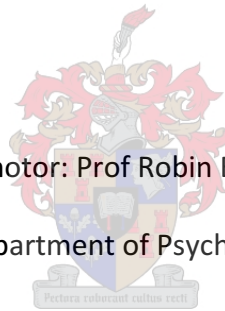# Measuring the impact of HIV on the fronto-striatal system

By

Stéfan du Plessis

Dissertation presented for a PhD degree in Psychiatry at Stellenbosch University

Promotor: Prof Robin Emsley

Department of Psychiatry

Faculty of Heath Sciences

Stellenbosch University

Co-Promotor: Dr Matthijs Vink

Departments of Experimental and Developmental Psychology

Faculty of Social Sciences

Utrecht University

Utrecht, The Netherlands

Declaration by the candidate:

With regard to chapters 2 through 5 the nature and scope of my contribution were as follows: S.D.P. conceived the study, performed the analysis and drafted the manuscript. S.D.P. also made changes to the manuscript as requested by the co-authors.

Authors Dr M Vink, Dr J Joska, Prof E Koutsilieri, Prof Dan Stein and Prof R Emsley contributed to chapters 2 through 5 and Dr A Bagadia contributed to chapters 3 through 5 as follow:

Name: Dr Matthijs Vink (m.vink2@uu.nl)

Address:

Departments of Experimental and Developmental Psychology, Faculty of Social Sciences, Utrecht University, Utrecht, The Netherlands

Nature of contribution: M.V. aided study conception, assisted in analysis, interpretation and participated in manuscript writing.

Name: Dr John Joska (john.joska@uct.ac.za)

Address: Department of Psychiatry, University of Cape Town, J-Block, Groote Schuur Hospital, Observatory, Cape Town, South Africa

Nature of contribution: J.A.J. provided feedback on the approach, interpretation and review of manuscript drafts.

Name: Prof Eleni Koutsilieri (eleni.koutsilieri@vim.uni-wuerzburg.de)

Address: Institute for Virology and Immunobiology, University of Würtzburg, Sanderring 2, 97070 Würtzburg, Germany

Nature of Contribution : E.K. aided interpretation of the findings as well as aiding in writing of the manuscript.

Name: Dr Asif Bagadia (asif@sun.ac.za)

Address: Department of Radiology, 5th Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town 7505, South Africa

Nature of Contribution: A.B inspected all the MRI scans for clinical abnormalities and provided input in this regard.

Name: Prof Dan Stein

Address: Department of Psychiatry, University of Cape Town, J-Block, Groote Schuur Hospital, Observatory, Cape Town, South Africa

Nature of Contribution: D.J.S provided feedback on study approach, interpretation of data as well as manuscript review.

Name: Prof Robin Emsley

Address: Department of Psychiatry, 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town 7505, South Africa

Nature of contribution: R.E provided feedback on study approach, interpretation of data as well as manuscript review.

Declaration with signature in possession of candidate and supervisor.

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the

candidate and the co-authors to chapters 2 through 5,

2. no other authors contributed to chapters 2 through 5 besides those specified above, and

3. potential conflicts of interest have been revealed to all interested parties and that the

necessary arrangements have been made to use the material in chapters 2 through 5 of this

dissertation.

Declaration with signatures in possession of candidate and supervisor.

Acknowledgements

1. My promotor, Professor Robin Emsley: His patience, knowledge and sound input brought the study together and ensured its conclusion.

2. My co-promotor, Dr Matthijs Vink, on who's large body of truly remarkable imaging work the present thesis depends.

3. Prof John Joska for making the seemingly impossible job of participant recruitment possible.

4. Professors Eleni Koutsilieri and Carsten Scheller for their sound input and advise on virology and neuro-chemistry, forming the basis of the present thesis.

5. Dr Paul Carey for his initial input that set the project into motion.

6. Dr Bruce Spottiswoode for helping me initially to get to grips with MRI imaging techniques.

7. Dr Laila Asmal for asking me to help her with her computer, which was the real start to my career in neuro-imaging.

8. Dr Hetta Gouse and Prof. Rob Paul for their assistance in determining the neuropsychological assessment battery as well as giving input into the neuropsychological assessments.

9. Mr Teboho Linda from the University of Cape Town for assisting in the recruiting of participants.

10. All my other co-authors for their invaluable support.

11. All my friends for their support for what amounted to grueling 6 year long academic marathon.

Funding

5

Dedication

I dedicate this work to both my parents, for their unwavering love, support and enthusiasm.

Table of Contents

Summary

HIV infection remains a major contributor to the global health burden despite the introduction of effective prevention strategies and the effectiveness of combined antiretroviral therapy (cART). Of particular importance is the impact of HIV on the brain. While cART has been successful in treating the more severe forms HIV induced cognitive impairment, the minor forms of impairment are now more prevalent. There remains some controversy with regard to the latter. Being diagnosed in the absence of other symptoms, there is some doubt that this category of cognitive impairment is valid at all. As such, investigating HIV induced functional brain changes may be helpful in the study of these forms of impairment. Although functional magnetic resonance imaging (fMRI) studies have thus far shown various forms of functional impairment in the brain, how these impairments relate to one another is unclear. Many key aspects of HIV's potential impact on the fronto-striatal system remain unexplored. Our overall objective was therefore to investigate the early impact of HIV on the brain using fMRI as an objective measurement tool.

First, we investigated the effects of HIV on the brain by performing a quantitative meta-analysis of all suitable fMRI data. Next, we proceeded to investigate the fronto-striatal network based on the results of the meta-analysis by performing fMRI imaging in a sample of HIV+ participants and matched HIV negative controls. Participants performed a stop-signal anticipation and a monetary incentive delay task to investigate the impact of HIV on important sub-networks of the fronto-striatal system. Finally, we investigated the relationship between striatal dysfunction with structural brain changes.

The results from the meta-analysis showed that HIV consistently affects the fronto-striatal system based on past fMRI studies. In subsequent studies, we demonstrated diminished functioning of the fronto-striatal networks involved in inhibition of voluntary movement as well as reward processing. Furthermore, this fronto-striatal dysfunction was also related to cortical atrophy often seen in HIV. Based on these findings, I therefore conclude that fronto-striatal dysfunction is a core component of HIV infection and needs to be considered in the assessment and management of all patients afflicted by this still very prevalent illness.

Opsomming

MIV-infeksie dra by tot die globale gesondheid las, ten spyte van die bekendstelling van effektiewe voorkomende strategieë en die doeltreffendheid van gekombineerde antiretrovirale terapie (ART). Van besondere belang is die impak van MIV op die brein. Terwyl ART suksesvol was in die behandeling van die meer ernstige klassifikasie van MIV geassosieerde kognitiewe inkorting, is die subtiele klassifikasie van hierdie inkorting nou nog meer algemeen. Die diagnose van meer ernstige kognitiewe inkorting is tans nog kontroversieel. Sedert dit gediagnoseer word in die afwesigheid van funksionele inkorting, is daar tans twyfel of hierdie kategorie van kognitiewe inkorting geldig is. Dit is dus nodig om te bepaal of MIV wel opsig self funksionele veranderinge in die brein veroorsaak. Alhoewel funksionele magnetiese resonansbeelding (fMRI) studies tot dusver verskeie manifestasies van funksionele inkorting in die brein toon, is die verband tussen die fMRI abnormaliteite en die virus nog onduidelik. Die spesifieke impak van MIV op die frontale-striatale stelsel is nog onbekend. Ons hoof doelwit was dus om die vroeë impak van MIV op die brein met behulp van fMRI as 'n objektiewe meetinstrument te ondersoek.

Eerstens het ons die gevolge van MIV infeksie op die brein ondersoek deur 'n kwantitatiewe meta-analise van alle geskikte fMRI data uit te voer. Gebaseer op die resultate van ons meta-analise, het ons voortgegaan om die frontale-striatale netwerk te ondersoek deur die toepassing van fMRI beelding in 'n groep MIV + deelnemers asook MIV negatiewe kontroles. Deelnemers het 'n stop-sein afwagtings taak en 'n monetêre afwagtings taak voltooi om die impak van MIV op belangrike sub-netwerke van die frontale-striatale stelsel te ondersoek.

Laastens, het ons die verhouding tussen striatale disfunksie en veranderinge in brein struktuur bestudeer.

Die resultate van die meta-analise het getoon dat MIV 'n beduidende invloed op die funksie van die frontale-striatale stelsel het. Verder het ons verminderde aktivering van die frontale-striatale netwerke wat betrokke is by die inhibisie van willekeurige beweging asook beloning prosessering gedemonstreer. Verder was hierdie fronto-striatale disfunksie ook verwant aan kortikale atrofie, 'n bekende bevinding in MIV infeksie. Op grond van hierdie bevindinge, kan ons dus aflei dat fronto-striatale disfunksie 'n hoofkomponent is van MIV-infeksie. Hierdie brein disfunksie moet dus oorweeg word in die assessering en behandeling van MIV.

Chapter 1

Introduction

Introduction

Background

Despite the effectiveness of combined antiretroviral therapy (cART) as well as prevention strategies, HIV remains a major contributor to the global health burden. This is especially relevant in sub-Saharan countries as the site of the highest prevalence rates [1]. Of particular concern is the impact of HIV on the brain. HIV can be considered a neurovirus as it is thought to enter the brain in the early stages of the illness via infected monocytes [2]. Consequently, the clinical presentation of untreated HIV dementia includes cognitive (e.g., memory impairment, with relative sparing of recognition)[3], neuropsychiatric (e.g., motivation, emotional control, social behavior)[4,5], and motor symptomatology (e.g., motor slowing) [6].

An estimated 50% of HIV+ individuals suffer from some degree of measureable cognitive impairment, referred to as HIV-associated neurocognitive disorder (HAND)[7]. While cART has been successful in treating the more severe forms of HAND, the minor forms have increased in prevalence [8]. This is attributed to the increased survival rate of patients treated with cART, with the resultant aging of patients being a key contributor to the increased prevalence of the minor forms of HAND [9,10].

There is some controversy regarding the diagnosis of the minor forms of HAND. Due in large part to the fact that the minor forms of HAND are diagnosed in the relative absence of functional impairment [11], doubt exists that these are valid categories of cognitive impairment. It has been suggested that the presence of mild neuropsychological

impairments may be better accounted for by premorbid conditions, other HIV-related comorbidities and normal ageing [12].

It remains, therefore, important to continue investigating the underlying functional brain disorders that ultimately result in these minor forms of impairment [7]. These are considered to represent early, potentially reversible pathological changes in HIV infection [13]. Key to addressing this issue is early diagnosis. However, to do so with more traditional forms of neurocognitive assessment presents a diagnostic challenge, as changes are subtle [3]. As such, utilizing a more sensitive measure such as functional and structural brain imaging may be helpful in the study of these forms of impairment. In order to do so, it is first necessary to consider the early underlying functional brain pathology thought to be common to the various forms of HAND.

As noted previously, the core clinical features of untreated HIV dementia are cognitive, behavioral as well as motor symptomatology, similar to that of Parkinson's disease. HIV was originally classified as a dementia potentially arising primarily from subcortical pathology [14]. Indeed, untreated HIV dementia has been associated with Parkinsonistic symptoms, indicating a potential dysfunction of the dopamine rich striatum [15]. Further investigation of post mortem studies in SIV infected macaque monkeys revealed striatal dopamine deficits early on in infection, particularly in the putamen [16]. Such findings have been corroborated in human post-mortem studies, where HIV has been found to pool early on mainly in striatal regions [17].

14

As striatal function is thought to play a key role in brain function [18], its potential role in HAND is still actively being investigated. Normal brain function depends on successful communication between the cortex and the striatum [18], which are connected in parallel, functionally segregated circuits [19]. One example of such a circuit connecting the prefrontal-regions with the striatum are the orbito-frontal-ventral striatal connections, considered important in goal directed activity or reward processing [20]. Another example is the prefrontal-striatal (i.e. inferior frontal gyrus – putamen) connection where cortico-striatal function is considered crucial in the inhibition of voluntary movement and therefore involved in executive functioning in general [21]. As the aforementioned core clinical features of HAND correspond with these functions, we propose that it is a result of the early impact of HIV on the striatum. This line of inquiry has yet to be investigated using neuro-imaging techniques.

Specifically, it remains to be demonstrated whether the impact of HIV on the fronto-striatal system is present in early untreated HIV infection, in the absence of well-known confounding factors. This would be a strong indication that HIV itself has an impact on the brain even in the absence of clinical symptomatology and subsequently implicate primary HIV infection in the minor form of HAND. It is therefore necessary to address the issue of the impact of HIV on the brain with the use of an objective measure.

There is a growing body of literature investigating functional brain changes attributable to HIV infection using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) [10]. fMRI could potentially be a sensitive measure of brain function loss due to HIV infection, as it has measured functional differences in HIV infected patients even in

the absence of neurocognitive impairment [22]. Measuring a subtle functional change in a brain region does not necessarily give an indication of its significance within the broader question of the impact of HIV on the brain. Volumetric brain imaging has also indicated prefrontal, and to a lesser extent subcortical atrophy, in HIV infection [23-25]. Furthermore, demonstrating a relationship between fronto-striatal function loss and prefrontal brain atrophy would serve as further confirmation of a substantial impact of HIV on this system. As such, our overall objective was to investigate the early impact of HIV on fronto-striatal function and its relationship with structural brain changes.

Research questions

The impact of HIV on brain function has been studied in terms of working memory and visual attention [22,26-28], episodic memory [29,30], simple motor function [31-33], visual stimulation [9,34], resting state [35-37] and mental rotation [38]. Although the findings from these studies showed some form of functional impairment, the relationship between these impairments were not addressed.

A number of key aspects regarding the potential impact of HIV on the fronto-striatal system remain unexplored, with several unanswered questions:

Firstly, does HIV have a consistent impact on the fronto-striatal system, or a more non-specific/generalized effect? No meta-analysis of fMRI findings had previously been performed to attempt to categorize the effect of the virus on brain function in a systematic way.

Secondly, given that HIV infection causes clinically measurable differences in motor responses [6], does it disrupt the primary GO (i.e. voluntary habituated responses) and STOP (i.e. The inhibition of a habituated voluntary responses) functions integral to large portions of the fronto-striatal network [39]? This would involve investigating the impact of HIV on the function of the inferior frontal gyrus and its associated striatal regions (i.e. the putamen). To date no study has investigated the impact of HIV on brain function during the inhibition of voluntary movement.

Thirdly, as it is thought that HIV primarily impacts striatal regions due to viral pooling early on in infection [40], is there a measurable impact on the non-motor fronto-striatal system as well? Specifically, does HIV have an impact on the ventral-striatal regions known to be involved in reward processing [41]? This would involve investigating the function of the orbito-frontal cortex and the ventral striatum.

Finally, while prefrontal cortical thinning is a well-known finding associated with HIV infection, it is still unclear whether this reflects a direct effect of the virus, whether it is related to disruption of subcortical function or whether it is better explained by epiphenomena, such as drug abuse or comorbid medical conditions.

Specific aims

This study had the following specific aims:

1. To systematically review the literature reporting fMRI findings in HIV-infected patients and to quantitatively investigate whether HIV has a predilection for the fronto-striatal system as proposed.

2. To investigate whether HIV infection disrupts the basic functional processes of the fronto-striatal system, by impairing the GO and STOP processes of the fronto-striatal network, using a Stop Signal Anticipation fMRI Task.

3. To investigate the effect of HIV infection on reward processing, a distinct function of the fronto-striatal system, crucial in goal-directed activity utilizing a monetary incentive delay task.

4. To investigate the potential relationship between prefrontal cortical thickness and subcortical function in HIV+ patients using a combination of brain morphometry and fMRI.

Hypotheses

Based on our review of the literature, we formulated the following research hypotheses:

1. In view of the fact that HIV infection consistently effects the fronto-striatal system, we hypothesized that meta-analysis of available data would demonstrate functional differences mainly in the frontal regions as well as the striatum.

2. Being a subcortical dementing process, HIV would demonstrate an impact on the putamen during reactive inhibition on the SSAT. A disruption of frontal functioning would be evident on proactive inhibition. An impact on the basic function of the primary motor cortex would be evident during timed GO responses.

18

3. HIV infection would affect the functioning of the ventral-striatum, active during reward cue processing. An effect on the cortex would be seen in the orbito-frontal cortex during reward outcome.

4. Being a subcortical dementing process, we predicted that functional differences in the striatum would be related to frontal atrophy commonly seen in HIV infection. Note that the relationship between striatal dysfunction and striatal atrophy is less predictable, as subcortical dysfunction in HIV has been demonstrated in the absence of subcortical atrophy.

General Methods

A full description of the study methodology is provided in the accompanying manuscripts. Briefly, the methodology that we employed was as follows:

Subject selection

The study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University and the Human Research Ethics Committee of the University of Cape Town, Cape Town, South Africa.

To address the specific impact of HIV on the brain, we recruited participants who had no history of substance abuse [33]; non-smokers [42] as well as no other significant psychiatric pathology that could have influenced our measurements of cognition, brain function and structure. Furthermore, we chose to primarily investigate untreated HIV+ participants, as cART treatment has been shown to have an impact on brain function in its own right [43].

19

Apathy

In the original proposal, we planned to investigate clinical symptoms of apathy, as measured by the Apathy Evaluation Scale (AES) by Marin et al. [44]. We could not however replicate findings of increased levels of apathy in our infected HIV population, with both groups scoring well below 38, the cut-off used for the diagnosis of apathy (Control: M = 25.58; HIV: M = 29.70; t(33) = -1.592; p = .121). We therefore chose to forgo the use of apathy as a measure of clinically measureable impairment and to focus on the objective measurement of HIV induced disruption of brain function with fMRI.

Systematic review and meta-analysis

In article 1, we followed the standardized PRISMA guidelines [45] to perform a systematic review of all the available literature in which fMRI was used to study brain function in patients with HIV infection. To determine the level of agreement across fMRI studies, that is an Activation Likelihood Estimate (ALE) [46], we utilized a tool specifically developed to test for above chance inter-experiment convergences (GingerALE)[46,47]. See methods section in article 1 [48], for further detail.

fMRI tasks used

In article 2 we performed the Stop Signal anticipation task (SSAT), based on work by Logan et al.[49]. During this task, subjects were required to give timed GO responses with occasional STOP signals occurring at fixed probabilities[50].

In article 3 we performed the monetary incentive delay task based on work by Knutson et al. [51]. This paradigm assesses behaviour as well as functional brain activity-associated reward anticipation and reward outcome [52].

Freesurfer structural data processing

In article 4, we performed a FreeSurfer structural analysis. Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite (http:// surfer.nmr.mgh.harvard.edu/). As FreeSurfer is computationally intensive, reconstructions were performed utilizing custom batching scripts, on the Centre for High Performance Computing (CHPC) Rosebank, Cape Town, Sun Intel Nehalem cluster (http://www.chpc.ac.za/). Scans were processed and analyzed using FreeSurfer stable release version 5.1. The technical details of these procedures are described in prior publications [53].

