University of Missouri South African Education Program

Proposal Application Form

Name/Title: Rob Paul / Brain Involvement in clade C HIV

Department/Campus: Psychology / UMSL

Campus Address: One University Blvd, Stadler 412, St. Louis

Telephone: 314 516-5398

Fax: 314 516-5392

E-mail: paulro@umsl.edu

I am applying for:

___ I. The UM/UWC Linkage Program

_X_ II. The South African Partnerships Program

Proposal Abstract (Include a brief statement of the problem or need being addressed, the intended outcomes/objectives of the project, the project methodology, and the project timeline.):

Please see attached aims

Proposed budget, including matching funding (use attached budget form):

Amount requested: $ 10,000 Matching: $ 0 TOTAL: $ 10,000

Attach a detailed narrative proposal (maximum of 10 pp.) and a detailed budget.

Signature of Applicant: Rob Paul

Date: 7/16/07

Signature of department chair and/or dean: Robert J Calyo

Date: _____

Please attach:
1) your curriculum vitae,
2) a letter of endorsement from your chair and/or dean, and
3) a letter of commitment from your South African collaborator.

Budget Summary

Item†	Amount Requested from UMSAEP	Amount funded by other sources‡	Total
1. Air fare	$ 2,200	$ 0	$ 2,200
2. Room/board	$ 400	$ 0	$ 400
3. Ground transportation including car rental	$ 150	$ 0	$ 150
4. Personnel	$ 1,650	$ 0	$ 1,650
5. Materials/printing	$ 500	$ 0	$ 500
6. Communications	$ 100	$ 0	$ 100
7. Other expenses MRIs	$ 5,000	$ 0	$ 5,000
Total	$ 10,000	$ 0	$ 10,000

† Attach a brief description justifying each budget item.
‡ Please identify the source(s) of matching funds.

Rough Guide to Expenses

UWC Visitors to Missouri

Airfare: 15,000 - 17,000 Rand
Food: $500/month
Lodging:
Columbia $600/month
Kansas City $59/day
Rolla $550/month
St. Louis $50/day

Car Rental:
Columbia $1,200/month
Kansas City $1,300/month
St. Louis $1,200/month
Rolla – depends if flying into KC or St. Louis & renting at one of these airports.

UM Visitors to UWC

	June-August	Rest of year
Airfare:	$2,200	$1,600
Food:	$500/month	$600/month
Lodging:	$1,300/month	$1,500/month
Car Rental:	$800/month*	$1000/month*

*Compact car w/out insurance

A detailed accounting of your expenditures must be submitted to Assistant Vice President Deborah Noble at the conclusion of your project.

You are encouraged to contact your campus coordinator for assistance in budget preparation.

A. Specific Aims

Human immunodeficiency virus (HIV) is a systemic disease that affects the brain within weeks of the initial infection. Brain involvement in HIV results in cognitive impairments in the majority of patients and these cognitive difficulties have significant functional impact on patients' abilities to maintain adherence to medications, maintain gainful employment, and their ability complete other aspects of activities of daily living (e.g., financial management, shopping ability, etc). Members of our group have shown that these limitations ultimately negatively impact perceived quality of life (Osoweicki et al., 2000).

At present the neurobiological substrates of HIV cognitive impairment are not well described. This is particularly the case for the genetic form of HIV known as clade C, since nearly all studies of cognitive function in HIV have been conducted in North America where the genetic strain of the virus is clade B. Early studies suggested that individuals infected with clade C may be less likely to develop cognitive impairments due to a biological defect in the tat protein present in clade C (Ranga et al., 2004). However, our group recently published data collected in India demonstrating that individuals infected with clade C exhibit significant cognitive impairment, suggesting that the brain is vulnerable among individuals with clade C HIV (Yepthomi et al., 2006). These findings are notable because clade C is the most common form of HIV in the world, as well as the dominant strain in South Africa. As such, it is important to understand the neurobiological substrates of cognitive impairment in this viral clade. The purpose of this present study is to develop a collaborative relationship between investigators based at the University of Missouri, St. Louis, investigators at the University of Cape Town (UCT) and investigators at the University of Western Cape (UWC) to begin a series of empirical studies to characterize the neural signatures of HIV brain involvement in patients infected with clade C HIV.

The investigative team has expertise in neuropsychology, psychiatry, neuroimaging, and international HIV work and we will draw on these strengths to establish the necessary infrastructure for collaborative studies across the three sites. In addition to developing the basic infrastructure to implement the collaborative work, the team will recruit 20 HIV-positive individuals with clade C HIV and 20 seronegative healthy controls matched on demographic characteristics. In addition, we will collect neuroimaging on a subset of the HIV patients, using diffusion tensor imaging (DTI). DTI is a novel neuroimaging methodology that examines the microstructural integrity of the white matter by defining the rate and direction of water diffusion in the brain. Studies have revealed that DTI is more sensitive than traditional structural neuroimaging techniques at identifying brain subtle brain abnormalities. Dr. Paul (PI) is currently funded by the National Institutes of Health to examine DTI abnormalities in the white matter of seronegative individuals. Further, a mulit-university Brain-Behaviour Initiative has recently been established between the Universities in the Cape, including installment of a high-power 3 Tesla neuroimaging unit (one of the few such machines in the developing world), and is focused on attempting to develop capacity in neuroimaging research that is relevant to local problems such as HIV/AIDS We will apply this innovative methodology to understand the microstructural abnormalities of the white matter in patients with clade C HIV in South Africa. Our study will be the first to integrate laboratory indices of HIV, diffusion tensor imaging, and neuropsychological outcome in patients infected with clade C HIV. The study will address the following aims within a 12 month period:

