLOWSHIPS, HONORS, AND AWARDS

2013 Compass Award, FIRST (For Inspiration and Recognition of Science and Technology) Tech Challenge, Massachusetts State Championship, Bridgewater, MA

2004 Travel Award, Organization for Human Brain Mapping, Budapest, Hungary

2004 Clara Mayo Memorial Fellowship, Boston University Department of Psychology fellowship awarded to support dissertation research
RESEARCH EXPERIENCE

Sept 2002 - May 2004  **Graduate Research Assistant**, Cognitive Neuroimaging Lab in the Center for Memory and Brain, Boston University, Boston, MA
Advisor: Chantal E. Stern, D.Phil
Research: Examined the neuropathology of Human Immunodeficiency Virus (HIV) infection using structural magnetic resonance imaging (MRI) techniques including diffusion tensor imaging (DTI), cortical thickness measurements, and subcortical volume analyses. Experimental studies focus on combining the above techniques with neuropsychological testing and functional MRI to define the cognitive and anatomical course of the disease.
Responsibilities:
• Recruited HIV+ and HIV- control participants
• Designed and implemented sMRI and DTI studies
• Instructed fellow lab members on MR scanning procedures and data analysis using the Freesurfer processing stream
• Prepared and managed Institutional Review Board documentation and grant progress reports

Jan 2000 - Aug 2002  **Research Assistant**, MGH/MIT/HMS Martinos Center for Biomedical Imaging, Charlestown, MA
Advisors: Eric Halgren, PhD and Anders Dale, PhD
Research: Analyzed MRI, DTI, MEG, and EEG data in a variety of Linux-based processing streams. Collected iEEG, MRI, and MEG data for patients with epilepsy, Huntington’s Disease, and control participants. Instructed other members in the Center on the usage of Freesurfer MR image processing tools.

Jun 1998 - Aug 2000  **Undergraduate Research Assistant**, Visual and Circulatory Biophysics Laboratory, Boston University Department of Biomedical Engineering, Boston, MA
Advisor: Mark Bitensky, M.D. and Tatsuro Yoshida, PhD
Research: Conducted assays on increasing the life span of red blood cells for longer term blood bank storage.

Jun 1997 - Aug 1997  **Summer Research Assistant**, NeuroMuscular Research Center, Boston University Department of Biomedical Engineering, Boston, MA
Advisor: Lars Oddsson, PhD
Research: Within the Injury Analysis and Prevention Lab, assisted in running digital simulations of people falling in real life situations for movement analysis.
TEACHING EXPERIENCE

Aug 2011- Present  Science Faculty, The Rivers School, Weston, MA
Responsibilities:
• Courses taught: A.P. Biology, Honors Biology and Chemistry 1, Biology and Chemistry 1, Biology and Chemistry 2, Neuroanatomy and Behavior, Chemistry, Robotics, Intro to Psych, Memory and the Brain
• Collaborated to develop an integrated 2 year course in Biology and Chemistry for both regular and honors levels
• Robotics Team Lead Mentor, 2012-2015
  o Guided team in building, programming, and engineering notebook construction in preparation for competition in the FIRST Tech Challenge and Massachusetts State Championship (invited 2013-2015)
  o Organized and hosted regional competitions
  o Managed team budget
• Assistant Varsity Softball Coach, 2012-2014, 2016-present
• Assistant Robotics Club Mentor, 2016-present

Sep 2015- Mar 2016  Evening Study Hall Proctor, Belmont Hill School, Belmont, MA
Responsibilities: Supervise weekly 2-hour study hall for boarding students in the dormitories

Aug 2006- July 2010  Science Faculty, Berkshire School, Sheffield, MA
Responsibilities:
• Courses Taught: Advanced Biology, Biology, Neuroanatomy and Behavior, Cognitive Neuroscience
• Coordinator of Health and Wellness Education, 2007-2010
  o Orchestrated all-school, form-specific, and small-group events on a wide range of topics related to the health and well being of both day and boarding students
  o Developed curricula and created a proposal for a mandatory health course
• House Head, 2007-2010
  o Dormitories: Godman, 50 female students; Spurr, 23 female students
• Coach, 2006-2009
  o Sports: Varsity Softball (Assistant), Boys’ Regatta Crew (Assistant), J.V. Girls’ Lacrosse (Head), Girls’ Regatta Crew (Assistant)
Jan 2005-May 2005  Adjunct Lecturer, Pine Manor College, Department of Psychology
Chestnut Hill, MA
Responsibilities:
• Taught the undergraduate course PY320, Physiological Psychology for the spring semester

Jan 2004-Dec 2005  Teaching Assistant, Department of Psychology, Boston University, Boston, MA
Responsibilities: Attended lectures, ran weekly discussion sections, and held office hours for the following undergraduate courses:
• Fall 2005, Neuropsychology (PS338)
• Spring 2005, Experimental Psychology: Physiological (PS322)
• Spring 2005, General Psychology (PS101)
• Fall 2004, Physiological Psychology (PS231)
• Spring 2004, Neuropsychology (PS338)

July 2000-Oct 2001  Software Instructor, Massachusetts General Hospital Nuclear Magnetic Resonance Center, Charlestown, MA
Responsibilities: Delivered hands on demonstration of the Freesurfer software package for graduate students and post-doctoral students at the Visiting Fellowship in fMRI.

PUBLICATIONS

Courtney MG, Salat DH, Melrose RJ, Castelo JMB, Stern CE. (submitted) Regional white matter alterations are related to performance measures of HIV-associated neurocognitive disorder.

Courtney MG, Salat DH, Melrose RJ, Castelo JMB, Stern CE. (in preparation) Thickness measures of the cerebral cortex associated with cognitively asymptomatic HIV infection.


Castelo JM, Courtney MG, Melrose RJ, Stern CE. (2007) Putamen hypertrophy in nondemented patients with human immunodeficiency virus infection and cognitive compromise. Arch Neurol, 64(9), 1275-80.


ORAL RESEARCH PRESENTATIONS

Courtney, MG. (2005) HIV-related cortical thinning. Talk presented at the Brain, Behavior, and Cognition weekly colloquia. Boston University Department of Psychology. November 4, 12 p.m. – 1 p.m.

ABSTRACTS


Melrose RJ, Poulin RM, Boer JM, Courtney MG, Stern CE (2006) Reasoning ability and fronto-striatal circuitry in HIV. Poster presented at the International Neuropsychological Society Meeting, Boston, MA