21

Chapter 2

HIV infection and the fronto–striatal system: a systematic review and meta-analysis of fMRI studies

HIV infection and the fronto–striatal system: a systematic review and meta-analysis of fMRI studies

Stéfan Du Plessis[a], Matthijs Vink[b], John A. Joska[e], Eleni Koutsilieri[c], Dan J. Stein[d,e] and Robin Emsley[a]

Functional MRI studies investigating the impact of HIV on the brain have implicated the involvement of fronto–striatal circuitry. However, to date there is no review and meta-analysis of this work. We systematically reviewed the literature and performed a meta-analysis of functional magnetic resonance imaging (fMRI) studies in HIV-infected individuals using a well validated tool recently developed for use in fMRI, 'GingerALE'. Twenty-one studies (468 HIV+, 270 HIV− controls) were qualitatively reviewed, of which six (105 HIV+, 102 controls) utilized fMRI paradigms engaging the fronto–striatal–parietal network, making a quantitative analysis possible. Our meta-analysis revealed consistent functional differences in the left inferior frontal gyrus and caudate nucleus between infected participants and controls across these studies. This fronto–striatal dysfunction was qualitatively related to cognitive impairment, disease progression and treatment effects. Although further work needs to be done to further delineate the potentially confounding influence of substance abuse and HIV-related comorbidities, as well as HIV's effect on functional haemodynamic vascular coupling, these findings indicate that further investigation of the fronto–striatal sub-networks in HIV-infected patients is warranted.

© 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

Introduction

The effects of HIV on the brain remain a concern even in the era of combination antiretroviral therapy (CART) as around 50% of infected individuals are estimated to suffer from some degree of cognitive impairment [1]. Currently, the effects of HIV on the brain are assessed by neurocognitive testing and are termed HIV-associated neurocognitive disorders (HAND) [2]. With the advent of CART, there has been some success in the treatment of HAND, as the more severe forms of these disorders have decreased in incidence [3,4]. However, with the resultant decrease in mortality there has been an increase in the overall prevalence of HAND [3–5]. Three categories of HAND based on neuropsychological assessment are currently recognized, namely HIV-1–associated asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-1–associated

[a]Department of Psychiatry, University of Stellenbosch, Cape Town, South Africa, [b]Rudolf Magnus Institute of Neuroscience, University of Utrecht, Utrecht ,The Netherlands, [c]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany, [d]Medical Research Council (Unit on Anxiety and Stress Disorders), and [e]Department of Psychiatry, University of Cape Town, Cape Town, South Africa.

Correspondence to Stéfan du Plessis, Department of Psychiatry, 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Rylaan, Tygerberg, Cape Town, 7505, South Africa.

E-mail: stefandup@sun.ac.za

dementia (HAD), which is the more severe form of the disorder [2].

It must be noted, however, that there is some debate regarding the ANI category. The absence of clinically detectable loss of function raises the issue of whether the category is clinically valid at all. The presence of mild neuropsychological impairments may be accounted for by premorbid conditions, other HIV-related comorbidities as well as normal ageing [6]. As a group, the milder forms of cognitive impairment (i.e. MND and ANI) are highly prevalent and may have a substantial impact on health-related outcomes and quality of life. There is some evidence that the presence of MND, and possibly ANI, could represent early, potentially reversible functional and pathological changes [7]. The problem of establishing mild-to-moderate loss of function in developing world settings is a particular challenge. Reasons include individuals frequently under-reporting self-rated impairment [8]. This makes clinical diagnosis difficult, and the need for reliable biomarkers an even more pressing concern [6,9].

Furthermore, because of a lack of biomarkers sensitive to early changes during HAND, little is known of the underlying neuropathological events leading up to impairment seen in HAND [9,10] which could potentially aid early diagnosis and treatment strategies.

To understand how the effects of HIV on the brain develop into HAND, it is necessary to know which brain structures are primarily involved, and how this relates to viral infection. The typical neuropsychiatric disorder associated with untreated HIV is best described as a 'sub-cortical' or 'fronto-sub-cortical' dementia [11], and this is supported by neuropathological findings, with the features of HIV-encephalitis (HIVE) being most commonly found in the putamen and caudate [12–14]. HIV is thought to disrupt the dopamine-rich striatum by means of neuroinflammation stimulated either by viral proteins such as Tat and gp120 [15,16] or by activated microglia [17].

One potential method of investigating the underlying neuropathological mechanisms leading to HAND is blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) [18]. This technique includes task-based fMRI, resting state fMRI [19], arterial spin labelling (ASL) [20] as well as contrast-aided 'perfusion' fMRI intended to measure regional cerebral blood flow (rCBF) [21] and cerebral blood volume (CBV) [22]. There is a growing literature investigating the effects of HIV on brain function utilizing these fMRI techniques. fMRI is potentially a sensitive and safe technique to detect functional deficits, even in the absence of overt neurocognitive impairment in HIV [23]. These functional changes could represent the earliest measurable effects of the virus on human brain function,

thereby providing a clear signal of involvement even before neuropsychological deficits are apparent. Showing suitable spatial and temporal resolution, as well as being related to specific functional processes, fMRI has the potential to explore these functional changes associated with HIV infection and to elucidate how these relate to disease severity [10].

Although several reviews of neuroimaging in HIV have been conducted [24–27], to our knowledge no meta-analysis has been performed specifically for fMRI studies, and particularly none have utilized recently developed quantitative methods for meta-analysis in neuroimaging [28]. The need for such a review is underscored by a previous review highlighting the potential of functional and structural neuroimaging techniques in HIV in early detection and treatment monitoring [25].

The present study aims to systematically review the literature reporting fMRI findings in HIV-infected patients and to quantitatively investigate the effects of HIV by means of an activation likelihood estimation (ALE) [28] on brain function, and to relate these effects qualitatively to measures of cognitive impairment (i.e. HAND), mechanisms of HIV pathogenicity, treatment effects as well as the effect of HIV on functional haemodynamic vascular coupling.

Methods

The qualitative systematic search method we used was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) [29]. A literature search was performed in October 2012 using two online search engines, the National Library of Medicine (PubMed) and Sciverse Scopus. The following search terms were used: fMRI, BOLD imaging, blood oxygen level dependent, fMRI, arterial spin labeling, resting state, resting state fMRI, functional connectivity, resting-state fMRI, HIV dementia, HIV-associated neurocognitive disorder, HIV-associated neurocognitive disorder, HIV brain injury, HIV brain injury, HIV cognitive impairment, HIV cognitive impairment, HIV brain function, HIV brain function, HIV brain activation, HIV brain function, abnormal brain activation, abnormal brain function. References of the identified studies were cross-checked for any additional relevant studies.

Data collection

All identified articles were captured in a standard electronic database. General study information (author names, publication dates, etc.), patient population demographics, clinical assessment instruments and diagnostic criteria used, all available neuropsychological test results, potential confounds as well as fMRI findings were captured (See Table 1 for summary).

Eligibility criteria for qualitative systematic review

BOLD fMRI studies in HIV+ CART treated/treatment-naive human adult (male/female) patients were included in the present review. As our primary aim was to investigate brain dysfunction associated with HIV infection, we did not limit our review to patients with documented HAND. Both prospective and longitudinal studies were included and no time frame was specified.

Only original studies with at least one HIV+ group were included. Duplicates were identified and removed. Studies that included a cohort that was previously reported were noted and not considered in the meta-analysis. Studies in all languages were considered.

Meta-analysis eligibility criteria

Although all variants of BOLD fMRI imaging where included in the systematic review, studies specifically examining HIV-related brain dysfunction using MRI imaging methods that would provide suitable peak activation co-ordinates as well as comparable fMRI paradigms were considered for inclusion in our ALE meta-analysis.

The subset of studies that provided local maximum activation co-ordinates of inter-group differences of a HIV+ group and a control group were grouped together according to the functional paradigms that were used. Where two or less studies that utilized similar fMRI paradigms were available, those paradigms were excluded, as we considered this too few to provide useful information in an ALE meta-analysis. Also, we only included studies that utilized paradigms examining higher cognitive functions. To be included, studies needed to address potential confounding chronic medical or neuropsychiatric illnesses as well as substance abuse. Studies that could not account for the effects of advanced age (> 65 years) were also excluded from the meta-analysis.

Meta-analysis

For assessing cross study agreement, a meta-analysis was performed using GingerALE version 2.1 (www.brain-map.org) [30] on selected studies. In brief, this method estimates the above-chance convergence of activation by testing the likelihood of a uniform spread throughout the brain. The GingerALE method seeks to delineate where the inter-experiment convergences are higher than would be expected if the results were independently distributed [31]. Peak activational co-ordinates are used to derive three-dimensional Gaussian probability distributions of the regional activation variability of a given co-ordinate. In the current meta-analysis, wherein activation differences between HIV+ and HIV− groups are investigated for each of the experiments, a patient number of the smaller group was used to provide the full-width-half-maximum (FWHM) value, giving a more conservative estimate. The resulting modelled activation probabilities are combined into a modelled activation map. ALE scores are then computed for each voxel based on the union of the modelled activation maps. It is assumed that functional activation occurs mainly in grey matter. The resulting ALE computation is therefore restricted to a broadly defined grey matter mask, based on a more than 10% chance of a voxel to be grey matter as defined in the International Consortium for Brain Mapping (ICBM) tissue probability maps [30,32]. This resulting ALE map is then tested against an empirically derived null-distribution. This random-effects inference is therefore based on the above-chance convergence between experiments, not between foci. Results were corrected for multiple comparisons using the false discovery rate (FDR) as is standard in GingerALE. Results are reported at a 0.05 FDR rate. The peak co-ordinates of comparable BOLD fMRI group differences between HIV and healthy controls were used, and if needed, converted into Talaraich space using the GingerALE conversion tool. We report the results of the meta-analysis first, followed by the complementary pertinent information gathered from the remainder of the studies included in the qualitative systematic review.

Results

In total, 42 matching articles were retrieved on PubMed and 236 on Scopus, from which 21 studies were found to satisfy eligibility criteria for this review, totalling 468 HIV+ participants and 270 controls. We included 19 cross-sectional studies [21−23,33−48], one longitudinal study [49] and one study reporting a posthoc analysis of an already included study [50]. Of the 21 studies included in the review, a subset of six BOLD fMRI studies utilizing similar fronto−parieto−striatal engaging tasks were included in the ALE meta-analysis [23,34,37,40,48,49]. These six studies included peak activation differences of 207 participants (105 HIV positive, 102 controls). Of the six studies included, all controlled for confounds. Five studies excluded participants with a past history of substance dependence [34,37,40,49], whereas one study excluded participants with recent substance abuse [48]. Four studies confirmed no current substance use by means of urinary drug tests [23,34,49,51]. One study included a patient with a history of past substance abuse, in the absence of dependence [40]. The study controlled for this by including a control matched for substance use. All six studies included patients below 65 years of age. Study information is summarized in Table 1.

Demographics

The 21 fMRI studies included in the systematic review consisted of 468 participants (73% male, 27% female) with weighted mean age of 42 ± 4 years. Virtually all of the

Table 1. Summary data of functional MRI studies performed in HAND.

Nr	1st author	fMRI type	Task name	Paradigm	Network	On cART	Neurocognitive impairment	Brief summary
1	Tracey I 1998	Contrast Aided CBV	NA	NA	NA	NA	77%	Mild–moderate	Patients with cognitive impairment showed increases in CBV in deep grey matter.
2	Chang L (2000)	Contrast Aided rCBF	NA	NA	NA	NA	NA	Mild–moderate	rCBF decreases in bilateral IFG and parietal regions. Increases were found in inferior parietal white matter.
3	Chang L (2001)	BOLD fMRI	NBACK	WM, visual attention	FSP	91%	Mild–moderate	Increased BOLD activation in frontal and parietal regions.
4	Ernst T (2002)*	BOLD fMRI	NBACK	WM, visual attention	FSP	90%	Unimpaired	Increased BOLD activation in lateral prefrontal cortex.
5	Ernst T (2003)	BOLD fMRI and MRS	NBACK	WM, visual attention	FSP	93%	Mild–moderate	Positive correlation between BOLD signal in parietal and lateral prefrontal cortex and metabolites predominant in glial cells.
6	Chang L (2004)*	BOLD fMRI	NBACK	WM, visual attention	FSP	83%	Mild–moderate	Decreased BOLD activation in VA with contra-lateral increases in VA.
7	Ances B (2006)	ASL	NA	NA	S	76%	All categories	Decreases in Caudate blood flow with increases in neurocognitive impairment.
8	Castelo J (2006)	BOLD fMRI	Episodic encoding task (pictures)	Episodic memory	FSPT	71%	Unimpaired	Decreased BOLD activation in right hippocampus, right inferior frontal gyrus and increases in lateral frontal/posterior parietal regions.
9	Chang L (2007)*	BOLD fMRI	Nonverbal visual attention task	Nonverbal visual attention	FSP	50%	Mild–moderate	Increased BOLD activation in right frontal regions.
10	Juengst SB (2007)	BOLD fMRI	Finger tapping	Motor task	MS	NA	All categories	Increased BOLD activation in right frontal regions in VA.
11	Ances BM (2008)	BOLD fMRI	Checkerboard and finger tapping	Visual and motor stimulation tasks	MS and V	100%	All categories	Increased BOLD activation in MS in low-CPE relative to high-CPE HAART.
12	Melrose R. (2008)*	BOLD fMRI	Semantic event sequencing	Semantic event sequencing	FS	91%	Unimpaired	BOLD hypoactivation in FS.
13	Ances BM (2009)	ASL	NA	NA	S and V	80%	Unimpaired	Reduced rCBF within LN and V.
14	Ernst T (2009)*	BOLD fMRI	Nonverbal visual attention	Nonverbal visual attention	FSP	100%	Unimpaired	Significant 1-year follow-up increases in BOLD activation in FSP.
15	Maki PM (2009)	BOLD fMRI	Episodic memory (verbal) task	Episodic memory	FSPT	42%	Unknown	Decreased BOLD activation during memory encoding and increased activation during delayed recognition.
16	Ances B (2010)	BOLD-fMRI	Checkerboard	Visual stimulaion task	V	83%	Unimpaired	Lower baseline CBF and BOLD activation in V. Increased test/re-test inter-subject variablity in HIV+ in baseline CBF.
17	Ances BM (2010)	BOLD fMRI and ASL	Checkerboard	Visual stimulation task	V	60%	Unknown	Functional BOLD signal and CBV decreases in visual cortex.
18	Ances BM (2011)	BOLD fMRI and ASL	Finger tapping	Motor task	MS	80%	Unknown	Increased BOLD signal in LN.
19	Meade CS (2011)	BOLD fMRI	Delay discounting task	Reward	DLPC/OFC/IPL/SMA	100%	Unknown	BOLD Deficits in executive network in HIV+ cocaine users.
20	Qiu W (2011)	BOLD fMRI	NA	Resting State: regional inhomogeneity	Whole Brain	NA	Mild–moderate	Decreased BOLD in ReHo in FSPT and occipital gyrus.
21	Schweinsburg BC (2012)*	BOLD fMRI	Mental rotation task	Mental rotation	FSP	91%	Unknown	Increased BOLD activation in FSP.

ASL, arterial spin labeling; BOLD fMRI, blood oxygen level dependant functional magnetic resonance imaging; DLPC, dorsolateral prefrontal cortex; FSP, fronto–striatal–parietal network; FSPT, fronto-striatal-parietal-temporal network; IPL, inferior parietal lobule; MS, motor netvor; NA, not applicable; OFC, orbitofrontal cortex; SMA, supplementary motor area; S, striatum; V, visual cortex; WM, working memory; *Study included in meta-analysis; CBV, cerebral blood volume; CBF, cerebral blood flow; CPE, Central nervous system penetration effectiveness; IFG; LN, lentiform nucleus; MRS, magnetic resonance spectroscopy; VA, visual attention network.
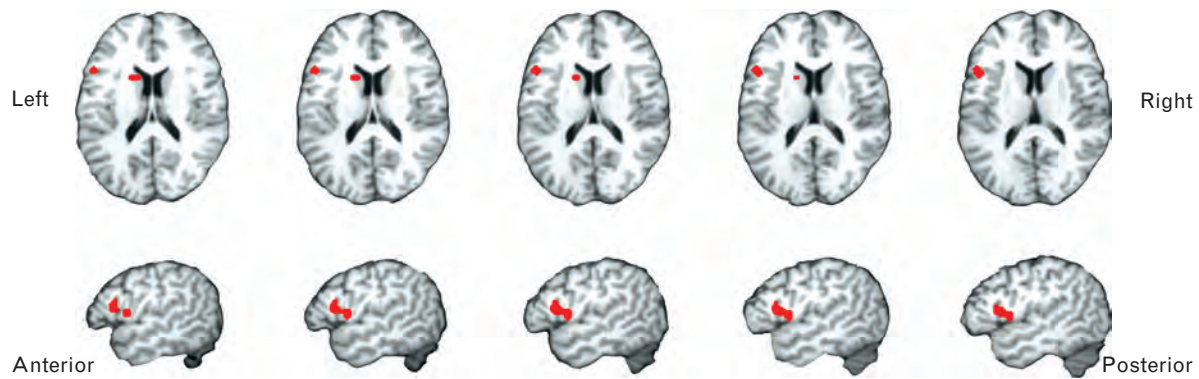
Fig. 1. Results of Activation Likelihood Estimation (ALE) meta-analysis showing significant overlap across studies of HIV-related dysfunction in the fronto–striatal system. Figure shows the summary results of the ALE meta-analysis [30]. Red represents above chance overlap of fMRI differences between HIV+ patients and seronegative controls across six studies that utilized similar tasks to activate the fronto–striatal–parietal system. HIV+ participants showed significant differences (FDR < 0.05) in the left IFG (−46,12,10) as well as in the ipsilateral caudate (−14,9,16) across studies.

participants were from the United States ($n = 20$), where the clade B viral subtype is most prevalent [52], and one study included participants from China. The weighted mean $CD4^+$ cell counts for all studies was 444.79 ± 137.43 cells/µl. The majority (82%) of HIV+ study participants were receiving CART.

Cognitive impairment

Cognitive impairment measurements ranged from no impairment to HAD: Only three studies included patients with all categories of cognitive impairment. Seven studies included patients with mild/moderate impairments and three included all cognitive impairment categories. Six studies were performed in relatively cognitively unimpaired populations and five studies did not report on the degree of cognitive impairment present in the study participants.