Aim 1. Establish a scientific partnership comprised of investigators at the University of Missouri, St. Louis, University of Cape Town, and the University of Western Cape. We will:

A. Conduct an investigator meeting at the University of Cape Town and the University of Western Cape during the initial months of the award. The meeting will provide a mechanism for introductions, development of materials for Human Subjects clearance, and completion of a needs assessment regarding neuropsychological and neuroimaging protocols at all three institutions. During these meetings we will also determine the appropriate (culturally-valid) neurocognitive tests and modules for brain morphometric analyses.

B. Develop a subsequent NIH grant proposal to extend our initial work as a collaborative team. During the first year the team will develop methodologies to incorporate novel biomarkers of HIV-related cognitive impairment including HIV proviral DNA, gene/protein expression studies, and related areas of proteonomics.

Aim 2. Determine the cognitive and neuroimaging status of individuals infected with clade C HIV residing in South Africa. We hypothesize that:

A. Individuals infected with HIV will exhibit significantly poorer cognitive performances on tests of thinking speed, executive function and psychomotor function compared to demographically-matched healthy controls.

B. Individuals infected with HIV will exhibit significantly poorer microstructural integrity of the white matter as determined by in vivo diffusion tensor imaging. We expect that values of fractional anisotropy, a measure of directional water diffusion, in the frontal white matter will be significantly lower among HIV patients compared to controls.

C. Lower FA measured in the frontal white matter will correlate significantly with HIV disease burden factors (viral load, CD4 cell count), and performance on cognitive tests.

B. Background, Significance

HIV crosses the blood-brain-barrier and infects the brain compartment within two weeks of initial infection. While individual neurons are not directly infected, the presence of the virus in the brain inititates and inflammatory cascade that results in impaired neuronal function. Most of our knowledge regarding HIV brain involvement is limited to the genetic strain of the virus present in North America, while little is known about the more prevalent genetic strains in the world. Clade C HIV is the dominant strain in South Africa, Asia, and the world, and early studies suggested that the brain was less vulnerable in patients with clade C infection. However, our recent empirical studies have demonstrated that the brain is highly vulnerable in this population. At present we do not have any data regarding the neurological signatures of HIV brain infection associated with clade C. The purpose of this present study is to develop a collaborative relationship between investigators based at the University of Missouri, St. Louis, investigators at the University of Cape Town (UCT) and investigators at the University of Western Cape (UWC) to begin a series of empirical studies to characterize the neural signatures of HIV brain involvement in patients infected with clade C HIV. Below we provide a brief review of the literature regarding HIV brain involvement, including studies of clade C.

HIV infects the CNS.

HIV enters the CNS soon after initial infection (Ho et al., 1985; Davis et al., 1992) and the virus directly infects supportive cells of the brain including mononuclear phagocytes such as microglial cells, astrocytes, and macrophages (Merrill & Chen, 1991; Brack-Werner, 1999; Zink et al., 1999). HIV infects the brain systemically, and especially high concentrations of the virus are found in subcortical regions. Neuropathological studies have demonstrated that HIV has a particular predilection for the caudate nucleus and white matter pathways (Budka et al., 1991; Wiley et al., 1999; Everall et al., 1993; Navia et al., 1986; Aylward et al., 1993; Dal Pan et al., 1992). Once the virus has entered the brain and infected mononuclear phagocytes, a paracrine-amplified inflammatory response begins and persists throughout the course of the infection (Anderson et al. 2002; Poluektova et al., 2001). A variety of neuropathologies develop once HIV enters the CNS (see Paul et al., 2002; 2003; Brack-Werner, 1999), including perivascular accumulations of microglia cells, monohistocytes and macrophages. In addition, leukoencephalopathy develops secondary to diffuse myelin loss, astroglial proliferation and infiltration by mono- and multinucleated macrophages.

HIV impacts cognition.

HIV produces a range of cognitive impairment from very mild symptoms to frank dementia. The prevalence of AIDS dementia complex (ADC) has declined since the introduction of ART, however there is some indication that the prevalence of HIV-related encephalopathy is increasing (Nuenberg et al., 2002). The neuropsychological profile associated with HIV was first described by Navia and colleagues (Jordan et al., 1985). HIV patients perform poorly on measures of complex attention, information processing speed and verbal memory. Since this seminal work, a number of studies have demonstrated that HIV patients perform more poorly than seronegative controls on tests of mental flexibility, motor and information processing speed, verbal fluency and verbal memory (Martin et al., 1992; 2001-2004; Becker et al., 1997; Van Gorp et al., 1989;

1994; Basso & Bornstein, 2003; Bornstein et al., 1993; Heaton et al., 1995; Cohen et al., 2001; Marcotte et al., 2003). This profile is typical of a "subcortical pattern" (Navia, 1997).

Cognition in clade C HIV.

Nearly all of the work that has been completed on cognition in HIV has focused on a specific genetic strain of the disease identified as clade B. However, clade C is the most common strain in the world (including South Africa). The importance of this distinction is that the two strains of the virus differ in terms of specific protein binding sites and binding characteristics, replicative capacity (Centlivre et al., 2005), and possibly in the development of treatment resistance (Grossman et al., 2001; Kantor et al., 2002), all of which suggests a potentially different outcome associated with the clade C viral strain.