Meta-analysis

Of the 21 studies included in the systematic review, six task-based BOLD fMRI studies reported activation of the fronto–parieto–striatal network in healthy controls [23,34,37,40,48,49]. We were therefore able to group all of these studies together, as they utilized similar information processing steps − that is, selective attention to visual information, retaining of relevant information in working memory and manipulation of this information to successfully perform the task [53,54].

The fMRI paradigms we identified as being suitable for our meta-analysis included a sequential working memory letter NBACK paradigm ($n = 1$) [23], nonverbal visual attention tasks ($n = 3$) [34,37,49], mental rotation ($n = 1$) [48] and a semantic event sequencing task ($n = 1$) [40].

Significant convergence of relative increases found in activation likelihood estimation analysis

Two significant clusters were found at the recommended cluster threshold of 112 mm³, based on the FDR threshold ($P = 0.05$), which calculates the number of potential false positives that could arise due to the multiple comparisons that were performed. The clusters were located on the left inferior frontal gyrus (IFG) and the left caudate. Although some increases were seen in the parietal cortices, these clusters were below the cluster threshold. The majority of convergence was therefore found in the fronto–striatal regions (Fig. 1; Table 2).

Discussion

This article reports the results of the first quantitative meta-analysis of BOLD fMRI literature on the effects of HIV on brain function. Of the 21 studies included, six contained sufficient data for an ALE meta-analysis [30]. Overall, the meta-analysis provides evidence to suggest that HIV infection causes hyperactivation in the IFG as

Table 2. Meta-analysis results: HIV-related differences in the fronto–striatal network.

Cluster Nr	Volume (mm³)	*x*	*y*	*z*	Location
1	952	−46	12	10	Left inferior frontal gyrus
2	176	−14	9	16	Left caudate nucleus

well as left caudate, implicating the fronto−striatal system in HAND. Although individual studies report on findings in other regions including the insula, parietal cortex, thalamus, supplementary motor area, precentral gyrus and occipital cortex (see Table 1), the majority of the convergence measured across studies was confined to the fronto-striatal network involving the left IFG and the left caudate.

This is not surprising, considering that while tasks requiring simple attention are often preserved in HIV, except in cases of severe HAD, it is well recognised that tasks requiring complex information processing and selective attention are often sensitive to the effects of HIV [55].

Apart from task related activity, which actively engages the cortex, several fMRI BOLD studies report baseline rCBF reductions in the fronto−striatal system. Chang et al. reported bilateral reductions of rCBF in bilateral inferior frontal gyri and Ances et al. found reductions in the lentiform nuclei [21,35,41]. Furthermore, a relationship was found between reduced caudate rCBF and greater neurocognitive impairment [35].

Relationships between functional magnetic resonance imaging fronto−striatal findings and HIV-associated neurocognitive disorders

Comparisons across studies investigating how brain functional differences relate to cognitive impairment are complicated by the different methods of neurocognitive assessment utilized across studies. Despite the fact that the majority of studies included participants with mild or no measurable cognitive impairment, measurable functional brain changes were demonstrated in these patients. This suggests that fMRI has the potential to detect early effects in HIV-infected patients in the absence of overt cognitive impairment, and that it is sensitive to detecting underlying early neurobiological effects of the virus [23,36,40,41,43,49]. Future studies should address whether these functional brain changes are precursors to HAND, or whether they perhaps even represent physiological responses to HIV infection that protect against the development of HAND.

Mechanism of fronto−striatal impairment

The exact mechanism of the reported fronto−striatal activation changes in HAND remains unclear. It is generally considered that HIV-mediated cognitive impairment is the result of viral-induced neuro-inflammation, as the degree of monocyte activation as well as the level of microglial activation has proven to be a more reliable correlate with HIV-D than viral load or even active viral replication [56,57]. One possibility, proposed by Ernst et al. [23] is that this HIV-induced inflammation impairs neural efficiency with resultant compensatory increases in neuronal activation with task demands. In support of their hypothesis they reported a positive correlation between BOLD signal strength in the lateral prefrontal cortex as well as the posterior parietal cortex, with elevated basal ganglia metabolites including myo-inositol and total creatine as measured with magnetic resonance spectroscopy that are putatively predominant in glial cells [50]. In addition, dopamine neurocircuitry may be involved. It is well recognized that dopamine is an important modulator of the activity of the fronto−striatal networks [58], thereby making it a likely candidate for involvement in dysfunction in these systems. In support of this possibility is the finding that altered dopaminergic neurotransmission takes place as early as 2 months following immunodeficiency infection [17,59−62], and in both HIV-infected humans and SIV-infected macaques increased dopamine availability was observed [63,64], whereas intracellular levels of dopamine were reduced [17,59,65]. Further validation studies examining such neurochemical and immuno-logical disturbances in conjunction with fMRI have yet to be performed.

Limitations of reviewed studies

The fMRI studies performed in HIV-positive patients thus far are limited by small sample sizes, ranging from 6 to 42 HIV-positive participants per study. Participants tend to be mostly male, therefore possible sex differences on fMRI activation are uncertain. The potential effect of HIV on functional haemodynamic vascular coupling (i.e. the basic assumption that changes in regional blood flow reflect neuronal activity) still needs to be fully explored, with studies to date reporting conflicting results [38,44]. More importantly, fMRI studies tend to include samples of patients mostly treated with CART. Two studies have specifically investigated the possible effects of CART, demonstrating potential CART-related signal increases in the frontal and parietal cortices [37,39]. More CART naive sample groups are therefore needed to explore the effects of HIV in the absence of CART.

As viral transmission is often associated with intravenous drug abuse, especially in the United States where up to 15−30% of intravenous drug abusers are HIV+ [6,66], care has to be taken to account for its effects. Specifically abuse of opiates and amphetamines has been associated with a more severe clinical course as well as a more rapid progression of HAND [67]. As drugs of abuse have their own direct effects on the fronto−striatal system [68], as well as potential immune-modulatory effects [69], there is a need to explore their confounding as well as synergistic effects in more detail [69,70]. Two fMRI studies included in the current review examined potential synergistic effects between HIV and drugs of abuse: Meade et al. [46] demonstrated HIV-positive patients who abuse cocaine have abnormal functioning in their executive networks relative to substance naive HIV-positive controls, when performing a delay-discounting task. Beau et al. [71] furthermore showed both HIV and a previous history methamphetamine dependence have potentially

independent effects on the lentiform nuclei function during a tapping task as well as cerebral blood flow. Although few in number, these studies underscore the necessity to control for the various forms of drug-related disorders. Importantly, most studies included in the current review controlled for past drug dependence ($n = 12$), with 11 studies performing urinary drug tests immediately prior to scanning.

The impact of other comorbidities such as opportunistic infections as well as head injuries need to be considered. Most studies in the current review excluded comorbid general medical conditions on history. However, only three studies performed serological tests for syphilis. The effects of hepatitis C as well as cardiovascular disease, considered potentially important comorbid conditions in HAND, have yet to be explored in conjunction with fMRI measurements [6,72,73].

Conclusion

This systematic review and meta-analysis shows convergence in findings of hyperactivation in the left IFG and left caudate in patients with mild-to-moderate HAND. This increased activation could potentially be due to compensation in the fronto–striatal–parietal network, and could be linked to abnormal striatal dopaminergic neurotransmission or perhaps due to HIV's general predilection for deep grey matter areas. Important confounding effects of HIV on functional haemodynamic vascular coupling, CART, drugs of abuse, co-infections such as neurosyphilis and hepatitis C as well as cardiovascular disease in an ageing HIV population need to be explored.

Acknowledgements

Conflicts of interest
The authors declare no conflicts of interest.

References

1. Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, *et al.* **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy.** *Neurology* 2010; **75**:2087–2095.
2. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, *et al.* **Updated research nosology for HIV associated neurocognitive disorders.** *Neurology* 2007; **69**:1789–1799.
3. Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, *et al.* **HIV-associated neurologic disease incidence changes: multicenter AIDS Cohort Study, 1990-1998.** *Neurology* 2001; **56**:257–260.
4. Sacktor N, McDermott MP, Marder K, Schifitto G, Selnes OA, McArthur JC, *et al.* **HIV-associated cognitive impairment before and after the advent of combination therapy.** *J Neurovirol* 2002; **8**:136–142.
5. McArthur JC. **HIV dementia: an evolving disease.** *J Neuroimmunol* 2004; **157**:3–10.
6. Bonnet F, Amieva H, Marquant F, Bernard C, Bruyand M, Dauchy F-A, *et al.* **Cognitive disorders in HIV-infected patients: are they HIV-related?** *AIDS* 2013; **27**:391–400.
7. Marcotte TD, Ghate M, Deutsch R, Letendre S, Meyer R, Godbole S, *et al.* **Earlier initiation of ART results in better neurocognitive functioning.** *19th Conference on Retroviruses and Opportunistic Infections.* 2012. http://www.retroconference.org/2012b/Abstracts/44698.htm [Accessed 24 May 2013].
8. Heaton RK, Marcotte TD, Mindt MR, Sadek J, Moore DJ, Bentley H, *et al.* **The impact of HIV-associated neuropsychological impairment on everyday functioning.** *J Int Neuropsychol Soc* 2004; **10**:317–331.
9. McGuire D. **CSF biomarkers in HIV dementia: through a glass darkly.** *Neurology* 2009; **73**:1942–1944.
10. Logothetis NK. **What we can do and what we cannot do with fMRI.** *Nature* 2008; **453**:869–878.
11. Navia BA, Jordan BD, Price RW. **The AIDS dementia complex: I. Clinical features.** *Ann Neurol* 1986; **19**:517–524.
12. Reyes MG, Faraldi F, Senseng CS, Flowers C, Fariello R. **Nigral degeneration in acquired immune deficiency syndrome (AIDS).** *Acta Neuropathol* 1991; **82**:39–44.
13. Navia BA, Cho ES, Petito CK, Price RW. **The AIDS dementia complex: II. Neuropathology.** *Ann Neurol* 1986; **19**:525–535.
14. Cornford ME, Holden JK, Boyd MC, Berry K, Vinters HV. **Neuropathology of the acquired immune deficiency syndrome (AIDS): report of 39 autopsies from Vancouver, British Columbia.** *Can J Neurol Sci* 1992; **19**:442–452.
15. Agrawal L, Louboutin J-P, Marusich E, Reyes BAS, Van Bockstaele EJ, Strayer DS. **Dopaminergic neurotoxicity of HIV-1 gp120: reactive oxygen species as signaling intermediates.** *Brain Res* 2010; **1306**:116–130.
16. Purohit V, Rapaka R, Shurtleff D. **Drugs of abuse, dopamine, and HIV-associated neurocognitive disorders/HIV-associated dementia.** *Mol Neurobiol* 2011; **44**:102–110.
17. Scheller C, Sopper S, Jenuwein M, Neuen-Jacob E, Tatschner T, Grünblatt E, *et al.* **Early impairment in dopaminergic neurotransmission in brains of SIV-infected rhesus monkeys due to microglia activation.** *J Neurochem* 2005; **95**:377–387.

18. Ogawa S, Lee TM, Kay AR, Tank DW. **Brain magnetic reso-nance imaging with contrast dependent on blood oxygenation.** *Proc Natl Acad Sci USA* 1990; **87**:9868–9872.

19. Cole D, Smith SM, Beckmann CF. **Advances and pitfalls in the analysis and interpretation of resting-state FMRI data.** *Front Syst Neurosci* 2010:1–15.

20. Detre JA, Wang J, Wang Z, Rao H. **Arterial spin-labeled perfu-sion MRI in basic and clinical neuroscience.** *Curr Opin Neurol* 2009; **22**:348–355.

21. Chang L, Ernst T, Leonido-Yee M, Speck O. **Perfusion MRI detects rCBF abnormalities in early stages of HIV-cognitive motor complex.** *Neurology* 2000; **54**:389–396.

22. Tracey I, Hamberg LM, Guimaraes AR, Hunter G, Chang I, Navia BA, *et al.* **Increased cerebral blood volume in HIV-positive patients detected by functional MRI.** *Neurology* 1998; **50**:1821–1826.

23. Ernst T, Chang L, Jovicich J, Ames N, Arnold S. **Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients.** *Neurology* 2002; **59**:1343–1349.

24. Holt JL, Kraft-Terry SD, Chang L. **Neuroimaging studies of the aging HIV-1-infected brain.** *J Neurovirol* 2012; **18**:291–302.

25. Tucker KA, Robertson KR, Lin W, Smith JK, An H, Chen Y, *et al.* **Neuroimaging in human immunodeficiency virus infection.** *J Neuroimmunol* 2004; **157**:153–162.

26. Paul R, Cohen R, Navia B, Tashima K. **Relationships between cognition and structural neuroimaging findings in adults with human immunodeficiency virus type-1.** *Neurosci Biobehav Rev* 2002; **26**:353–359.

27. Avison MJ, Nath A, Greene-Avison R, Schmitt FA, Greenberg RN, Berger JR. **Neuroimaging correlates of HIV-associated BBB compromise.** *J Neuroimmunol* 2004; **157**:140–146.

28. Turkeltaub PE, Eickhoff SB, Laird AR, Fox M, Wiener M, Fox P. **Minimizing within-experiment and within-group effects in activation likelihood estimation meta-analyses.** *Hum Brain Mapp* 2011; **33**:1–13.

29. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. **Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement.** *Open Med* 2009; **3**:e123–e130.

30. Eickhoff SB, Laird AR, Grefkes C, Wang LE, Zilles K, Fox PT. **Coordinate-based activation likelihood estimation meta-ana-lysis of neuroimaging data: a random-effects approach based on empirical estimates of spatial uncertainty.** *Hum Brain Mapp* 2009; **30**:2907–2926.

31. Eickhoff SB, Bzdok D, Laird AR, Kurth F, Fox PT. **Activation likelihood estimation meta-analysis revisited.** *NeuroImage* 2012; **59**:2349–2361.

32. Evans AC, Kamber M, Collins DL, MacDonald D. *An MRI-based probabilistic atlas of neuroanatomy, vol. 264.* United States: Springer; 1994. pp. 263–274.

33. Chang L, Speck O, Miller EN, Braun J, Jovicich J, Koch C, *et al.* **Neural correlates of attention and working memory deficits in HIV patients.** *Neurology* 2001; **57**:1001–1007.

34. Chang L, Tomasi D, Yakupov R, Lozar C, Arnold S, Caparelli E, *et al.* **Adaptation of the attention network in human immuno-deficiency virus brain injury.** *Ann Neurol* 2004; **56**:259–272.

35. Ances BM, Roc AC, Wang J, Korczykowski M, Okawa J, Stern J, *et al.* **Caudate blood flow and volume are reduced in HIV+ neurocognitively impaired patients.** *Neurology* 2006; **66**:862–866.

36. Castelo JMB, Sherman SJ, Courtney MG, Melrose RJ, Stern CE. **Altered hippocampal-prefrontal activation in HIV patients dur-ing episodic memory encoding.** *Neurology* 2006; **66**:1688–1695.

37. Chang L, Yakupov R, Nakama H, Stokes B, Ernst T. **Antiretro-viral treatment is associated with increased attentional load-dependent brain activation in HIV patients.** *J Neuroimmune Pharmacol* 2007; **3**:95–104.

38. Juengst SB, Aizenstein HJ, Figurski J, Lopez OL, Becker JT. **Alterations in the hemodynamic response function in cogni-tively impaired HIV/AIDS subjects.** *J Neurosci Methods* 2007; **163**:208–212.

39. Ances BM, Clifford DB. **HIV-associated neurocognitive disor-ders and the impact of combination antiretroviral therapies.** *Curr Neurol Neurosci Rep* 2008; **8**:455–461.

40. Melrose RJ, Tinaz S, Castelo JMB, Courtney MG, Stern CE. **Compromised fronto-striatal functioning in HIV: an fMRI in-vestigation of semantic event sequencing.** *Behav Brain Res* 2008; **188**:337–347.

41. Ances BM, Sisti D, Vaida F, Liang CL, Leontiev O, Perthen JE, *et al.* **Resting cerebral blood flow: a potential biomarker of the effects of HIV in the brain.** *Neurology* 2009; **73**:702–708.

42. Maki PM, Cohen MH, Weber K, Little DM, Fornelli D, Rubin LH, *et al.* **Impairments in memory and hippocampal function in HIV-positive vs HIV-negative women: a preliminary study.** *Neurology* 2009; **72**:1661–1668.

43. Ances BM, Vaida F, Yeh MJ, Liang CL, Buxton RB, Letendre S, *et al.* **HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging.** *J Infect Dis* 2010; **201**:336–340.

44. Ances BM, Roc AC, Korczykowski M, Wolf RL, Kolson DL. **Combination antiretroviral therapy modulates the blood oxy-gen level-dependent amplitude in human immunodeficiency virus-seropositive patients.** *J Neurovirol* 2008; **14**:418–424.

45. Ances B, Vaida F, Ellis R, Buxton R. **Test-retest stability of calibrated BOLD-fMRI in HIV− and HIV+ subjects.** *Neuro-Image* 2011; **54**:2156–2162.

46. Meade CS, Lowen SB, MacLean RR, Key MD, Lukas SE. **fMRI brain activation during a delay discounting task in HIV-positive adults with and without cocaine dependence.** *Psychiatry Res: Neuroimaging* 2011; **192**:167–175.

47. Qiu W, Yan B, Li J, Tong L, Wang L, Shi D. *A resting-state fMRI study of patients with HIV infection based on regional homo-geneity method.* Shanghai: Institute of Electrical and Electronics Engineers (IEEE and partners); 2011. pp. 997–1000.

48. Schweinsburg BC, Scott JC, Schweinsburg AD, Jacobus J, Theil-mann RJ, Frank LR, *et al.* **Altered prefronto-striato-parietal network response to mental rotation in HIV.** *J Neurovirol* 2012; **18**:74–79.

49. Ernst T, Yakupov R, Nakama H, Crocket G, Cole M, Watters M, *et al.* **Declined neural efficiency in cognitively stable human immunodeficiency virus patients.** *Ann Neurol* 2009; **65**:316–325.

50. Ernst T, Chang L, Arnold S. **Increased glial metabolites predict increased working memory network activation in HIV brain injury.** *NeuroImage* 2003; **19**:1686–1693.

51. Chang L, Alicata D, Ernst T, Volkow N. **Structural and meta-bolic brain changes in the striatum associated with metham-phetamine abuse.** *Addiction* 2007; **102**:16–32.

52. Hemelaar J. **The origin and diversity of the HIV-1 pandemic.** *Trends Mol Med* 2012; **18**:182–192.

53. Goldman-Rakic PS. *Comprehensive physiology.* Hoboken, NJ, USA: John Wiley & Sons, Inc; 2011 doi:10.1002/cphy.cp010509.

54. Zald DH. **Orbital versus dorsolateral prefrontal cortex: anato-mical insights into content versus process differentiation mod-els of the prefrontal cortex.** *Ann N Y Acad Sci* 2007; **1121**:395–406.

55. Grant I. **Neurocognitive disturbances in HIV.** *Int Rev Psychiatry* 2008; **20**:33–47.

56. Anthony IC, Bell PJE. **The neuropathology of HIV/AIDS.** *Int Rev Psychiatry* 2008; **20**:15–24.

57. Glass JD, Fedor H, Wesselingh SL, Mcarthur JC. **Immunocyto-chemical quantitation of human immunodeficiency virus in the brain: correlations with dementia.** *Ann Neurol* 1995; **38**:755–762.

58. Mink JW. **The basal ganglia: focused selection and inhibition of competing motor programs.** *Prog Neurobiol* 1996; **50**:381–425.

59. Czub S, Koutsilieri E, Sopper S, Czub M, Stahl-Hennig C, Müller JG, *et al.* **Enhancement of central nervous system pathology in early simian immunodeficiency virus infection by dopaminer-gic drugs.** *Acta Neuropathol* 2001; **101**:85–91.

60. Koutsilieri E, Sopper S, Scheller C, Meulen ter V, Riederer P. **Involvement of dopamine in the progression of AIDS Dementia Complex.** *J Neural Transm* 2002; **109**:399–410.

61. Koutsilieri E, Sopper S, Scheller C, Meulen ter V, Riederer P. **Parkinsonism in HIV dementia.** *J Neural Transm* 2002; **109**:767–775.

62. Koutsilieri E, Scheller C, Sopper S, Meulen ter V, Riederer P. **The pathogenesis of HIV-induced dementia.** *Mech Ageing Dev* 2002; **123**:1047–1053.

63. Scheller C, Sopper S, Jassoy C, Meulen ter V, Riederer P, Koutsilieri E. **Dopamine activates HIV in chronically infected T lymphoblasts.** *J Neural Transm* 2000; **107**:1483–1489.

64. Koutsilieri E, Götz ME, Sopper S, Stahl-Hennig C, Czub M, Meulen ter V, *et al.* **Monoamine metabolite levels in CSF of SIV-infected rhesus monkeys (Macaca mulatta).** *Neuroreport* 1997; **8**:3833–3836.

65. Kumar AM, Fernandez JB, Singer EJ, Commins D, Waldrop-Valverde D, Ownby RL, *et al.* **Human immunodeficiency virus type 1 in the central nervous system leads to decreased dopamine in different regions of postmortem human brains.** *J Neurovirol* 2009; **15**:257–274.

66. Rabkin JG, McElhiney MC, Ferrando SJ. **Mood and substance use disorders in older adults with HIV/AIDS: methodological issues and preliminary evidence.** *AIDS* 2004; **18 (Suppl 1)**:S43–S48.

67. Nath A, Maragos WF, Avison MJ, Schmitt FA, Berger JR. **Acceleration of HIV dementia with methamphetamine and cocaine.** *J Neurovirol* 2001; **7**:66–71.

68. Volkow ND, Wang GJ, Tomasi D, Baler RD. **Unbalanced neuronal circuits in addiction.** *Curr Opin Neurobiol* 2013; **23**:639–648.

69. Nath A, Schiess N, Venkatesan A, Rumbaugh J, Sacktor N, Mcarthur J. **Evolution of HIV dementia with HIV infection.** *Int Rev Psychiatry* 2008; **20**:25–31.

70. Nath A. **Human immunodeficiency virus-associated neurocognitive disorder.** *Ann N Y Acad Sci* 2010; **1187**:122–128.

71. Ances BM, Vaida F, Cherner M, Yeh MJ, Liang CL, Gardner C, *et al.* **HIV and chronic methamphetamine dependence affect cerebral blood flow.** *J Neuroimmune Pharmacol* 2011; **6**:409–419.

72. Sulkowski MS, Moore RD, Mehta SH, Chaisson RE, Thomas DL. **Hepatitis C and progression of HIV disease.** *JAMA* 2002; **288**:199–206.

73. Verucchi G, Calza L, Manfredi R, Chiodo F. **Human immunodeficiency virus and hepatitis C virus coinfection: epidemiology, natural history, therapeutic options and clinical management.** *Infection* 2004; **32**:33–46.

Chapter 3

HIV Infection Is Associated with Impaired Striatal Function during Inhibition with Normal Cortical Functioning on Functional MRI

HIV Infection Is Associated with Impaired Striatal Function during Inhibition with Normal Cortical Functioning on Functional MRI

Stéfan du Plessis,[1] Matthijs Vink,[2] John A. Joska,[3] Eleni Koutsilieri,[4] Asif Bagadia,[5] Dan J Stein,[3,6] AND Robin Emsley[1]

[1]Department of Psychiatry, 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town, South Africa
[2]Brain Center Rudolf Magnus, CG Utrecht, The Netherlands
[3]Department of Psychiatry, University of Cape Town, J-Block, Groote Schuur Hospital, Observatory, Cape Town
[4]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany
[5]Department of Radiology, 5th Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town, South Africa
[6]Medical Research Council (Unit on Anxiety and Stress Disorders), 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Tygerberg, Cape Town, South Africa

Abstract

The aim of the present study was to investigate the effect of HIV infection on cortical and subcortical regions of the frontal-striatal system involved in the inhibition of voluntary movement. Functional MRI (fMRI) studies suggest that human immunodeficiency virus (HIV) infection is associated with frontostriatal dysfunction. While frontostriatal systems play a key role in behavioral inhibition, there are to our knowledge no fMRI studies investigating the potential impact of HIV on systems involved during the inhibition of voluntary movement. A total of 17 combined antiretroviral therapy (cART) naïve HIV+ participants as well as 18 age, gender, ethnic, education matched healthy controls performed a modified version of the stop-signal paradigm. This paradigm assessed behavior as well as functional brain activity associated with motor execution, reactive inhibition (outright stopping) and proactive inhibition (anticipatory response slowing before stopping). HIV+ participants showed significantly slower responses during motor execution compared to healthy controls, whereas they had normal proactive response slowing. Putamen hypoactivation was evident in the HIV+ participants based on successful stopping, indicating subcortical dysfunction during reactive inhibition. HIV+ participants showed normal cortical functioning during proactive inhibition. Our data provide evidence that HIV infection is associated with subcortical dysfunction during reactive inhibition, accompanied by relatively normal higher cortical functioning during proactive inhibition. This suggests that HIV infection may primarily involve basic striatal-mediated control processes in cART naïve participants. (*JINS*, 2015, *21*, 722–731.)

Keywords: Brain, Inhibition, Dementia, fMRI, Putamen, HIV

INTRODUCTION

It is well recognized that the human immunodeficiency virus (HIV) invades the brain early in the course of infection, and is thought to cause neurocognitive impairment in up to 52% of patients (An, Groves, Gray, & Scaravilli, 1999; Heaton et al., 2010). The typical neuropsychological disorder associated with untreated HIV infection is best described as a "subcortical" or "fronto-subcortical" dementia, as it involves cognitive (e.g., memory impairment, with relative sparing of recognition)

(Grant, 2008), neuropsychiatric (e.g., motivation, emotional control, social behavior) (Castellon, Hinkin, Wood, & Yarema, 1998; Paul et al., 2005), and motor symptomatology (e.g., motor slowing) (Hardy & Hinhn, 2002). Postmortem studies show that HIV has a predilection for the striatum as well as the white matter tracts connecting for the striatum with the cortex (Langford et al., 2002; Wiley et al., 1998).

Past studies have consistently shown impaired function associated with frontostriatal circuits involved in visual attention and working memory (Chang et al., 2001; Ernst, Chang, Jovicich, Ames, & Arnold, 2002), episodic memory (Castelo, Sherman, Courtney, Melrose, & Stern, 2006), delay discounting (Meade, Lowen, MacLean, Key, & Lukas, 2011), and a monetary incentive delay task (Plessis et al., 2015). Furthermore, frontostriatal function loss is already

Correspondence and reprint requests to: Stéfan du Plessis, Department of Psychiatry, 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town, 7505 South Africa. E-mail: stefandup@sun.ac.za

observed during rest (Ortega, Brier, & Ances, 2015; Thomas, Brier, Snyder, Vaida, & Ances, 2013) and during simple motor paradigms (Ances et al., 2011). However, to date the impact of inhibitory control over voluntary movement has not been investigated. Such control entails concerted activation of both frontal regions and the striatum (Zandbelt & Vink, 2010). As HIV is known to involve the motor system clinically (Hardy & Hinhn, 2002), it is important to investigate how HIV impacts the various subsystems of this network involved in the control over the motor system.

Response inhibition represents a major component of frontostriatal functioning (Aron, 2011). Although cognitive neuropsychological measures have demonstrated potential abnormalities of response inhibition in terms of Stroop task performance (Hinkin, Castellon, Hardy, Granholm, & Siegle, 1999), the potential impact of HIV on the function of frontostriatal brain systems related to response inhibition has yet to be studied. Inhibition involves several sub processes, among which are (1) motor execution (i.e., GO responses), (2) outright stopping as an immediate reaction to a STOP signal (i.e., reactive inhibition) (Mink, 1996; Vink et al., 2005), and (3) higher order cortical functions involved in proactive anticipation of stopping (i.e., proactive inhibition) (Aron, 2011; Vink et al., 2005; Zandbelt & Vink, 2010).

In a previous study in healthy volunteers, a modified version of a Stop Signal Anticipation Task (SSAT) was used to investigate cortical and subcortical functions involved in the inhibition of voluntary movement (see Figure 1). During this task, subjects were required to give timed GO responses with occasional STOP signals occurring at fixed probabilities. The probability of a STOP signal occurring was explicitly stated to allow participants to adjust their responses proactively. GO responses in the absence of STOP signals served as a baseline, and they mainly activated the primary motor cortex. The striatum was found not to be active during these baseline responses, as these involve simple button presses. The putamen, however, was found to be active bilaterally during successful STOP trials (i.e., reactive inhibition) (Zandbelt & Vink, 2010). Furthermore, it was found that proactive inhibition evoked activation in both frontal and striatal regions to facilitate STOP performance by slowing down GO responses. Proactive inhibition specifically engaged the right inferior frontal gyrus, bilateral parietal gyri, and the right striatum (Zandbelt & Vink, 2010).

We chose to investigate a cART naïve cohort for two reasons. First, it allowed us to investigate the impact of the illness on brain function without the confounding effects of medication. Indeed, cART has been shown to potentially confound the effects of HIV on the frontostriatal system, leaving its true impact uncertain (Chang, Yakupov, Nakama, Stokes, & Ernst, 2007). Second, unmedicated HIV-positive patients continue to form an important part of patients seen in clinical settings in sub-Saharan countries (Reda & Biadgilign, 2012). For example individuals in Sub-Saharan Africa are generally only eligible for cART with CD4 counts of 350 or 500 cells/mL. Also, many eligible individuals do not access care for various reasons and a substantial number default form care after 12 months (15–20%) (Fox & Rosen, 2010). Therefore, the effect of illness in the absence of cART remains an important question in these settings.

The aim of the present study was to investigate the effect of HIV infection on cortical and subcortical regions of the frontal-striatal system involved in the inhibition of voluntary movement. To this end, 22 cART naive HIV+ and 18 matched controls performed a stop signal anticipation task while being scanned with functional MRI.

We hypothesized that HIV infection would have the following effects: (1) motor execution as measured by baseline timed GO responses would be increased, as HIV infection often involves psychomotor slowing (Hardy & Hinhn, 2002; Navia, Jordan, & Price, 1986); (2) reactive inhibition time as measured by the speed of inhibition, that is, STOP signal reaction time (SSRT) (Logan, Cowan, & Davis, 1984) would be increased, similar to findings in other subcortical dementia's such as Parkinson's disease (Gauggel, Rieger, & Feghoff, 2004); (3) subcortical dysfunction as evidenced by hypofunction in the putamen during reactive inhibition would be demonstrated (Zandbelt, van Buuren, Kahn, & Vink, 2011); and (4) compromised higher cortical functioning would be reflected in the participants' inability to proactively increase their response times in anticipation of
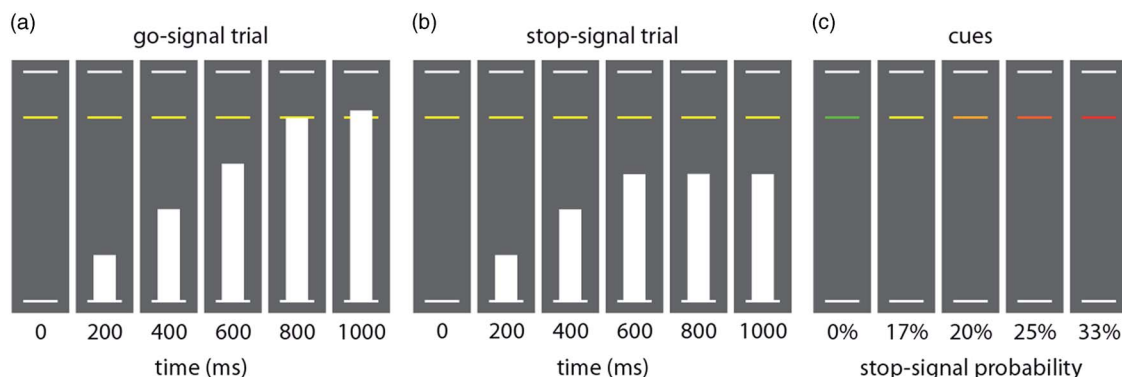
Fig. 1. The Stop Signal Anticipation Task. There are two types of trials: (a) GO signal trials interspersed with (b) occasional STOP signal trials. (c) A changing color cue indicates the stop-signal probability, which varies from trial to trial.

STOP signals, as well as in associated cortical activation deficits in the inferior frontal gyrus during proactive inhibition (Zandbelt et al., 2011).

METHOD

Participants

The study was approved by the Health Research Ethics Committee of Stellenbosch University and the Human Research Ethics Committee of the University of Cape Town, Cape Town, South Africa. Before enrolment, all participants provided written consent after receiving a full description of this study. Our sample was recruited from a medically stable clinic-attending population during routine HIV care and testing at Site C primary health care clinic, in Khayelitsha, Cape Town, South Africa. A total of 22 HIV+ participants were included in the study together with 18 gender, education, ethnicity, and age-matched healthy controls. The controls were HIV negative as confirmed by the enzyme-linked immunosorbent assay. All HIV+ patients included in the study were cART naïve.

Participants were screened using the Mini International Neuropsychiatric Interview (MINI) 6.0.0/MINI-PLUS 6.0.0. (Sheehan et al., 1998). All HIV+ participants received a full medical examination by a clinician and were excluded if there was a current comorbid psychiatric/neurological or general medical condition that could confound the diagnosis of HIV associated neurocognitive disorders (HAND); any history of substance use/abuse as assessed by the Substance Abuse and Mental Illness Symptoms Screener (SAMISS) screening questionnaire (Pence et al., 2005); a score for smoking greater than 1 as confirmed by the Kreek-McHugh-Schluger-Kellogg (KMSK) scale (Kellogg et al., 2003); were pregnant as confirmed by a urine pregnancy test; or were currently receiving treatment for tuberculosis. All participants were right handed as confirmed by the Edinburgh Handedness Inventory (Oldfield, 1971).

HIV+ participants underwent detailed neuropsychological assessment within two weeks of neuroimaging and controls within 1 year for characterization purposes only. The test battery assessed the following cognitive domains: abstraction/ executive function, memory, learning, speed of information processing, verbal fluency, motor and sensory/ perception (Grant, 2008). From these scores, a Global Deficit composite score was derived (Carey et al., 2004) using normative data from a larger parent study (Joska et al., 2010).

The following laboratory measures were performed in the HIV+ participants within 2 weeks of neuroimaging: CD_4 count, HIV viral load, Rapid Plasma Reagin for syphilis and thyroid stimulating hormone level. All participants received a urinary drug screen before being scanned. In view of the fact patient reports of duration of infection are highly unreliable in our present population we relied on pre-treatment CD4 count (as a proxy for nadir) and viral load as an estimation of disease progression as all participants were cART naive and being treated for the first time. While hepatitis C co-infection has been associated with increased risk and severity of cognitive impairment in HAND, participants were not screened as it is not endemic to the region (Amin et al., 2004). An experienced radiologist reviewed all of the scans for intracranial pathology that could potentially confound functional imaging measurement results.

Functional MRI

All scans were acquired on a 3 Tesla Siemens Allegra at the Combined Universities Brain Imaging Centre (CUBIC). During MRI image acquisition, 622 whole-brain two-dimensional-echo planar imaging images [repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip-angle: 72.5 degrees; field of view (FOV): 256×256; 30 slices; 4 mm isotropic voxels) were acquired. Trial-by trial variability was accounted for by setting the total task length to 17 min. Excess scans were discarded.

For image registration, a T1 ME-MPRAGE weighted structural scan was acquired (TR = 2530 ms; TE_1 = 1.53 ms; TE_2 = 3.21, ms; TE_3 = 4.89 ms; TE_4 = 6.57 ms; flip-angle: 7 degrees; FOV: 256 mm; 128 slices; 1 mm isotropic voxel size) (van der Kouwe, Benner, Salat, & Fischl, 2008).

Stop-Signal Anticipation Functional MRI Task

During the functional MRI (fMRI) experiment, participants performed the STOP signal anticipation task (Zandbelt & Vink, 2010). The experiment was performed using Presentation® software (Version 14.6, www.neurobs.com). The task is based on original work by Logan et al. (1984) who proposed a horse-race model, suggesting that a response, either GO or STOP, is a result from a race between the GO process and the STOP process. The response is stopped when the STOP process finished before the GO process reaches execution threshold (Logan & Cowan, 1984). The task and experimental procedures were identical to those described before (Zandbelt & Vink, 2010). All participants received standardized training in task performance before scanning by a trained technician in their first language (isiXhosa).

During task performance, participants were presented with three background lines. In each trial, a bar moved at a constant speed from the bottom line to the top line, passing the middle line within 800 milliseconds (ms). On GO trials, participants were required to stop the bar with a button press using their right index finger, as close to the middle/colored line as possible. Should the bar reach the top line after 1000 ms, the GO trial was considered a failure. The inter-trial interval was kept at 1000 ms. On STOP signal trials the bar stopped on its own, indicating a STOP signal. During STOP trials the participant was required to withhold the button press (reactive response inhibition).

To measure proactive response inhibition, the probability of the stop signal was explicitly indicated to the participant by the color of the middle line. This allowed a participant to proactively anticipate a STOP signal in each STOP trial, by taking the stop-signal probability into account. There were

five stop-signal probability levels: 0% (green), 17% (yellow), 20% (amber), 25% (orange), and 33% (red). In total, 414 go trials (0%, $n = 234$; 17%, $n = 30$; 20%, $n = 48$; 25%, $n = 54$; 33%, $n = 48$) and 60 stop trials (17%, $n = 6$; 20%, $n = 12$; 25%, $n = 18$; 33%, $n = 24$) were presented in a single run in pseudorandom order.