One interesting clinical outcome associated with the clade C virus is the reportedly low prevalence of HIV-associated dementia (HAD) in India. Two studies (Satishchandra et al., 2000; Wadia et al., 2001) have reported that the prevalence of HAD prior to HAART was less than 3%, compared to 15-30% in the United States (Grant et al.,1995; Simpson, 1999). Most recently, Ranga et al. (2004) demonstrated an important natural variation in the dicysteine motif of the Tat protein (C31S) that was conserved only in clade C virus. Further, the authors demonstrated that the variation was functionally significant, as the mutation diminished monocyte chemokine migration properties. Since the Tat protein promotes viral replication directly and it reduces HIV-resistance in uninfected cells, a functional change in the Tat protein could have a significant impact on the virulence of the infection. Indeed, studies have demonstrated that Tat is selectively involved in the migration of monoctyes into the brain via upregulation of inflammatory cytokines and adhesion molecules (Pu et al., 2003). Tat also has been shown to disrupt the tight-junction proteins that support the blood-brain-barrier (Andras et al., 2003), possibly allowing for greater brain involvement via inflammatory processes. Given the important role that Tat plays in brain disruption secondary to HIV, Ranga et al. (2004) concluded that the Tat variation may in part explain the low prevalence of HAD in India.

Our recent studies conducted in a small sample of patients in India, however, have demonstrated that cognition is impaired among patients with clade C infection. As such, the low prevalence of dementia associated with clade C may be due to the fact that most other studies did not employ standardized cognitive tests to determine the presence of impairment rates, and therefore the prevalence of significant impairment may be higher than previously reported. It is also possible that less severe, but still clinically meaningful, difficulties on cognitive tests are present among individuals with clade C virus. Clearly, more comprehensive studies are needed, particularly those that incorporate neuroimaging as described below.

Diffusion tensor imaging of HIV brain involvement

White matter abnormalities have been described in HIV, but structural MRI may lack the sensitivity to detect pathological changes (i.e., normal appearing white matter). A more sensitive approach is diffusion tensor imaging (DTI). DTI measures the random thermal motion of water in brain tissue (Tuch et al., 2003). Water diffusion along directional pathways (e.g., white matter) occurs preferentially along the axis of the pathway (anisotropic diffusion) compared to equally distributed movement (isotropic diffusion) in nondirectional regions (e.g., grey matter). Alterations in myelin (e.g., MS) or changes to the microstructure of axonal projections reduce fractional anisotropy (Beaulieu, 2002) and therefore DTI metrics are useful biomarkers of white matter integrity. More than 200 published studies have demonstrated the utility of DTI to examine brain dysfunction in clinical populations (see Lim & Halpern, 2002; Sundgren et al., 2004; Taylor et al., 2004 for partial reviews), and the results are consistent with the pathologies associated with the disorders (Beaulieu, 2002; Aherns, Laidlaw et al., 1998).

Recent studies have applied DTI in HIV. Fillipi et al. (2001) reported that HIV patients with high plasma viral loads demonstrated decreased anisotropy in the corpus callosum, and increased diffusion in the subcortical white matter. Overall, patients with the highest diffusion constant elevations and largest anisotropy decreases had the most advanced systemic HIV disease. Similar findings were reported by Pomara et al. (2001). In this study, HIV patients and healthy control subjects underwent DTI, proton density MRI and T2 weighted images to compare the relative sensitivity of the three sequences to frontal white matter abnormalities. The two groups did not differ in terms of neuroimaging abnormalities on the conventional proton density and T2-weighted

images, but the HIV patients exhibited significant alterations in fractional anisotropy compared to the controls. In a recent study, Ragin et al., (2004) reported strong relationships between whole brain fractional anisotropy (FA) and severity of dementia among a small cohort of HIV patients (n=6). Finally, Cloak et al. (2004; applied DTI in a small study of HIV patients (n = 11) and reported significant differences in diffusion in the frontal white matter in HIV, which correlated significantly with performance on cognitive tests and glial markers in the frontal white matter. These studies demonstrate the utility of DTI to examine brain integrity associated with HIV and related conditions. To date, no study has examined DTI signatures of brain involvement among individuals infected with clade C HIV.

Summary of Background.
Studies have now demonstrated that cognitive function is impaired in patients with clade C HIV, however, no study has identified the neural signatures of these effects. Through the University of Missouri South Africa Education Program we have a unique opportunity to combine scientific strengths from investigators at multiple institutions to address these issues. South Africa has a substantial burden in terms of the prevalence of HIV and cognitive impairment associated with the virus will soon become a prominent healthcare concern in this region of the world. The genetic diversity of HIV in South Africa provides an outstanding opportunity for us to answer key questions about the impact of HIV on the brain. Below we provide results from preliminary studies that provide further support for this application.

C. Preliminary Studies

We have obtained solid pilot data derived from studies directed by Dr. Paul (PI) and other members of the collaborative team. These studies have addressed neurocognitive function in HIV-positive and HIV-negative substance abusers, and relationships between HIV disease markers and CNS injury. We also have obtained data from our neuroimaging studies of HIV and from our NIH-funded study of cognition in patients infected with clade C HIV in southern India. Results from these studies are described below.

1. International Studies of HIV and cognitive impairments in clade C

Our group was the first group to publish a comprehensive study of cognitive function in an international setting. With funding from the National Institutes of Health we completed a study of 30 HIV-positive individuals infected with clade C virus in Chennai, India. We also recruited 30 healthy controls from family members of seropositive individuals as well as individuals examined at the clinic who were seronegative. The healthy controls were matched to the HIV group according to sex, age, and education.

All subjects were administered a battery of neuropsychological measures that had been adapted from traditional US-based tests. The tests were translated and back-translated and adapted for cultural relevance according to International Test Commission guidelines. To determine if patients with clade C HIV exhibit cognitive impairments compared to healthy controls we contrasted performances on the neuropsychological tests using t-tests. Comparisons of HIV-positive patients and the seronegative controls demonstrated significant differences between the two groups for verbal list learning total recall ($t(58)=-2.4$, $p=0.016$), verbal list learning delayed recall ($t(58)=-2.6$, $p=0.012$), BVMT-R total immediate recall ($t(58)=-2.3$, $p=0.023$), BVMT-R delayed recall ($t(58)=-2.2$, $p=0.026$), Grooved Pegboard Non-Dominant Time to completion ($t(58)=3.12$, $p=0.003$), Color Trails-1 ($t(55)=2.53$, $p=0.014$), and Color Trails-2 ($t(55)=2.6$, $p=0.012$). Group contrasts on the Stroop Word Trial, Stroop Color Trial, Stroop Color/Word Trial, and Grooved Pegboard Dominant Hand Time were not statistically significant ($ps > 0.12$).

Overall, the range of impairment was 4% (Stroop incongruent) to 40%, with the largest percentages evident on the test of visual memory test (total learning) and Color Trails 2. We also determined the percentage of patients that were impaired on two or more tests using the same criterion for impairment. Results from this analysis revealed that 46% of the HIV participants with advanced HIV met the criterion for impairment on two tests.

Results from our study revealed that HIV-positive individuals infected with clade C performed significantly more poorly than healthy controls on verbal and visual measures of memory, fine motor speed and dexterity and visual scanning/cognitive flexibility. This pattern is consistent with observations of HIV-related cognitive

impairment in the United States (Heaton et al., 2004). The observation that HIV patients with clade C performed below expectations on multiple cognitive tests raises the possibility that the frequency of dementia associated with clade C remains higher than previously reported. We cannot address this issue directly since the test battery did not consist of sufficient breadth and depth to fully examine cognitive performances across domains (e.g., attention, visuospatial function, etc) and we did not collect information related to functional abilities. We will address these issues in the proposed study.

2. Diffusion tensor imaging of HIV-related cognitive impairment.

Our team has extensive experience with neuroimaging of HIV-related cognitive impairment. We have published studies describing structural brain abnormalities associated with HIV in patients infected with clade B. We also have obtained data using DTI to describe the white matter abnormalities associated with HIV infection. These studies are described below and demonstrate our ability to collect these data in the proposed study. As noted previously, our collaborators in South Africa have a new 3.0 tesla magnet to facilitate these neuroimaging studies.

Diffusion tensor imaging of brain dysfunction in HIV (Paul et al.). Recently our group has been collecting diffusion tensor MRI in HIV patients with mild cognitive difficulties. As part of this study, all individuals were scanned during a single session, and cognitive function was assessed on a separate day, typically within one week. The cognitive battery included computerized versions of the Stroop test, N-back (1-back), auditory verbal learning, trail making, verbal fluency, and an executive maze task. Relationships between the structural scans and the DTI measures (fractional anisotropy) were examined using correlation analyses.

Performances on the cognitive battery.

Measure	HIV	HIV seronegative
FAS	38.2 (13.4)	42.9 (9.1)
Trail Making B- modified	50.4 (12.8)	53.0 (8.8)
Stroop incongruent/correct	11.1 (3.3)	12.9 (4.1)
Executive mazes (errors)	45.0 (19.8)	48.9 (14.8)
N-back /ms correct targets	933.0 (88.6)	924.0 (75.9)
Total verbal learning	26.6 (6.2)	29.5 (3.4)

Note. *P < .05. Higher scores on Trail Making, Mazes errors, and N-back reflect poorer performance. ^Power calculations reveal this difference would be significant with the proposed sample size.

The images below depict the diffusion maps.

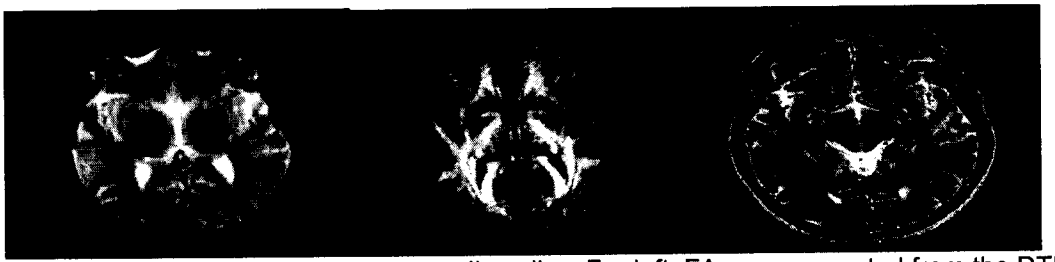

Figure. Three images of the corresponding slice. Far left: FA map generated from the DTI data set, Middle: Trace ADC image, Far right: spin-echo image showing greater anatomical detail.