The STOP signal delay (SSD), the interval between start of a trial and the STOP signal, was initially 550 ms and varied for each STOP signal according to a staircase procedure. That is, should a STOP trial be successful, the trial difficulty was increased by increasing the STOP signal delay by 25 ms. Should a STOP trial be unsuccessful, trial difficulty was decreased in the same manner. This technique assured an equal amount of successful and unsuccessful STOP trials. For more details on the SSAT, see Zandbelt and Vink (2010).

Motor Execution: Baseline GO Reaction Time

Timed baseline GO responses were measured in the absence of the possibility of a STOP signal. To explore speeded responses in terms of simple reaction time we included the California Computerized Assessment Package (CALCAP) in our neuropsychological assessment battery (Miller, Satz, & Visscher, 1991).

Reactive Inhibition

Reactive inhibition was studied in terms of the SSRT (Logan et al., 1984; Zandbelt & Vink, 2010), which was calculated according to the integration method (Logan & Cowan, 1984) and pooled across all STOP signal probability levels.

Proactive Inhibition

In keeping with previous studies (Vink et al., 2005, 2014; Zandbelt & Vink, 2010), proactive inhibition was measured as the effect of STOP signal probability on GO signal response time. Impaired proactive inhibition is evidenced by a reduced effect of STOP signal probability on GO signal response time (Vink et al., 2005, 2014; Zandbelt & Vink, 2010). This indicates a weaker anticipation of a STOP signal. Statistical analysis of proactive inhibition consisted of a repeated measures analysis of variance on mean GO signal response times, with STOP signal probability and HIV serostatus as factors.

fMRI Data Analysis

Image data were modeled using SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8/). Preprocessing and first-level statistical analyses were performed as previously described (Zandbelt & Vink, 2010). Preprocessing involved correction for slice timing differences by resampling all slices in time relative to the middle slice using Fourier interpolation. Re-alignment to the mean image was performed using fourth-degree B-spline interpolation to correct for head motion. Head motion parameters were analyzed to ensure that the maximum motion did not exceed a predefined threshold (scan-to-scan >3 mm) (Van Dijk, Sabuncu, & Buckner, 2012). Spatial normalization was performed using linear and non-linear deformations to the Montreal Neurological Institute template brain, and spatial smoothing using a 6-mm full-width at half-maximum Gaussian kernel to accommodate inter-individual differences in neuro-anatomy.

The fMRI data were modeled voxel-wise, using a general linear model, in which the following events were included as regressors: Timed GO signal trials with STOP signal probability >0%, successful STOP signal trials and failed STOP signal trials. For GO signal trials with a STOP probability above 0%, we also included a parametric regressor modelling the STOP signal probability level as well as variations in response time. GO baseline (0% STOP probability) as well as activity during rest was explicitly modelled.

The fMRI data were high-pass filtered (cut-off 128 Hz) to remove low-frequency drifts. For each participant, we computed four contrast images: (1) Baseline GO-activation, to measure motor response initiation, (2) activation during successful STOP signal trials *versus* failed STOP signal trials (to assess reactive inhibition), (3) activation during successful STOP signal trials *versus* GO signal trials in the 0% STOP signal probability context (also to assess reactive inhibition), and (4) the parametric effect of STOP signal probability on GO signal activation (to assess proactive inhibition). We computed two contrasts for reactive inhibition because there is no consensus currently, on which contrast is most appropriate for investigating reactive inhibition.

We assessed group activation differences in predefined *a priori* regions of interest (ROIs), based on activation maps acquired in a previous experiment (Zandbelt & Vink, 2010), in which an independent sample of healthy volunteers performed the same task. These ROIs were defined using a cluster-level threshold (cluster-defining threshold $p < .001$, cluster probability of $p < .05$, family-wise error corrected for multiple comparisons). They included the primary motor cortex as activated during baseline GO responses; the right striatum during reactive inhibition and the right inferior frontal gyrus during proactive inhibition. We chose the right inferior frontal gyrus as our primary region of interest during proactive inhibition, as the inferior frontal regions have been shown to be affected by HIV in tasks of visual attention and working memory (Plessis et al., 2014). An exploratory voxel wise whole-brain analysis was also performed for each of the above contrasts, testing for group differences using independent sample t-tests (family-wise error corrected for multiple comparisons).

RESULTS

Demographics

Forty participants were initially recruited into the study. We excluded five patients for the following reasons: Tested positive for benzodiazepines ($n = 1$); Did not receive a full physical examination ($n = 1$); Problems with response box

Table 1. Demographic characteristics of the diagnostics groups.

	HIV ($N = 17$)	HC ($N = 18$)	Test Statisticstatistic	p-Value
Gender (M/F)	2/15	3/15	$\chi^2 = 0.172$	0.679
Mean Age age (years)	31 ± 4.1	28 ± 5.3	$T = -2.00$	0.054
Education (years)	12 (10.8–12)	$12 \pm (11–12)$	$U = 128$	0.424
Viral Load load (copies/ml)	51065.4 ± 62034.4			
CD$_4$ (cells/μl)	392 ± 211.8			
Participants with AIDS defining CD$_4$ count	7			
GDS (Summary summary Scorescore)	0.21 (0.07–0.36)	0.14 (0.07–0.3)	$U = 275$	0.44

Note. Age, viral load, and CD$_4$ data represent mean $\pm$ SD. Education and GDS data represent median and interquartile range between 25th and 75th centiles. AIDS defining CD$_4$ count of 350 cells/μl used.
F, female; M, male; GDS: Global Deficit Score (22); HC: Hhealthy Ccontrols, HIV: HIV+ participants. AIDS defining CD$_4$ count of 350 cells/μl used.

($n = 2$); Poor scan quality leading to failure in pre-processing ($n = 1$). One healthy control was excluded to excessive movement (6.7 mm between scan movement) with a notable drop in signal to noise as assessed with in house quality assessment software (Geissler et al., 2007; Stöcker et al., 2005). Therefore, 17 HIV+ participants and 18 healthy controls were included in the final analysis (see Table 1).

The groups did not differ with regard to age, gender, ethnicity, or education level. All HIV participants were ambulant and healthy enough to participate in the experiment. No significant pathology was found on the structural MRI scans. As expected, most of the present sample is female as the HIV+ population of sub-Saharan Africa consists mostly of women infected by heterosexual contact (UNAIDS, 2012).

Behavioral Results

Motor execution

Response times for baseline GO trials (a STOP signal probability of 0%) were close to the target response time of 800 ms (794 ± 7 ms), for controls, whereas HIV+ participants were significantly slower (M $= 827 \pm 8$; $t(33) = -3.067$; $p = .004$; $r = 0.471$). Reaction time assessment according to CALCAP confirmed that HIV participants showed significant signs of motor slowing (Control: M $= 295 \pm 16$ ms; HIV: M $= 367 \pm 19$ ms; $t(32) = -2.818$; $p = .008$; $r = 0.446$). Despite this slow baseline response speed, there was no difference in baseline GO accuracy between groups (Control: M $= 96 \pm 0.6$; HIV: M $= 96 \pm 0.7$; $t(33) = 0.673$; $p = .506$; $r = 0.116$), indicating that all subjects were able to perform the basic task regardless of baseline response speed.
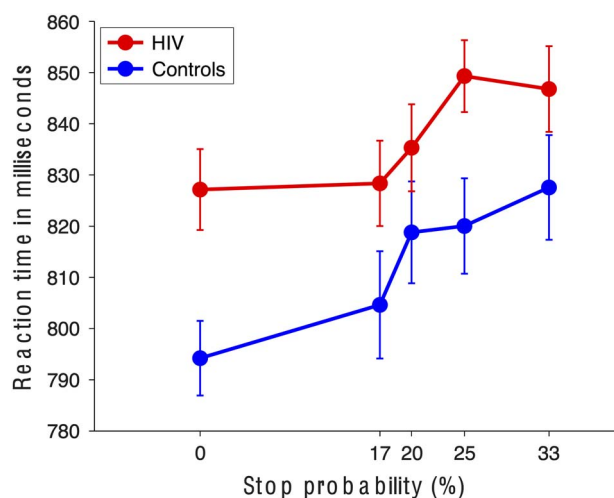
Reactive inhibition

The speed of reactive inhibition (SSRT) did not differ between the groups (Control: M $= 270 \pm 3$ ms; HIV: M $= 267 \pm 6$ ms; $t(33) = 0.429$; $p = .670$; $r = 0.075$). As STOP errors were manipulated according to subject performance as previously described (Pence et al., 2005; Zandbelt & Vink, 2010), there was no difference in STOP related errors ($t(33) = -1.191$; $p = .242$; $r = 0.203$).
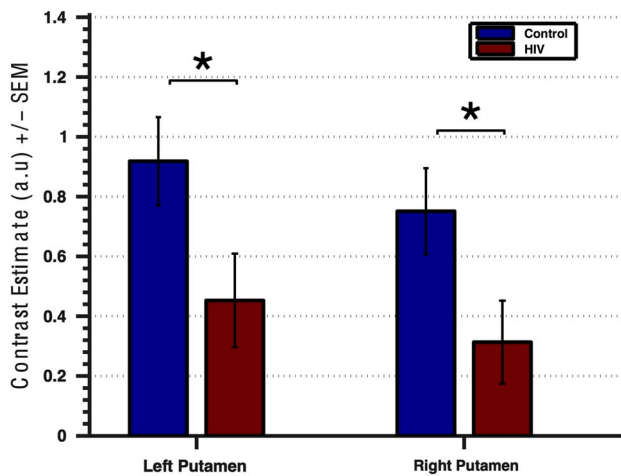
Proactive inhibition

There was a significant main effect of STOP probability on reaction time regardless of disease status, showing response slowing as the STOP probability increased ($F(2.1,70.4) = 16.808$; $p < .001$) in trials where a STOP signal could occur (see Figure 2). Although there was no significant group by STOP probability interaction ($F(2.1,70.4) = 1.516$; $p = .226$), there was a trend toward a group effect ($F(1,33) = 3.235$; $p = .081$). This shows that both groups were able to slow down their responses proactively during GO trials in which a STOP signal could occur, with the HIV+ group being on average slower than controls.

On further analysis, both groups showed, as expected, a main effect of response slowing relative to baseline ($F(1,33) = 31.171$; $p < .001$). There was no group by response speed interaction, indicating that both groups showed an equal amount of proactive slowing relative to their own baseline ($F(1,33) = 2.42$; $p = .129$). Finally, HIV+ had the same accuracy on GO trials where a STOP signal could occur (Control: M $= 97 \pm 0.5$%; HIV: M $= 95 \pm 0.8$%; $t(33) =1.745$;

Fig. 2. Effect of stop-signal probability on go-signal response time across groups with error bars indicating the standard error of the mean.

37

Fig. 3. Putamen activation during reactive inhibition in both HIV+ participants and seronegative controls.

$p = .090; r = 0.291$). Both groups had a STOP accuracy close to the target of 50%, indicating that the stepwise difficulty adjustment during STOP trials were successful (Control: M $= 51 \pm 1.5\%$; HIV: M $= 54 \pm 1\%$; $t(33) = -1.522$; $p = .137$). It should be noted that, as the difficulty was adjusted according to individual performance, we expected no difference in STOP accuracy.

fMRI Results

Motor execution

Both groups showed equal activation in the motor cortex on baseline GO responses, indicating normal motor cortical function during the timed responses ($t(33) = 0.320$; $p = .751$; $r = 0.056$).

Reactive inhibition

When comparing successful STOPs *versus* unsuccessful STOPs, we found hypo-activation in the right putamen ($t(33) = 2.157$; $p = .038$; $r = 0.352$) in the HIV+ group during this task. Further exploratory ROI analysis revealed the same effect to be present in the left putamen ($t(33) = 2.136$; $p = .040$; $r = 0.348$) (see Figure 3). No further differences were found on an exploratory whole brain analysis (see Figure 4). An exploratory regression analysis with putamen activation as dependent variable and global deficit score, viral load, and age as predictors, revealed no further significant results in the HIV+ group. We did not find any significant correlations between cognitive domain scores and reactive inhibition activation (see Table 2).

Proactive inhibition

We found no difference in function associated with proactive inhibition between groups in the inferior frontal gyrus ($t(33) = -0.036$; $p = .972$; $r = 0.006$). An exploratory whole-brain analysis was also performed, which also showed no group differences. Exploratory analysis revealed no relationship between frontal task activation and cognitive domain scores.

DISCUSSION

In this study, we investigated frontostriatal functioning during an inhibition task that assessed motor execution,
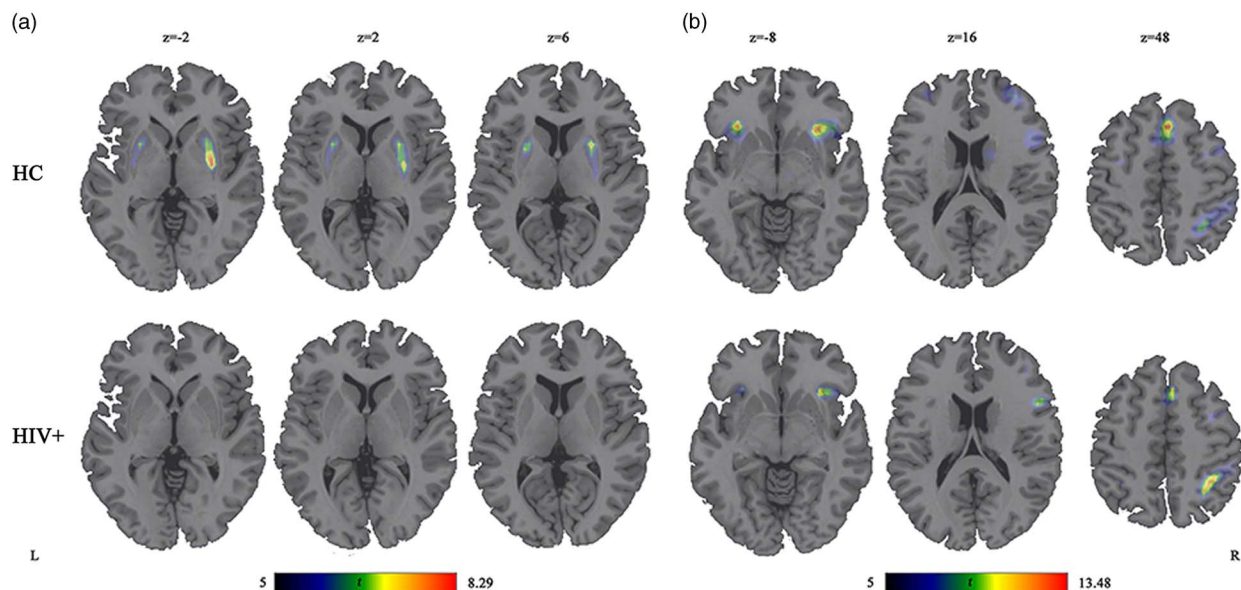
Fig. 4. Whole-brain activation during reactive inhibition in HIV+ participants and healthy control subjects (HC). One-sample *t*-tests of (a) successful STOP signal *versus* failed STOP signal activation and (b) successful STOP signal *versus* GO signal 0% activation, for HIV+ and healthy control subjects. Significant activation clusters ($p < .05$, family-wise error-corrected) are displayed on the normalized and skull-stripped group-average brain (neurological orientation). HC, control subject; L, left; R, right; HIV+, HIV-positive participant.

Table 2. Cognitive domains tested (Mean *Z* value corrected *via* normal control group) as well as Pearson's correlations performed for the left as well as the right putamen activations during reactive inhibition.

Cognitive domain	HIV (*N* = 17)		*SE*	Left putamen	*p*-Value	Right putamen	*p*-Value
Abstraction/executive function	−.286	+/−	0.235	−.216	.390	−.274	.271
Memory	−.424	+/−	0.202	.061	.810	.059	.815
Learning	.301	+/−	0.183	.072	.776	−.115	.650
Speed of information processing	−.218	+/−	0.193	−.276	.268	−.258	.301
Verbal fluency	.027	+/−	0.173	.050	.845	−.121	.633
Motor	−.205	+/−	0.226	−.311	.209	−.291	.241
Sensory/perception	−.686	+/−	0.391	.037	.888	.129	.623

SE = standard error.

reactive inhibition and proactive inhibition, in 17 cART naïve HIV+ participants and 18 matched healthy controls. The HIV + participants had significantly slower baseline GO reaction times indicating impaired baseline motor execution. Both groups demonstrated similar responses on behavioral measures of reactive inhibition as well as proactive inhibition. During fMRI, however, in HIV+ participants the putamen was found to be hypo-active during reactive inhibition. There were no significant fMRI differences between groups in the cortex during proactive inhibition. This could indicate a relative sparing of higher cortical function with a specific dysfunction of the more basic functions of the basal ganglia in a cART naïve population during reactive inhibition.

We investigated motor execution in terms of timed GO responses and found HIV+ participants to be significantly slower. HIV+ participants displayed a normal inhibition process in terms of speed of inhibition. Given that their timed GO responses were abnormally slow, these findings suggest that the SSRT and the GO process are dependent on different fronto-striatal pathways. This is consistent with animal studies reporting that SSRT is sensitive to cortical lesions and GO reaction time is disrupted by subcortical lesions (Eagle, Baunez, et al., 2007).

The HIV+ group proactively reduced their response speed similar to controls. This indicates that, despite abnormalities found in their baseline GO responses, HIV+ participants could still anticipate STOP signals and slow down their responses to facilitate stopping. As proactive inhibition requires higher cortical regions to appropriately respond to complex environmental cues and successfully communicate this information to the striatum (Zandbelt et al., 2011; Zandbelt & Vink, 2010), it suggests higher cortical functions are largely intact in the present sample.

Our finding of no differences between HIV+ participants and controls in the function of the motor cortex during timed GO responses differs from a previous study in a cART treated group. In the latter study, increased motor cortical activation was found in HIV infected participants using a motor-tapping paradigm (Ances, Roc, Korczykowski, Wolf, & Kolson, 2008). Our finding suggests that the cortex is relatively spared in cART naïve HIV+ individuals (Chang et al., 2007) as seen here during response execution.

As predicted by findings of both clinical (Navia et al., 1986) and post mortem studies (Langford et al., 2002; Wiley et al., 1998), we found subcortical regions to be primarily affected as evidenced by putamen hypofunction during reactive inhibition. Although task based fMRI studies have reported subcortical involvement with HIV infection (Ances et al., 2011; Melrose, Tinaz, Castelo, Courtney, & Stern, 2008) studies differ with regards to the directionality of this involvement. For example, increased activation in the striatum has been reported during a fMRI motor tapping paradigm (Ances et al., 2011) and decreased activity during a semantic event-sequencing task (Melrose et al., 2008). A possible explanation for these differences from the present study could be that behavioral tasks differed between studies. Additionally, a positron emission tomography (PET) study, reported baseline hypometabolism in the basal ganglia in HIV+ participants with moderate motor-slowing, whereas basal ganglia hypermetabolism was found in HIV+ participants with normal motor performance (Giesen et al., 2000). As fMRI studies in HIV often include elderly subjects (Plessis et al., 2014), differences in age across studies could also be a confounding factor, as HIV+ effects on the brain is thought to co-vary with increased age (Ances, Ortega, Vaida, Heaps, & Paul, 2012; Chang, Holt, Yakupov, Jiang, & Ernst, 2013; Holt, Kraft-Terry, & Chang, 2012). Another possible explanation for the directional difference of our data is the effect of cART (Chang et al., 2007), as previous fMRI studies included samples mostly on treatment (Plessis et al., 2014). Furthermore, two arterial spin labelling studies have reported reduced baseline regional cerebral blood flow in the striatum of HIV+ participants, which further supports our finding of hypo-activity of these subcortical structures at baseline (Ances et al., 2006, 2009). Our findings therefore confirm the impact of HIV on the function of the striatum and extend these results by demonstrating striatal hypofunction in a cART naïve sample during reactive inhibition.

We found no difference in cortical activity during proactive inhibition. This is seemingly in contrast to functional studies performed in the past that do indeed find relative cortical hyperactivity in HIV (Chang et al., 2004; Melrose et al., 2008; Plessis et al., 2014). These studies largely included participants on cART treatment, however. As cART

treatment has been shown to be associated with increased cortical activity (Chang et al., 2007), this could serve as a possible explanation for this difference.

We did not find any significant correlations between cognitive test scores and fMRI activation. This is not surprising given that fMRI has been shown to be more sensitive to cerebral pathology than cognitive testing (Ernst et al., 2002). Also, the study may be underpowered due to the small sample size. Further investigation is, therefore, warranted.

The slow GO processes and relatively normal STOP processes found in HIV infection in the present study could be explained on the basis of dopaminergic deregulation: Supportive evidence is forthcoming from animal studies suggesting that dopamine deregulation could result in an abnormally slow motor responses, leaving the speed of inhibition spared relative to controls (Bari, Eagle, Mar, Robinson, & Robbins, 2009; Eagle, Tufft, Goodchild, & Robbins, 2007). Studies performed in rats revealed that neither the administration of the mixed D_1/D_2 receptor antagonist *cis*-flupenthixol (Eagle, Tufft, et al., 2007) nor dopamine-associated transport blockade by GBR-12909 influenced SSRT in a modified version of the SSAT (Bari et al., 2009). However, in the same studies GO reaction time was found to speed up in response to dopamine re-uptake blockade as well as methylphenidate administration (Bari et al., 2009; Eagle, Tufft, et al., 2007). Our finding of a slow GO process in the presence of a normal speed of inhibition indicates that the exact biochemical nature of striatal dysfunction in HIV infection requires further elucidation. As the striatum is modulated by dopaminergic activity (Frank, 2005), the present finding of striatal hypofunction could relate to dopamine deregulation. This could be further investigated by PET using dopamine ligands in conjunction with fMRI functional measurements. Prospective pre- and post-cART prospective studies would help to clarify the striatal and cortical effects of cART (Chang et al., 2007).

A strength of our study is the exclusion of important confounds that could influence fronto-striatal function such as cART (Chang et al., 2007), comorbid depression and substance abuse. The effects of age should also be minimal (Ances et al., 2010; Holt et al., 2012), due to the relatively young age of our cohort. We could not demonstrate a link between slow GO processes and brain function. Although we found normal motor cortical activation in HIV+ participants, we could not rule out differences in other parts of the motor system, as our task involved only simple timed motor responses. We also could not demonstrate a behavioral difference in terms of reactive STOP accuracy, which related to putamen activation. This was due to an inherent limitation of our task design: As putamen activation relies on balanced correct *versus* incorrect STOP factors, task difficulty was adjusted to achieve equal successful and unsuccessful STOP for both groups. We did however demonstrate that the putamen is hypoactive in HIV infection when controlling for task performance in this way. Our study is further limited by a small sample size due to the carefully selected nature of our sample.

Taken together, our findings support the hypothesis that HIV infection primarily affects the basic functions of the putamen during reactive inhibition, with relative sparing of cortical function during proactive inhibition.

ACKNOWLEDGMENTS

REFERENCES

Amin, J., Kaye, M., Skidmore, S., Pillay, D., Cooper, D.A., & Dore, G.J. (2004). HIV and hepatitis C coinfection within the CAESAR study. *HIV Medicine*, *5*(3), 174–179. http://doi.org/10.1111/j.1468-1293.2004.00207.x

An, S.F., Groves, M., Gray, F., & Scaravilli, F. (1999). Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *Journal of Neuropathology and Experimental Neurology*, *58*(11), 1156–1162

Ances, B.M., Ortega, M., Vaida, F., Heaps, J., & Paul, R. (2012). Independent effects of HIV, aging, and HAART on brain volumetric measures. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *59*(5), 469–477. http://doi.org/10.1097/QAI.0b013e318249db17

Ances, B.M., Roc, A.C., Korczykowski, M., Wolf, R.L., & Kolson, D.L. (2008). Combination antiretroviral therapy modulates the blood oxygen level–dependent amplitude in human immuno-deficiency virus-seropositive patients. *Journal of Neurovirology*, *14*(5), 418–424. http://doi.org/10.1080/13550280802298112

Ances, B.M., Roc, A.C., Wang, J., Korczykowski, M., Okawa, J., Stern, J., … Detre, J.A. (2006). Caudate blood flow and volume are reduced in HIV+ neurocognitively impaired patients. *Neurology*, *66*(6), 862–866. http://doi.org/10.1212/01.wnl.0000203524.57993.e2

Ances, B.M., Sisti, D., Vaida, F., Liang, C.L., Leontiev, O., Perthen, J.E., … Ellis, R.J. (2009). Resting cerebral blood flow: A potential biomarker of the effects of HIV in the brain. *Neurology*, *73*(9), 702–708. http://doi.org/10.1212/WNL.0b013e3181b59a97

Ances, B.M., Vaida, F., Cherner, M., Yeh, M.J., Liang, C.L., Gardner, C., … Buxton, R.B. (2011). HIV and chronic methamphetamine dependence affect cerebral blood flow. *Journal of Neuroimmune Pharmacology*, *6*(3), 409–419. http://doi.org/10.1007/s11481-011-9270-y

Ances, B.M., Vaida, F., Yeh, M.J., Liang, C.L., Buxton, R.B., Letendre, S., … McCutchan, J.A. (2010). HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging. *The Journal of Infectious Diseases*, *201*(3), 336–340. http://doi.org/10.1086/649899

Aron, A.R. (2011). From reactive to proactive and selective control: Developing a richer model for stopping inappropriate responses. *Biological Psychiatry*, *69*(12), e55–e68

Bari, A., Eagle, D.M., Mar, A.C., Robinson, E.S.J., & Robbins, T.W. (2009). Dissociable effects of noradrenaline, dopamine, and serotonin uptake blockade on stop task performance in rats. *Psychopharmacology*, *205*(2), 273–283. http://doi.org/10.1007/s00213-009-1537-0

Carey, C.L., Woods, S.P., Gonzalez, R., Conover, E., Marcotte, T.D., Grant, I., … Heaton, R.K. (2004). Predictive validity of global deficit scores in detecting neuropsychological impairment in HIV infection. *Journal of Clinical and Experimental Neuropsychology*, *26*(3), 307–319. http://doi.org/10.1080/13803390490510031

Castellon, S.A., Hinkin, C.H., Wood, S., & Yarema, K.T. (1998). Apathy, depression, and cognitive performance in HIV-1 infection. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *10*(3), 320–329

Castelo, J.M.B., Sherman, S.J., Courtney, M.G., Melrose, R.J., & Stern, C.E. (2006). Altered hippocampal-prefrontal activation in HIV patients during episodic memory encoding. *Neurology*, *66*(11), 1688–1695. http://doi.org/10.1212/01.wnl.0000218305.09183.70

Chang, L., Holt, J.L., Yakupov, R., Jiang, C.S., & Ernst, T. (2013). Lower cognitive reserve in the aging human immunodeficiency virus-infected brain. *Neurobiology of Aging*, *34*(4), 1240–1253. http://doi.org/10.1016/j.neurobiolaging.2012.10.012

Chang, L., Speck, O., Miller, E.N., Braun, J., Jovicich, J., Koch, C., … Ernst, T. (2001). Neural correlates of attention and working memory deficits in HIV patients. *Neurology*, *57*(6), 1001–1007

Chang, L., Tomasi, D., Yakupov, R., Lozar, C., Arnold, S., Caparelli, E., & Ernst, T. (2004). Adaptation of the attention network in human immunodeficiency virus brain injury. *Annals of Neurology*, *56*(2), 259–272. http://doi.org/10.1002/ana.20190

Chang, L., Yakupov, R., Nakama, H., Stokes, B., & Ernst, T. (2007). Antiretroviral treatment is associated with increased attentional load-dependent brain activation in HIV patients. *Journal of Neuroimmune Pharmacology*, *3*(2), 95–104. http://doi.org/10.1007/s11481-007-9092-0

Eagle, D.M., Baunez, C., Hutcheson, D.M., Lehmann, O., Shah, A.P., & Robbins, T.W. (2007). Stop-signal reaction-time task performance: Role of prefrontal cortex and subthalamic nucleus. *Cerebral Cortex*, *18*(1), 178–188. http://doi.org/10.1093/cercor/bhm044

Eagle, D.M., Tufft, M.R.A., Goodchild, H.L., & Robbins, T.W. (2007). Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology*, *192*(2), 193–206. http://doi.org/10.1007/s00213-007-0701-7

Ernst, T., Chang, L., Jovicich, J., Ames, N., & Arnold, S. (2002). Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. *Neurology*, *59*(9), 1343–1349

Fox, M.P., & Rosen, S. (2010). Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: Systematic review. *Tropical Medicine & International Health*, *15*, 1–15. http://doi.org/10.1111/j.1365-3156.2010.02508.x

Frank, M.J. (2005). Dynamic dopamine modulation in the basal ganglia: A neurocomputational account of cognitive deficits in medicated and nonmedicated Parkinsonism. *Journal of Cognitive Neuroscience*, *17*(1), 51–72. http://doi.org/10.1016/S0028-3932(02)00068-4

Gauggel, S., Rieger, M., & Feghoff, T.-A. (2004). Inhibition of ongoing responses in patients with Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *75*(4), 539–544

Geissler, A., Gartus, A., Foki, T., Tahamtan, A.R., Beisteiner, R., & Barth, M. (2007). Contrast-to-noise ratio (CNR) as a quality parameter in fMRI. *Journal of Magnetic Resonance Imaging*, *25*(6), 1263–1270. http://doi.org/10.1002/jmri.20935

Giesen, von, H.J., Antke, C., Hefter, H., Wenserski, F., Seitz, R.J., & Arendt, G. (2000). Potential time course of human immunodeficiency virus type 1-associated minor motor deficits: Electrophysiologic and positron emission tomography findings. *Archives of Neurology*, *57*(11), 1601–1607

Grant, I. (2008). Neurocognitive disturbances in HIV. *International Review of Psychiatry*, *20*(1), 33–47. http://doi.org/10.1080/09540260701877894

Hardy, D.J., & Hinhn, C.H. (2002). Reaction time performance in adults with HIV/AIDS. *Journal of Clinical and Experimental Neuropsychology*, *24*(7), 912–929

Heaton, R.K., Clifford, D.B., Franklin, D.R. Jr, Woods, S.P., Ake, C., Vaida, F., … CHARTER group (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy. *Neurology*, *75*, 2087–2095

Hinkin, C.H., Castellon, S.A., Hardy, D.J., Granholm, E., & Siegle, G. (1999). Computerized and traditional Stroop task dysfunction in HIV-1 infection. *Neuropsychology*, *13*(2), 306–316

Holt, J.L., Kraft-Terry, S.D., & Chang, L. (2012). Neuroimaging studies of the aging HIV-1-infected brain. *Journal of Neurovirology*, *18*(4), 291–302. http://doi.org/10.1007/s13365-012-0114-1

Joska, J.A., Westgarth-Taylor, J., Myer, L., Hoare, J., Thomas, K.G.F., Combrinck, M., … Flisher, A.J. (2010). Characterization of HIV-associated neurocognitive disorders among individuals starting antiretroviral therapy in South Africa. *AIDS and Behavior*, *15*(6), 1197–1203. http://doi.org/10.1007/s10461-010-9744-6

Kellogg, S.H., McHugh, P.F., Bell, K., Schluger, J.H., Schluger, R.P., LaForge, K.S., … Kreek, M.J. (2003). The Kreek-McHugh-Schluger-Kellogg scale: A new, rapid method for quantifying substance abuse and its possible applications. *Drug and Alcohol Dependence*, *69*(2), 137–150

Langford, T.D., Letendre, S.L., Marcotte, T.D., Ellis, R.J., McCutchan, J.A., Grant, I., … HNRC Group (2002). Severe, demyelinating leukoencephalopathy in AIDS patients on anti-retroviral therapy. *AIDS*, *16*(7), 1019–1029

Logan, G.D., & Cowan, W.B. (1984). On the ability to inhibit thought and action: A theory of an act of control. *Psychological Review*, *91*(3), 295–327. http://doi.org/10.1037/0033-295X.91.3.295

Logan, G.D., Cowan, W.B., & Davis, K.A. (1984). On the ability to inhibit simple and choice reaction time responses: A model and a method. *Journal of Experimental Psychology: Human Perception and Performance*, *10*(2), 276–291. http://doi.org/10.1037/0096-1523.10.2.276

Meade, C.S., Lowen, S.B., MacLean, R.R., Key, M.D., & Lukas, S.E. (2011). fMRI brain activation during a delay discounting task in HIV-positive adults with and without cocaine dependence. *Psychiatry Research: Neuroimaging*, *192*(3), 167–175. http://doi.org/10.1016/j.pscychresns.2010.12.011

Melrose, R.J., Tinaz, S., Castelo, J.M.B., Courtney, M.G., & Stern, C.E. (2008). Compromised fronto-striatal functioning in HIV: An fMRI investigation of semantic event sequencing. *Behavioural Brain Research*, *188*(2), 337–347. http://doi.org/10.1016/j.bbr.2007.11.021

Miller, E.N., Satz, P., & Visscher, B. (1991). Computerized and conventional neuropsychological assessment of HIV-1-infected homosexual men. *Neurology*, *41*(10), 1608–1616

Mink, J.W. (1996). The basal ganglia: Focused selection and inhibition of competing motor programs. *Progress in Neurobiology*, *50*(4), 381–425

Navia, B.A., Jordan, B.D., & Price, R.W. (1986). The AIDS dementia complex: I. Clinical features. *Annals of Neurology*, *19*(6), 517–524. http://doi.org/10.1002/ana.410190602

Oldfield, R.C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113

Ortega, M., Brier, M.R., & Ances, B.M. (2015). Effects of HIV and combination antiretroviral therapy on cortico-striatal functional connectivity. *AIDS*, *29*(6), 703–712. http://doi.org/10.1097/QAD.0000000000000611

Paul, R., Flanigan, T., Tashima, K., Cohen, R., Lawrence, J., Alt, E., … Hinkin, C. (2005). Apathy correlates with cognitive function but not CD4 status in patients with human immunodeficiency virus. *Journal of Neuropsychiatry and Clinical Neurosciences*, *17*(1), 114

Pence, B.W., Gaynes, B.N., Whetten, K., Eron, J.J., Ryder, R.W., & Miller, W.C. (2005). Validation of a brief screening instrument for substance abuse and mental illness in HIV-positive patients. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *40*(4), 434–444

Plessis, du, S., Vink, M., Joska, J.A., Koutsilieri, E., Bagadia, A., Stein, D.J., & Emsley, R. (2015). HIV infection results in ventral-striatal reward system hypo-activation during cue processing. *AIDS*, *29*(11), 1335–1343. http://doi.org/10.1097/QAD.0000000000000680

Plessis, du, S., Vink, M., Joska, J.A., Koutsilieri, E., Stein, D.J., & Emsley, R. (2014). HIV infection and the fronto-striatal system: A systematic review and meta-analysis of fMRI studies. *AIDS*, *28*, 803–811. http://doi.org/10.1097/QAD.0000000000000151

Reda, A.A., & Biadgilign, S. (2012). Determinants of adherence to antiretroviral therapy among HIV-infected patients in Africa. *AIDS Research and Treatment*, *2012*(4), 1–8. http://doi.org/10.1155/2012/574656

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., … Dunbar, G.C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33; quiz 34–57

Stöcker, T., Schneider, F., Klein, M., Habel, U., Kellermann, T., Zilles, K., … Shah, N.J. (2005). Automated quality assurance routines for fMRI data applied to a multicenter study. *Human Brain Mapping*, *25*(2), 237–246. http://doi.org/10.1002/hbm.20096

Thomas, J.B., Brier, M.R., Snyder, A.Z., Vaida, F.F., & Ances, B.M. (2013). Pathways to neurodegeneration: Effects of HIV and aging on resting-state functional connectivity. *Neurology*, *80*(13), 1186–1193. http://doi.org/10.1212/WNL.0b013e318288792b

UNAIDS (2012). *UNAIDS report on the global AIDS epidemic* (pp. 1–106). Geneva: Joint United Nations Program on HIV/AIDS (UNAIDS)

van der Kouwe, A.J.W., Benner, T., Salat, D.H., & Fischl, B. (2008). Brain morphometry with multiecho MPRAGE. *Neuroimage*, *40*(2), 559–569. http://doi.org/10.1016/j.neuroimage.2007.12.025

Van Dijk, K.R.A., Sabuncu, M.R., & Buckner, R.L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage*, *59*(1), 431–438. http://doi.org/10.1016/j.neuroimage.2011.07.044

Vink, M., Kahn, R.S., Raemaekers, M., van den Heuvel, M., Boersma, M., & Ramsey, N.F. (2005). Function of striatum beyond inhibition and execution of motor responses. *Human Brain Mapping*, *25*(3), 336–344. http://doi.org/10.1002/hbm.20111

Vink, M., Zandbelt, B.B., Gladwin, T., Hillegers, M., Hoogendam, J.M., van den Wildenberg, W.P.M., … Kahn, R.S. (2014). Frontostriatal activity and connectivity increase during proactive inhibition across adolescence and early adulthood. *Human Brain Mapping*, *35*(9), 4415–4427. http://doi.org/10.1002/hbm.22483

Wiley, C.A., Soontornniyomkij, V., Radhakrishnan, L., Masliah, E., Mellors, J., Hermann, S.A., … Achim, C.L. (1998). Distribution of brain HIV load in AIDS. *Brain Pathology*, *8*(2), 277–284

Zandbelt, B.B., & Vink, M. (2010). On the role of the striatum in response inhibition. *PLoS One*, *5*(11), e13848. http://doi.org/10.1371/journal.pone.0013848

Zandbelt, B.B., van Buuren, M., Kahn, R.S., & Vink, M. (2011). Reduced proactive inhibition in schizophrenia is related to corticostriatal dysfunction and poor working memory. *Biological Psychiatry*, *70*, 1151–1158

Chapter 4

HIV infection results in ventral–striatal reward system
hypo-activation during cue processing

43

HIV infection results in ventral–striatal reward system hypo-activation during cue processing

Stéfan du Plessis[a], Matthijs Vink[b], John A. Joska[e], Eleni Koutsilieri[c], Asif Bagadia[f], Dan J. Stein[d,e] and Robin Emsley[a]

Objective: Functional MRI has thus far demonstrated that HIV has an impact on frontal–striatal systems involved in executive functioning. The potential impact of HIV on frontal–striatal systems involved in reward processing has yet to be examined by functional MRI. This study therefore aims to investigate the effects of HIV infection on reward processing by examining the function of the ventral–striatal reward system during a monetary incentive delay task.