Results of the DTI analyses.

Measure	HIV	HIV seronegative
FA genu corpus callosum	.81 (.06)	.86 (.05)
FA splenium corpus callosum	.80 (.07)	.86(.01) ^
FA genu of internal capsule	.63 (.02)	.72 (.03)
FA anterior limb internal capsule	.47 (.08)	.54 (.06)
FA posterior limb internal capsule	.58 (.04)	.64 (.05)^

FA ranges from 0-1, with higher ratios reflecting greater anisotropy. ^Power calculations reveal this difference would be significant with the proposed sample size.

HIV studies conducted in South Africa.
In clinical studies on patients with HIV in the Cape region, our collaborators have used structured diagnostic interviews to establish psychiatric morbidity and disability in patients recently diagnosed with HIV/AIDS. We have noted that major depression (34.9%), dysthymic disorder (21.5%), post-traumatic stress disorder (14.8%), and alcohol dependence (10.1%) as prevalent in this population. In these studies, female gender, the perceived impact of negative life events, and increased disability were found to predict current major depression. Our collaborators also have an extensive history related to translation of psychological measures into local languages, and the cross-cultural validity of our measures. As such the team is well equipped to carry-out the proposed study.

Summary of the preliminary studies. The data presented above demonstrate our ability to address the specific aims of the proposal, as well as provide a solid foundation for the specific aims. We have shown significant impact of HIV clade C on cognition among individuals residing in India, and relationships between neuroimaging indices and cognitive function among individuals infected with clade B HIV. We have significant expertise in the neurocognitive, neuroimaging and laboratory aspects of HIV and as such we are confident in our ability to complete the study. Our preliminary findings suggest that clade C HIV is associated with abnormalities in brain function and we believe that damage to the white matter underlies these effects. In the present application we will test this model directly by measuring neuroimaging markers of clade C HIV infection. This initial grant award will be important for us to establish the scientific team and develop a history of collaboration. Once established, we will be ideally positioned to seek additional external funding.

D. Research Design and Methods

Aim 1. Create a collaborative research relationship between the University of Missouri, University of Western Cape and the University of Cape Town.

The first phase of the award period will be focused on a needs assessment at both collaborating sites including the University of Cape Town and the University of Western Cape. It is important to note that we have explicit support from both universities including support from Dr. Charles Malcolm at the University of Western Cape. In the first year we will work to formally strengthen this relationship in order to include both universities in the project and follow-up proposals. The primary review will focus on three core elements: 1) neuropsychological measures, 2) neuroimaging acquisition and analysis capacity, 3) HIV laboratory assessment facilities. The primary focus of each is described below.

Neuropsychological measures: Studies conducted in the US, including studies from our laboratory, have consistently reported that measures purportedly tapping frontal-subcortical brain regions are sensitive measures to document cognitive difficulties associated with HIV (Paul et al. 2003). The theoretical basis for this finding is that HIV typically aggregates in subcortical regions of the brain and in turn disrupts flow of information within the dorsolateral prefrontal circuit (Paul et al. 2003). Most tests for HIV have been developed, and for the most part, normed within the US and other developed countries. As such one of the first goals of the developmental application will be to modify cognitive measures sensitive to HIV-related difficulties to account for sociocultural differences including language and literacy skills.

Neuroimaging acquisition and analysis: The University of Cape Town has access to a new 3.0 Tesla MRI capable of acquiring the pulse sequence optimal for the proposed brain morphometric analyses. In the first visit to the clinic, we will review the scanner facilities. We will also review the computer hardware and software support available for quantification of MRI morphometrics. Our laboratory has particular expertise with NIH Image, a software program available for semi-automated quantification of volumetrics and DTI.

Clinical laboratory facilities: The clinics are well-equipped for collection, storage and analysis of clinical laboratory data including viral load, lymphocyte cell count and HIV genotyping. In the first phase of visits to the clinic, the collaborators and associated personnel will review the laboratory setup and identify the necessary equipment and laboratory space necessary to conduct the proposed study. We will establish

fiber optic internet connections for uploading of image files and a firewall security system will be installed. An encryption code will protect documents; personal identifiers will not be transferred between clinics.

Aim 2. Determine the cognitive and neuroimaging status of individuals infected with clade C HIV residing in South Africa

Overview of Study Design.

This is a two-group cross-sectional study of cognition and neuroimaging associated with clade C HIV. Laboratory indices of HIV disease burden, neuroimaging and cognitive function will be collected from 20 seropositive individuals infected with clade C HIV and 20 health seronegative controls matched for age, education, and sex. Neurocognitive function will be measured with tests sensitive to cognitive impairment in co-infection. Substance abuse status, neuropsychiatric status, fatigue, and history of HAART will be recorded. Neuroimaging will consist of DTI of the white matter, and fractional anisotropy will serve as the primary neuroimaging index.

Study Participants.