Design: This is a cross-sectional case-control study.

Methods: Eighteen combined antiretroviral therapy-naive HIV-positive (HIV+) participants, as well as 16 matched healthy controls, performed a monetary incentive delay task. This paradigm assesses behaviour as well as functional brain activity-associated reward anticipation and reward outcome.

Results: HIV+ participants showed a general decrease in activation associated with both neutral as well as potentially rewarding cues in their ventral striatum. We found normal activity related to reward outcome in the orbito-frontal cortex. Despite HIV+ participants' reaction times being significantly slower when independently measured from the reward paradigm, this performance deficit normalized during the performance of the reward task.

Conclusion: HIV caused a decrease in activity during cue processing in the ventral striatum, with normal cortical functioning during reward outcome processing. Our results therefore suggest that HIV not only has an impact on fronto-striatal systems involved in executive functioning, but also has a direct impact on the function of the ventral–striatal reward system.   Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

Introduction

HIV enters the brain in the early stage of infection [1], and even in the era of combined antiretroviral therapy (cART), HIV causes neurocognitive impairment in up to half of the individuals [2]. Functional imaging studies have demonstrated that HIV affects the functioning of the fronto-striatal network, a key system involved in executive functioning [3,4]. The effect of HIV on the fronto-striatal network could, at least in part, be due to high concentrations of viral RNA accumulating in the striatum and the surrounding areas early on during infection [5]. More specifically, striatal dopamine neurons may be particularly sensitive to viral effects [6,7]. This could either be caused directly by toxic effects of viral proteins such as GP120 [8] or tat [9], or indirectly by means of the de-regulation of the host immune system [10].

As striatal function plays an important role in reward processing [11−14], HIV could potentially interfere with the function of the ventral−striatal reward system. Behavioural evidence thus far has indicated that HIV may affect reward processing [15,16]. For example, HIV-positive (HIV+) substance-dependent individuals exhibit greater risky decision-making behaviour on a gambling task [16]. HIV+ participants favoured relatively larger payoffs, which incurred infrequent large penalties. While controls also selected these payoffs, they quickly learned to avoid them. HIV infection was thought to play a role in this behavioural deficit, as although half of the participants were classified as having past drug dependence, few met criteria for current drug dependence. Furthermore, controlling for these factors did not affect the final results. Although such behavioural studies provide evidence for an affect of HIV on reward processing, the effect of HIV in the absence of drug abuse on the function of the ventral−striatal reward system remains to be demonstrated.

Here, we investigated the effects of HIV on the function of the ventral−striatal reward system. Eighteen drug and non-smoking medication (cART)-naive HIV+ participants and 16 matched controls performed a modified version of the monetary incentive delay task while being scanned with functional MRI (fMRI) [17,18]. This task has been shown to reliably activate the ventral−striatum during reward anticipation and the orbito-frontal cortex (OFC) during reward outcome [18−20].

In healthy controls, there is an increase in the ventral−striatal activity during the presentation of reward cues relative to the activity present during neutral cues [14,18]. HIV infection has been associated with hypoactive functional striatal responses, as well as a decreased baseline cerebral blood flow on past neuroimaging studies [21,22, unpublished data]. We therefore predicted that HIV infection is more likely to result in a general decrease in activity in the ventral striatum during reward neutral and reward cues, rather than a specific effect on any given cue.

We also predicted a normal cortical response during reward processing, as we have previously found evidence for an isolated effect of HIV on the striatal regions in our sample population [unpublished data]. As reward outcome tends to activate primarily cortical regions such as the OFC [17], we therefore predicted normal activity in this region in HIV+ participants during reward outcome.

Methods and materials

Participants
Participants were drawn from a larger study cohort described elsewhere [unpublished data]. The participants were recruited from a community primary health clinic in Khayelitsha, Cape Town, South Africa. They provided written informed consent after having received a complete description of the study in their first language (Xhosa), in accordance with procedures approved by the Health Research Ethics Committee (HREC) of Stellenbosch University and the University of Cape Town, Cape Town, South Africa.

Controls were confirmed to be HIV-negative on enzyme-linked immunosorbent assay (ELISA). Both participants and controls were screened using the Mini−International Neuropsychiatric Interview (MINI) 6.0.0/MINI-PLUS 6.0.0 [23]. Participants were excluded if they had a general medical condition that could confound the diagnosis of HIV-associated neuro-cognitive disorder (HAND), a history of substance abuse on the Substance Abuse and Mental Illness Symptoms Screener (SAMISS) questionnaire [24], a Kreek-McHugh-Schluger-Kellogg (KMSK) score for smoking greater than 1 [25], or if they were currently receiving treatment for tuberculosis. All participants were right-handed as confirmed by the Edinburgh Handedness Inventory [26].

Laboratory measures were performed within 2 weeks of neuroimaging. HIV+ participants received a $CD4^+$ cell count, HIV viral load, rapid plasma reagin for syphilis (RPR) and thyroid-stimulating hormone (TSH) level. HIV-ELISA or high plasma viral load confirmed the HIV serostatus. Controls were confirmed negative with HIV-ELISA performed within 2 weeks of neuroimaging.

All the scans were examined by a radiologist for intra-cranial pathology that could potentially confound results. Neuropsychological testing assessed the following cognitive domains: motor ability, memory function, learning, attention, speed of information processing, abstract thinking, executive function and working memory. Raw test scores were converted to t scores by means of a normative control group to calculate a global deficit score (GDS) to ascertain the level of HIV-associated neurocognitive impairment in the present sample [27].

Monetary incentive delay task
The task used [18,28,29] in the present study is based on the original monetary incentive delay (MID) task by Knutson et al. [14] (see Fig. 1). Potentially rewarding trials ($n = 30$) were indicated with a smiling face, and neutral trials ($n = 30$) were indicated with a neutral face at the onset of each trial. A fixation star followed the reward cue. Following the anticipation cue, a target was presented, requiring patients to react as fast as possible by button press using their right (dominant) index finger. All participants were instructed to respond to a target regardless of the trial type. Reward outcome was presented at the end of each trial. During a practice session prior to the main task, the participant's quickest reaction time was recorded to act as a baseline in order to
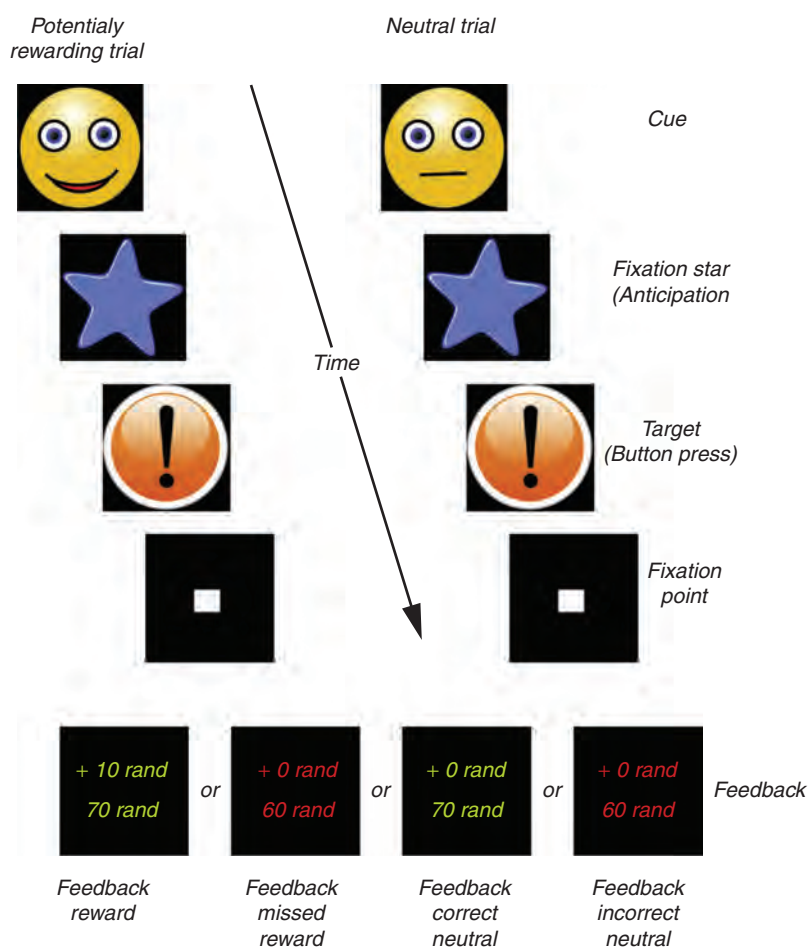
Fig. 1. Schematic representation of the Monetary Incentive Delay task [17,18].

vary the task difficulty. In 50% of the trials, the target was presented for the duration of the individual time limit + 200 ms, enabling participants to be successful in these trials. In the other trials, the time limit was decreased with 150 ms, to make sure that participants could not respond in time.

This ensured adequate power to compare rewarded and non-rewarded trials, as well as ensuring all participants received an equal reward amount (Target R150 ZAR).

To reduce collinearity between anticipation of reward and reward outcome, the anticipation cue time and the inter-trial interval time were varied (mean duration 3286 ms, range 779–6729 ms; mean duration 3535 ms, range 1029–6979 ms, respectively). In this way, the blood oxygen level-dependent (BOLD) signal in response to reward anticipation could be modelled independently from that of the reward outcome [18,20]. The complete task therefore consisted of 60 trials, with a mean duration of 9571 ms (range 4946–16107 ms), resulting in a total task duration of 9 min 35 s.

A response time independent of the reward paradigm was also obtained using the simple response time from the California Computerized Assessment Package (CAL-CAP) [30].

Behavioural data analysis

Repeated-measures analysis of variance (ANOVA) was performed to test for effects of condition (reward trials and neutral trials) and group (HIV+ participants and controls) on response time, as well as response accuracy. The reward amount was compared between groups using a two-sample *t* test.

Functional MRI

Measurements

Measurements   Scans were acquired on a 3T Siemens Allegra at the Combined Universities Brain Imaging Centre (CUBIC). Six hundred and twenty-two whole-brain 2D-EPI images [repetition time (TR) = 1600 ms, echo time (TE) = 23 ms, flip angle 72.5 degrees, field of view (FOV) 256 × 256, 30 slices, 4 mm isotropic voxels] were acquired in about 16 min. For image registration, a T1 ME-MPRAGE-weighted structural scan was acquired (TR = 2530 ms, TE_1 = 1.53 ms, TE_2 = 3.21 ms, TE_3 = 4.89 ms, TE_4 = 6.57 ms, flip angle 7 degrees, FOV 256 mm, 128 slices, 1 isotropic voxel size) [31].

Image pre-processing
Image data were analysed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Pre-processing and first-level statistical analyses were performed as previously described [18]. In brief, pre-processing involved correction for slice timing differences, re-alignment to correct for head motion, spatial normalization to the Montreal Neurological Institute template brain and spatial smoothing to accommodate inter-individual differences in neuro-anatomy. Head motion parameters were analysed to ensure that the maximum motion did not exceed a predefined threshold (scan-to-scan >3 mm).

Individual analyses
The pre-processed time-series data for each individual were analysed using a general linear model (GLM) analysis. The model consisted of six factors of interest, representing haemodynamic changes time-locked to anticipation during and after the presentation of the reward cue (reward anticipation), anticipation during and after a neutral cue (neutral anticipation), feedback reflecting a positive monetary reward outcome (reward outcome), feedback reflecting no reward, feedback reflecting a correct response in a neutral trial (neutral correct outcome) and feedback reflecting an incorrect response in a neutral trial (Fig. 1). The onset of the factors modelling anticipation (duration range 1529−7479 ms) was at the presentation of the cue, whereas the onset of the factors modelling feedback (duration 2000 ms) was at the presentation of the target, including the button press to the target and the subsequent feedback (Fig. 1). Motion parameters from the realignment procedure were included as factors of no interest. Low-frequency drifts were removed from the signal by applying a high-pass filter with a cut-off frequency of 128 s.

For each participant, we generated statistical maps for each of the conditions, as well as the following contrasts: reward anticipation versus neutral anticipation and reward outcome versus neutral correct outcome.

Region-of-interest analyses
Primary analyses were performed in one region of interest (ROI): the combined bilateral ventral striatum for anticipation and combined bilateral OFC for reward outcome, on the basis of previous findings by Knutson *et al.* [17]. These regions were defined using the Automated Anatomical Labeling-Atlas [32] and the Oxford-GSK-Imanova Striatal Connectivity Atlas for the ventral striatum [33]. For each participant, the mean activation level (expressed as percentage signal change) during the contrasts of interest specific to reward anticipation and reward outcome (reward anticipation, neutral anticipation, reward outcome and neutral correct outcome) was calculated over all the voxels of each ROI.

These values were used in a repeated-measures ANOVA, testing for main and group effects in activation levels between neutral versus potentially rewarding trials, reward anticipation versus reward outcome, as well as correct neutral trials versus positive reward outcome.

To determine whether activity was related to clinical measures, we performed a regression analysis in HIV+ participants with activation as a dependent variable, and GDS, viral load, age and sex as predictors.

Whole-brain analysis
In addition to the ROI analysis, whole-brain group-wise analyses were performed, to test for differences outside the predefined ROIs. Group-activation maps were thresholded at a family-wise error (FWE)-corrected cluster level of P equal to 0.05 (cluster-defining threshold of $P = 0.001$, critical cluster size of 26 for reward outcome and 33 for reward anticipation, respectively). These parameters were determined using SPM5 and a script (CorrClusTh.m, to be found on http://www2.warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/scripts/spm), which uses estimated smoothness [estimated full width at half maximum (FWHM): $3.56 \times 3.65 \times 3.46$ voxels] and random field theory to determine the corrected thresholds.

Results

Demographics
Twenty-two HIV+ participants and 18 matched controls were included in the present study. Two HIV+ participants were excluded due to poor task comprehension. One HIV+ participant and two controls were excluded due to poor scan quality as assessed by motion parameters (>3 mm movement), as well as in-house quality assessment software checking for regional signal-to-noise dropout [34−36]. One HIV+ participant screened positive for benzodiazepines prior to scanning. After these exclusions, 18 HIV+ participants and 16 healthy controls were included in the final analysis (see Table 1).

The groups did not differ with regards to sex, age or education level. All participants were from a similar socio-economic background. All HIV+ participants were ambulant and healthy enough to participate in the fMRI task. No potentially confounding intra-cranial pathology was found on inspection of the MRI scans.

Behaviour results
As expected, there was a main effect of condition on reaction time, with both groups reacting significantly faster on potentially rewarding trials than neutral trials [$F(1, 32) = 4.25$, $P = 0.04$]. There was no group-by-condition interaction effect during the performance of

Table 1. Demographic characteristics of the diagnostics groups.

	HIV ($N = 18$)	HC ($N = 16$)	Test statistic	P^*
Sex (M/F)	2/16	1/15	$\chi^2 = 0.249$	1.000
Mean age (years)	32 ± 4.6	28 ± 5.2	$T = -2.363$	0.096
Education (years)	11 (10–12)	12 (11–12)	$U = 111$	1.000
Viral load (copies/ml)	24936.3 ± 31877.4			
CD4$^+$ (cells/µl)	433 ± 199			
Participants with AIDS-defining CD4$^+$ cell count	7			
GDS (summary score)	0.21 (0.07–0.36)	0.17 (0.07–0.36)	$U = 86$	1.000

Age, viral load and CD4$^+$ data represent mean ± SD. Education and GDS data represent median and inter-quartile range between the 25th and 75th centiles. AIDS-defining CD4$^+$ cell count of 350 cells/µl used. F, female; GDS, Global Deficit Score [22]; HC, healthy controls; HIV, HIV-positive participants; M, male.
*P values reported are adjusted using the Bonferroni correction for multiple comparisons.

the reward task [$F(1, 32) = 0.71$, $P = 0.40$], indicating that both groups had a similar decrease in response time during potentially rewarding trials. Finally, there was no main effect of group [$F(1, 34) = 0.97$, $P = 0.33$], indicating that both groups responded equally fast. This was unexpected as we have previously reported that these HIV+ participants had significantly slower simple response times on CALCAP [$t(27) = -2.937$, $P = 0.007$].

As expected, there was a main effect of condition on task accuracy [$F(1, 32) = 32.79$, $P < 0.001$], with both groups showing significantly more accurate responses on potentially rewarding trials, compared to neutral trials. There was no significant group-by-condition interaction effect [$F(1, 32) = 0.451$, $P = 0.507$] or a main effect of group [$F(1, 32) = 0.042$, $P = 0.839$], confirming similar task performance of the HIV+ participants compared to the controls. As task difficulty was adjusted according to individual performance levels, both groups received an equal amount of reward [HIV: $M = 137 ± 15$, control: $M = 140 ± 12$, $t(32) = 0.613$, $P = 0.544$].

Imaging results
Reward processing in the ventral striatum
As expected, there was a significant main effect of condition in the ventral striatum [$F(1, 32) = 4.927$, $P = 0.034$], indicating an increase of ventral–striatal activity from neutral to reward cues. This did not differ between the two groups [group-by-condition interaction: $F(1, 32) = 0.287$, $P = 0.596$]. There was, however, a significant main group effect of condition in the ventral striatum [$F(1, 32) = 5.81$, $P = 0.02$] (see Fig. 2), with HIV+ patients showing less activation during both neutral and reward cues.