A total of 40 individuals will be recruited including 20 seropositive individuals and 20 seronegative individuals matched for age and education. **Inclusion criteria:** 1) Age between the years of 21 and 50. This age band was selected to avoid complications with neurodevelopment (< 18 years of age) and increased risk for age-related CNS changes (>50 years). The groups will be matched on a case-by-case basis in age bands below and above 35 years of age across the two groups. We will review this procedure once we have achieved 50% accrual. 2) ethnic/racial background consistent with NIH policy. 3) Male or female – total numbers consistent with NIH policy. 3) HIV serostatus documented by ELISA and confirmed by Western blot (for the HIV patients), plasma HIV RNA or a second antibody test for the HIV patient groups; 4) CD4 cell count < 500 for HIV-positive individuals to control for severity of HIV disease and to ensure recruitment of individuals at risk for cognitive impairment. **Exclusion criteria:** 1) Schizophrenia or bipolar disorder; 2) Confounding neurological disorders including MS and other CNS conditions. Careful attention will be focused on head injury since many individuals with substance abuse histories have co-morbid head injuries of various severity. In order to provide an efficient means to screen individuals for this factor, we will rely on duration of loss of consciousness (LOC) as our benchmark for injury severity. In particular, we will exclude any individual with LOC greater than 30 minutes); 3) Individuals with clinical evidence of opportunistic CNS infections (toxoplasmosis, progressive multifocal leukoencephalopathy, neoplasms).

General Design

Study Logistics. Fliers advertising the study will be posted at the university clinics. One research assistant will be devoted to subject recruitment at each primary site. Once potential participants contact the research assistants via phone (phone number will be provided on a pull-tab on the fliers), the RA will describe the study over the phone including the significant inclusion and exclusion criteria. Participants will not be asked to reveal personal information over the phone. Rather, they will be given information about the study, and subsequently asked if they are still interested in participating. Interested participants will then schedule a visit with the RA, who will complete a comprehensive consent process and this will be followed by a detailed medical and demographic history. CD4 count will also be obtained at this first visit to ensure that participants meet inclusion criteria; nadir CD4 count will also be recorded. On a separate day participants will be asked to return to the clinic to complete the psychiatric (described in detail below), KMSK drug use severity assessment, and neurological evaluation. Within one week of this visit, participants will be scheduled for the neuropsychological assessment and laboratory collection. Study participation will be voluntary and individuals will be free to withdraw from the study at any point. Subjects will receive compensation at each assessment period.

Summary of assessments and dependent variables – **Visit 1 and 2 will be completed within 1 week.**

Measure	Visit 1	Visit 2
Consent	X	
Demographic questionnaires	X	
CD4 cell count/viral load (for inclusion criteria)	X	
Psychiatric/Drug abuse assessment	X	X

Neuroimaging assessment		X
Neuropsychological assessment		X

Consent and Demographic Questionnaires.
Once the informed consent process has been completed, all participants will complete demographic and health questionnaires. These questionnaires will provide general background and health information, and will be useful factors to insure application of the inclusion criteria, and subsequently for data classification. The questionnaires will include information regarding participants' age, education, developmental history (neurodevelopment and educational achievement), ethnicity, race, mode of HIV transmission, time since HIV diagnosis, current medications, time since onset of ART and adherence to ART, Short-Form 36 (Ware & Sherborne, 1992; a questionnaire of health-related quality of life), early life stress questionnaire, the Sheehan Disability Scale and Fatigue Severity Index (Krupp et al., 1989).

Depression will be quantified using the Center for Epidemiology Scale for Depression (CES-D). The CES-D is a widely used measure of depression in HIV research, and it was the principle measure of mood disturbance incorporated into the CDC-funded HERS protocol. The measure is reliable and valid and our group has extensive experience using this measure, including preliminary data from HIV patients in South Africa. The dependent measure will be the total score. Apathy and related frontal neuropsychiatric symptoms will be examined using the Frontal Systems Behavior Scale (FrsBe; Grace and Malloy, 2003). The Lawton and Brody Activities of Daily Living questionnaire (Lawton and Brody, 1969) will be administered to assess capacity to complete basic and instrumental ADLs. This scale has been used extensively in studies of dementia. We currently administer the above measures in our ongoing studies, and have been able to complete these instruments within approximately 1 hour. Manuals will be developed for each of the two sites and Dr. Paul will be responsible for the initial training and standardization of the protocol.

Psychiatric and Drug Use Assessment.
The Structured Clinical Interview (SCID) will be administered to assess psychiatric and substance abuse behaviors. The SCID is a reliable and valid assessment of psychopathology that was developed using diagnostic criteria consistent with the DSM-IV. The SCID covers mood syndromes, psychotic syndromes, psychoactive substance abuse disorders, anxiety syndromes, somatoform disorders, substance abuse, and adjustment disorder. . All primary modules will be included. The SCID will be used to ensure that all participants meet inclusion criteria for both mental health parameters (e.g., schizophrenia, bipolar disorder) as well as substance abuse/dependence diagnoses. The Kreek-McHugh-Schluger-Kellogg scale (KMSK; Kellogg et al., 2003) will be administered to provide a quantified measure of drug use severity determined by frequency, duration, and amount of use for individual drugs. The scale has been modified to include marijuana as an additional drug, but no other modifications are needed.

HIV-RNA Quantification.
Plasma will be separated from cells by centrifugation at 400 x g within 6 hours of collection. For RT-PCR, two-1 ml aliquots of plasma will be prepared, frozen at -70C. HIV RNA quantitation in plasma will be performed using the Roche Monitor Ultra Sensitive RT-PCR assay and the assay, respectively. Appropriate viral RNA controls will be incorporated into each run (0, 150, 1500, 15000) viral RNA copies/ml. Lower limits of detection have been reported at 50 RNA copies/ml. Results will be exported electronically to the data manager using an electronic file format. Pre-post counseling will be available for individuals unaware of their HIV status prior to HIV testing. HIV serostatus will be determined by plasma ELISA.

Neuropsychological Evaluation.
A battery of cognitive tests sensitive to deficits associated with aging will be administered. Testing will be monitored by a highly trained research assistant working under the direction of the project coordinator. This battery is comprised of tests that are sensitive to HIV-related cognitive changes (Paul et al., 2007). The computerized battery requires approximately 60 minutes to complete. The battery is both valid (Paul et al., 2005) and reliable (Williams et al., 2005).

Sensori-motor domain. Simple motor tapping task: Participants are required to tap a circle on the touch-screen with their index finger, as fast as possible for 60 seconds. The dependent variable is total number of

taps with the dominant hand. Choice reaction time task: Participants are required to attend to the computer screen as one of four target circles is illuminated in pseudo random sequence over a series of trials. For each trial, the subject is required to place their index finger in preparation on a start circle displayed on the touch-screen. On each trial, the subject then had to touch the illuminated circle as quickly as possible following presentation. Twenty trials are administered with a random delay between trials of 2-4 seconds. The dependent variable is the mean reaction time across trials.

Attention domain. Span of Visual Memory: This test is a computerized visual analogue of a digit span test. Participants are presented with squares arranged in a random pattern on the computer screen. The squares are highlighted in a sequential order on each trial. Participants are required to repeat the order in which the squares are highlighted by touching the squares with their forefinger. Both forward and reverse trials are conducted. The total correct is the dependent variable. Digit Span: Participants are presented with a series of digits on the touch-screen (500ms presentation), separated by a one second interval. The subject is then immediately asked to enter the digits on a numeric keypad on the touch-screen. In the first part of the test, subjects are required to recall the digits in forward order (Digits forwards); in the second part, they are required to recall them in reverse order (Digits backwards). The dependent measure is total correct for forward and backward conditions. Continuous Performance task: To tap sustained attention, a series of letters (B, C, D or G) are presented to the subject on the computer screen (for 200ms), separated by an interval of 2.5 seconds. If the same letter appeared twice in a row, the subject is asked to press buttons with the index finger of each hand. Speed and accuracy of response are equally stressed in the task instructions. The dependent variables are the number of hits and omissions. Switching of attention task: this test is a computerized adaptation of the Trail Making test. It consists of two parts. In the first part, the subject is presented with a pattern of 25 numbers in circles and asked to touch them in ascending numerical sequence. As each number is touched in correct order, a line is drawn automatically to connect it to the preceding number in the sequence. The second part of the test is described below. The dependent variable is time to completion.

Executive function domain. Switching of attention task; part 2: In the second part of this task, the subject is presented with a pattern of 13 numbers (1-13) and 12 letters (A-L) on the screen and is required to touch numbers and letters alternatively in ascending sequence. The dependent variable is time to completion. Verbal Interference: This task taps the ability to inhibit automatic and irrelevant responses and has similarities to the Stroop task (1978). The subject is presented with colored words presented serially, one at a time. Each word is drawn from the following set of lower case words: red, yellow, green and blue. The color of each word is drawn from the following set of colors: red, yellow, green and blue. Below each colored word is a response pad with the four possible words displayed in black and in fixed format. The test has two parts. In part 1, the subject is required to identify the name of each word as quickly as possible after it is presented on the screen. In part 2, the subject is required to name the color of each word as quickly as possible. Responses are made on the screen by touching the appropriate word on the response pad. The dependent variable in each part is the number of words correctly identified. Maze Task: This task is a computerized adaptation of the Austin Maze (Walsh, 1985). The subject is presented with a grid (8x8 matrix) of circles on the computer screen. The object of the task is to identify the hidden path through the grid, from the beginning point at the bottom of the grid to the end point at the top. The subject is able to navigate around the grid by pressing arrow keys (up, down, left, right). The subject is presented with one tone (and a red cross at the bottom of the screen) if they made an incorrect move, and a different tone (and a green tick at the bottom of the screen) if they made a correct move. The trial ends when the subject completed the maze twice without error or after 10 minutes has elapsed. The dependent variable is the total maze time.

Language domain. Letter Fluency: Participants are required to generate by speech words that began with the letters F, A and S. 60 seconds are allowed for each letter and proper nouns were not allowed. The total number of correct words generated across the three trials is the dependent measure. Animal Fluency: Participants are required to name animals as quickly as possible for 60 seconds. Total correct serves as the dependent variable.

Memory domain. Verbal List-learning: The participants are read a list of 12 words, which they are asked to memorize. The list contained 12 concrete words from the English language. Words are closely matched on concreteness, number of letters and frequency. The list is presented 4 times in total and the subject is required to recall as many words as possible after each presentation. The subject is then presented with a list of distracter words and asked to recall them after presentation. Immediately following this, the subject is then

asked to recall the 12 words from the original list (short-delay recall trial). A long delayed recall trial is completed approximately 20 minutes later after a number of intervening tasks. A recognition trial is then completed after the delayed trial, also separated by a number of intervening tasks. The dependent variables are the number of words correctly recalled across the four learning trials.

Neuroimaging.

Diffusion tensor imaging methods. We will collect co-registered sagittal double spin-echo, echo-planar diffusion-weighted images as follows: 3 acquisitions with offset in slice direction by 0.0mm, 1.7mm and 3.4 mm, 2.5mm thick slices, 0.1mm inter-slice spacing, 30 slices per acquisition, 128x128 matrix, 21.7cm FOV (by interleaving the three, we get pseudo 1.7mm^3 resolution images), TR=7200, TE=156. Diffusion gradients will be applied in 12 non-collinear diffusion directions with 2 b magnitudes: 0, 1000 mm/s^2, NEX=3. We will use Siemen's MDDW protocol with partial echos and with interpolation on. The double-echo acquisition is not sensitive to eddy-current effects because it unwinds them during the second echo (Reese et al., 2003). The B0 field map acquisition will be: TR=500ms (1000ms with phased array), TE1: minimum possible, TE2 = TE1 + DeltaTE. DeltaTE = 4.92ms for 1.5T, Flip angle: 65 for 1.5T, FatSat: Off, Bandwidth: maximum, slice prescription: FOV, thickness, skip, pixel resolution, matrix size same as DTI. The entire brain will be imaged. Time per acquisition = 4:48 min (x3 runs). The diffusion tensor will be calculated per voxel using a nonlinear constrained fitting procedure. Fractional anisotropy (FA) and mean diffusivity (MD) will be computed from the diffusion tensor. Estimates of proton density and T2 will be computed using a two-point fit of the anatomical dual echo image data. **ROI analysis** - Using Analyze, MD and FA maps will be reoriented along the ACPC axis. ROIs will be sampled in this axial slice and two adjacent axial slices. Small 3 mm by 3 mm wide ROIs will be placed in the frontal white matter, genu and splenium of the corpus callosum, and left anterior limb of the internal capsule, and right and left genu of the internal capsule. MD and FA for each of the ROIs will be back calculated and used in the analyses as dependent variables. All neuroimaging data will be saved to CD, and backed up on the operating system. Script for unwarping:http://www.nmr.mgh.harvard.edu/~greve/fbirn/b0/.

Statistical Analyses.

Descriptive analyses will be conducted prior to the primary analytic plan in order to characterize the clinical and demographic features of the four patient groups. This initial examination will involve separate ANOVAs for demographic, psychosocial, and disease variables (infection duration, CD4 cell count, plasma viral load). MANOVAs will not be used in these preliminary analyses because we do not expect significant group effects on each of the dependent variables (e.g., ADLs).

Aim1: Note specific aim 1 is focused on development of the infrastructure and scientific collaborations and as such is not considered in terms of statistical analyses.

Aim 2:

The primary goal of the analyses for Aim2 is to determine if the HIV patients exhibit poorer cognitive performance and lower indices of white matter integrity compared to the healthy control subjects. The biomarker analyses will be carried out using analysis of variance (essentially a regression model), where group membership is the primary independent variable and estimated IQ is the covariate. For the group comparison, we will compare HIV mono-infection and the control group on cognitive function. The ANCOVA model specification is $cognition_{ik} = \mu_k + X_i\theta + e_{ik}$, where cognitive performance$_{ik}$ is the score for the ith patient in kth group (k=1,2). The indices are as follows: k=1 refers to HIV infection , k=2 for the uninfected control group. The X_i are covariates for which we will adjust; these will always include IQ estimate and will include other covariates that are significantly different between the groups, as determined by the exploratory analysis described above. The fitted model will permit hypothesis testing for all parts of Aim 2. In particular, we will use the fitted model to test hypotheses about cognitive performance across the groups, making appropriate adjustments for multiple comparisons. Beyond simple hypothesis testing for each group contrast, we will summarize the information in the biomarker using 95% confidence intervals for each contrast. Pearson correlations will be conducted to examine the degree of shared variance between HIV disease variables, DTI metrics (FA) and performance on the cognitive tests.

Resources in South Africa

Brain imaging facilities are housed in the Cross University Brain Imaging Centre (CUBIC). This is located in the Faculty of Health Sciences of the University of Stellenbosch, and is managed in collaboration with the University of Cape Town. The Siemens 3T Magnetom Allegra, is a compact, small bore, dedicated brain MRI scanner and is the only dedicated research MRI scanner of its kind in Africa. The Allegra is capable of gradient speeds with a slew rate of up to 400 T/m/ms. With the brain-optimised 22 cm field of view, these gradients allow coverage of the entire brain at a speed of 20 slices per second with concomitant advantages in respect of signal-to-noise ratio (SNR) over 1.5T. This increase in SNR allows greater spatial resolution and faster scanning. The platform is also equipped with specialized T1-based techniques such as time-of-flight MR angiography, very high contrast T2-weighted acquisitions, multidirectional diffusion weighted imaging from which ADC maps and diffusion tensors can be derived, BOLD imaging and finally multivoxel proton spectroscopy.

The Allegra system is equipped with a CP single channel head coil and a four channel phased array head coil. The four channel head coil can be used with integrated parallel acquisition techniques (iPAT) to further reduce the scan time without a proportional sacrifice in SNR. The Allegra is running on the Syngo MR2004A platform, and includes the BOLD, spectroscopy, 3D Basic and 3D Angio post-processing packages. The MRI suite is also equipped with a dedicated projector, measurement equipment and a stimulus-presentation computer for functional MRI studies, and a separate "mock" scanner facility.

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