In a further exploratory analysis, we investigated activity related to reward outcome, comparing activity during neutral correct and successful reward outcome trials in the ventral striatum. As expected, there was no main effect of condition in the ventral striatum [$F(1, 32) = 1.938$, $P = 0.174$]. There was no group-by-condition interaction effect [$F(1, 32) = 0.311$, $P = 0.581$], nor a main effect of group [$F(1, 32) = 0.051$, $P = 0.822$]. This

demonstrates that HIV has a specific impact on ventral–striatal reward cue processing and not a general effect on striatal blood flow.

Reward processing in the orbito-frontal cortex
As expected, there was a significant main effect of condition in the OFC [$F(1, 32) = 48.924$, $P < 0.001$], indicating an increase from neutral correct outcome to reward outcome in the OFC. There was no interaction effect between group and condition [$F(1, 32) = 0.779$, $P = 0.384$]. There was also no main effect of group [$F(1, 32) = 1.457$, $P = 0.236$].

Whole-brain analysis
To investigate whether there were activation differences outside the predefined regions, we performed whole-brain analyses (see Fig. 3). Whole-brain analysis revealed no results that survived multiple comparisons correction for reward anticipation (contrast: reward anticipation
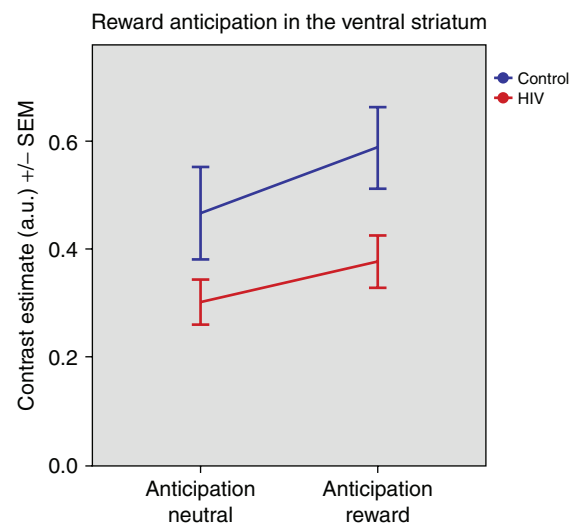
Fig. 2. Line graph showing repeated measures analysis of activity in the ventral striatum in the anticipatory period during neutral and rewarded trials in HIV+ participants and controls. HIV+, HIV-positive.

versus neutral anticipation). On a more liberal threshold [$P = 0.001$ (uncorrected)], reward anticipation-related activity was found in the striatum, thalamus and several frontal, temporal and parietal areas. Reward outcome-related activity (contrast: reward outcome versus neutral correct outcome) was mainly found in the bilateral OFC, anterior cingulate gyrus, left hippocampal and para-hippocampal gyri (FWE-corrected $P = 0.05$, cluster-defining threshold of 26). This is comparable to what has been previously found in the literature [14,18]. No between-group differences were found on whole-brain analysis.

Regression analyses

None of the clinical variables was found to significantly predict activity during reward anticipation or reward outcome in the ventral striatum or OFC.

Discussion

Here, we investigated brain activity during reward processing in 18 HIV+ substance and cART-naive participants and 16 matched controls using fMRI. To our knowledge, this is the first study to investigate the effects of HIV on fronto-striatal functioning during reward processing. Importantly, both groups were substance and cART-naive. As predicted, HIV+ participants showed a general decrease in ventral–striatal activity during anticipation for both neutral and potentially rewarding trials, when compared to controls. The increase in activity related to reward outcome in the OFC did not differ between the two groups. Despite HIV+ participants' reaction times being significantly slower at baseline, this performance deficit normalized during the performance of the reward task. These findings suggest that HIV has an impact on the function of the ventral striatum during cue processing, with a relative sparing of the cortical function. Striatal dysfunction during cue processing was present despite HIV+ participants still being able to speed up their responses in anticipation of a potential reward, indicating that the impact of HIV is not limited to striatally mediated executive function, but extends to reward processing.

We found a decrease in ventral–striatal activity during cue processing in HIV+ participants. It is well known that HIV's impact on brain function often results in a clinical fronto-striatal dementia [37]. Past functional studies have shown general striatal dysfunction, with hypo-activity in the putamen during reactive inhibition [unpublished data], caudate hypo-activity during semantic event sequencing [22], as well as a general decrease in resting cerebral blood flow in the striatum on arterial spin labeling [21]. Our data extend these findings by showing an HIV-related decrease in activity in the ventral–striatal reward system during cue processing. Given the limitations of BOLD fMRI [38], a potential explanation

for a general decrease in regional BOLD signal is that the consequences of HIV infection, such as neuro-inflammation [10], does not affect neural activity *per se*, but rather causes a general change in haemodynamics in the striatum [39]. This is unlikely to be the case, given that we find no decrease in the ventral–striatal activity during reward feedback in HIV. We therefore have shown that HIV does not only affect striatal functions associated with executive functioning such as the inhibition of voluntary movement [unpublished data] or semantic event sequencing [22], but also reward processing. Previous studies in the behavioural impact of HIV have been potentially confounded by past drug dependence [16]. Our findings further support behavioural studies reporting abnormal behaviour on gambling tasks [16] in HIV infection in the absence of illicit drug use/abuse, as cue processing is vital in predicting future rewards. For example, hypo-activation during ventral–striatal anticipatory activity has been associated with impulsive decision-making in alcohol dependence, as well as pathological gambling [40,41].

Our data show that HIV does not result in a specific deficit during the anticipation of reward cues, but also during activity related to neutral cues. Nevertheless, it is likely that a disruption in general cue processing could potentially result in abnormal reward-based decision-making. This could include risky decision-making with respect to sexual behaviour, aggression, substance abuse and potentially a decreased capacity to appreciate the long-term benefits of using and staying on treatment. Importantly, as our sample has no reported drug use, abnormal reward-related behavior in the HIV+ population is unlikely to be explained by the effects of drug abuse alone [15,42].

We found normal activity in the cortical regions known to be active during reward outcome [17]. This finding is consistent with our previous work which suggested that sub-cortical function is primarily affected in the present sample population [unpublished data]. This negative finding is seemingly at odds with other functional studies, which generally demonstrate HIV-induced hyper-activation in the cortex during working memory, as well as during visual attention tasks [4,43–45]. A possible explanation for why some studies found cortical dysfunction when we have not is that these studies differ from our present sample in terms of patient age and cART use. The additional effect of cART, as well as the effect of aging, has been associated with increases in cortical activation in HIV infection in their own right [46,47]. More importantly, participants from our sample population were all newly diagnosed, just prior to the initiation of treatment. This would suggest that HIV could have a different impact on cortical function after treatment [46]. This will have to be confirmed with further prospective studies utilizing fMRI tasks that reliably engage the cortex and the striatum.
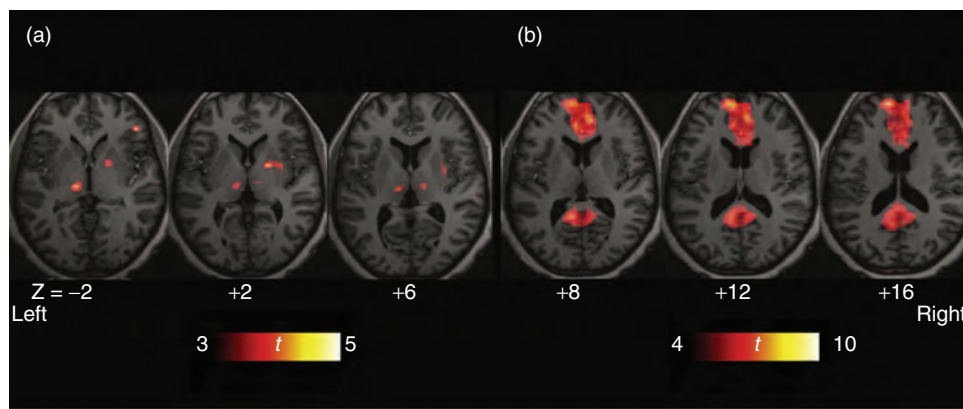
Fig. 3. Whole-brain activation during reward anticipation and reward outcome in HIV+ as well as HIV− controls while performing a Monetary Incentive Delay task [17,18]. One-sample *t* tests of (a) reward anticipation versus neutral anticipation are displayed at an activation threshold of $P = 0.001$ (uncorrected). (b) Reward outcome versus neutral correct outcome are FWE-corrected ($P = 0.05$) with a cluster-defining threshold of 26. FWE, family-wise error; HIV+, HIV-positive; HIV−, HIV-negative.

Both controls and HIV+ participants showed significantly faster response times as well as increased response accuracy between neutral trials and potentially rewarding trials. This is in keeping with behavioural responses previously reported in healthy control populations [18]. This indicates task comprehension in both the groups. Furthermore, the HIV+ participants are still able to anticipate potentially rewarding trials. The fact that HIV+ participants did not show generally slower responses than controls is surprising, given that we did indeed find slower simple response times acquired independent of the fMRI task. Although it is well known that HIV infection is associated with reaction time slowing [48], here we show that this response slowing could normalize during situations with a positive motivational valence. This implicates at least, in part, deficient reward processing in response time slowing in HIV.

As we have used a relatively simple reward task that utilizes only one level of reward, we cannot rule out the possibility that HIV+ participants will start showing slower behavioural responses with increased task complexity with multiple levels of reward, as well as the inclusion of punishment trials. We chose to utilize a simpler task to ensure that reward anticipation would not be influenced by a lack of task comprehension. As the present task was successfully utilized in children as young as 10 years, who demonstrated no measurable differences in response accuracy from those of adults, we believe that the task was simple enough not to confound performance in the present study [18]. As our task involved on an average a 50% failure rate, factors such as participant frustration could potentially have differed between the groups. Task accuracy was, however, not significantly different in the present sample, and therefore differences in levels of group frustration should have been minimal.

As our sample largely consisted of female participants, special mention should be made on the possible influence of sex on our findings. It has been postulated that women are differentially at risk for the development of HAND, due to potentially different prevalence of risk factors such as poverty, differences in substance abuse and prevalence of mental health disorders [49–52]. Studies performed thus far have indeed found a small HIV serostatus-by-sex interaction on cognition, with HIV-related comorbidities having a much larger impact [50,52]. The only functional study performed thus far in a largely female cohort did indeed report hippocampal dysfunction in women, but could potentially have been confounded by substance abuse [51]. In the present study, we confirm an impact of HIV on brain function in HIV+ women, and extend these results by demonstrating a functional impact of HIV in the absence of a history of drug use, severe comorbid psychopathology (i.e. major depression), as well as differences in demographic variables, which we controlled for with a strict sample selection.

HIV caused a decrease in activity during cue processing in the ventral striatum, with normal cortical functioning during reward outcome processing. Our results therefore suggest that HIV not only has an impact on the fronto-striatal systems involved in executive functioning, but also has a direct impact on the function of the ventral–striatal reward system.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1. An SF, Groves M, Gray F, Scaravilli F. **Early entry and wide-spread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals.** *J Neuropathol Exp Neurol* 1999; **58**:1156–1162.
2. Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, et al. **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy.** *Neurology* 2010; **75**:2087–2095.
3. Grant I. **Neurocognitive disturbances in HIV.** *Int Rev Psychiatry* 2008; **20**:33–47.
4. Plessis du S, Vink M, Joska JA, Koutsilieri E, Stein DJ, Emsley R. **HIV infection and the fronto-striatal system: a systematic review and meta-analysis of fMRI studies.** *AIDS* 2014; **28**:803–811.
5. Kumar AM, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. **Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction.** *J Neurovirol* 2007; **13**:210–224.
6. Koutsilieri E, Sopper S, Scheller C, Meulen ter V, Riederer P. **Involvement of dopamine in the progression of AIDS dementia complex.** *J Neural Transm* 2002; **109**:399–410.
7. Purohit V, Rapaka R, Shurtleff D. **Drugs of abuse, dopamine, and HIV-associated neurocognitive disorders/HIV-associated dementia.** *Mol Neurobiol* 2011; **44**:102–110.
8. Agrawal L, Louboutin J-P, Marusich E, Reyes BAS, Van Bockstaele EJ, Strayer DS. **Dopaminergic neurotoxicity of HIV-1 gp120: reactive oxygen species as signaling intermediates.** *Brain Res* 2010; **1306**:116–130.
9. Bruce-Keller AJ, Chauhan A, Dimayuga FO, Gee J, Keller JN, Nath A. **Synaptic transport of human immunodeficiency virus-Tat protein causes neurotoxicity and gliosis in rat brain.** *J Neurosci* 2003; **23**:8417–8422.
10. Desplats P, Dumaop W, Smith D, Adame A, Everall I, Letendre S, et al. **Molecular and pathologic insights from latent HIV-1 infection in the human brain.** *Neurology* 2013; **80**:1415–1423.
11. Schultz W, Dayan P, Montague PR. **A neural substrate of prediction and reward.** *Science* 1997; **275**:1593–1599.
12. Schultz W. **Predictive reward signal of dopamine neurons.** *J Neurophysiol* 1998; **80**:1–27.
13. Schultz W. **Multiple dopamine functions at different time courses.** *Annu Rev Neurosci* 2007; **30**:259–288.
14. Knutson B, Adams CM, Fong GW, Hommer D. **Anticipation of increasing monetary reward selectively recruits nucleus accumbens.** *J Neurosci* 2001; **21**:RC159.
15. Martin EM, Pitrak DL, Weddington W, Rains NA, Nunnally G, Nixon H, et al. **Cognitive impulsivity and HIV serostatus in substance dependent males.** *J Int Neuropsychol Soc* 2004; **10**:931–938.
16. Hardy DJ, Hinkin CH, Levine AJ, Castellon SA, Lam MN. **Risky decision making assessed with the gambling task in adults with HIV.** *Neuropsychology* 2006; **20**:355–360.
17. Knutson B, Fong GW, Adams CM, Varner JL, Hommer D. **Dissociation of reward anticipation and outcome with event-related fMRI.** *Neuroreport* 2001; **12**:3683–3687.
18. Hoogendam JM, Kahn RS, Hillegers MHJ, van Buuren M, Vink M. **Different developmental trajectories for anticipation and receipt of reward during adolescence.** *Dev Cogn Neurosci* 2013; **6**:113–124.
19. van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn RS, Ramsey NF. **Chronic effects of cannabis use on the human reward system: an fMRI study.** *Eur Neuropsychopharmacol* 2010; **20**:153–163.
20. Luigjes J, Smolders R, Valencia-Alfonso C-E, van Wingen G, de Kwaasteniet B, Mantione M, et al. **Deep brain stimulation restores frontostriatal network activity in obsessive-compulsive disorder.** *Nat Neurosci* 2013:1–4. 2014 **17**:1286.
21. Ances BM, Roc AC, Wang J, Korczykowski M, Okawa J, Stern J, et al. **Caudate blood flow and volume are reduced in HIV+ neurocognitively impaired patients.** *Neurology* 2006; **66**:862–866.
22. Melrose RJ, Tinaz S, Castelo JMB, Courtney MG, Stern CE. **Compromised fronto-striatal functioning in HIV: an fMRI investigation of semantic event sequencing.** *Behav Brain Res* 2008; **188**:337–347.
23. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. **The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10.** *J Clin Psychiatry* 1998; **59 (Suppl 20)**:22–33[quiz 34–57].
24. Pence BW, Gaynes BN, Whetten K, Eron JJ, Ryder RW, Miller WC. **Validation of a brief screening instrument for substance abuse and mental illness in HIV-positive patients.** *J Acquir Immune Defic Syndr* 2005; **40**:434–444.
25. Kellogg SH, McHugh PF, Bell K, Schluger JH, Schluger RP, LaForge KS, et al. **The Kreek-McHugh-Schluger-Kellogg scale: a new, rapid method for quantifying substance abuse and its possible applications.** *Drug Alcohol Depend* 2003; **69**:137–150.
26. Oldfield RC. **The assessment and analysis of handedness: the Edinburgh inventory.** *Neuropsychologia* 1971; **9**:97–113.
27. Carey CL, Woods SP, Gonzalez R, Conover E, Marcotte TD, Grant I, et al. **Predictive validity of global deficit scores in detecting neuropsychological impairment in HIV infection.** *J Clin Exp Neuropsychol* 2004; **26**:307–319.
28. Vink M, Kleerekooper I, van den Wildenberg WPM, Kahn RS. **Impact of aging on frontostriatal reward processing.** *Hum Brain Mapp* Published Online First: 20 February 2015. doi: 10.1002/hbm.22771.

29. de Leeuw M, Kahn RS, Vink M. **Fronto-striatal dysfunction during reward processing in unaffected siblings of schizophrenia patients.** *Schizophr Bull* 2015; **41**:94–103.

30. Albert S, Weber C, Todak G, Polanco C. **An observed performance test of medication management ability in HIV: Relation to neuropsychological status and medication adherence outcomes.** *AIDS Behav* 1999; **3**:121–128.

31. van der Kouwe AJW, Benner T, Salat DH, Fischl B. **Brain morphometry with multiecho MPRAGE.** *NeuroImage* 2008; **40**:559–569.

32. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. **Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.** *NeuroImage* 2002; **15**:273–289.

33. Tziortzi AC, Haber SN, Searle GE, Tsoumpas C, Long CJ, Shotbolt P, et al. **Connectivity-based functional analysis of dopamine release in the striatum using diffusion-weighted MRI and positron emission tomography.** *Cereb Cortex* 2014; **24**:1165–1177.

34. Stöcker T, Schneider F, Klein M, Habel U, Kellermann T, Zilles K, et al. **Automated quality assurance routines for fMRI data applied to a multicenter study.** *Hum Brain Mapp* 2005; **25**:237–246.

35. Geissler A, Gartus A, Foki T, Tahamtan AR, Beisteiner R, Barth M. **Contrast-to-noise ratio (CNR) as a quality parameter in fMRI.** *J Magn Reson Imaging* 2007; **25**:1263–1270.

36. Friedman L, Glover GH. **Report on a multicenter fMRI quality assurance protocol.** *J Magn Reson Imaging* 2006; **23**:827–839.

37. Navia BA, Jordan BD, Price RW. **The AIDS dementia complex: I. Clinical features.** *Ann Neurol* 1986; **19**:517–524.

38. Logothetis NK. **What we can do and what we cannot do with fMRI.** *Nature* 2008; **453**:869–878.

39. Juengst SB, Aizenstein HJ, Figurski J, Lopez OL, Becker JT. **Alterations in the hemodynamic response function in cognitively impaired HIV/AIDS subjects.** *J Neurosci Methods* 2007; **163**:208–212.

40. Beck A, Schlagenhauf F, Wüstenberg T, Hein J, Kienast T, Kahnt T, et al. **Ventral striatal activation during reward anticipation correlates with impulsivity in alcoholics.** *Biol Psychiatry* 2009; **66**:734–742.

41. Balodis IM, Kober H, Worhunsky PD, Stevens MC, Pearlson GD, Potenza MN. **Diminished frontostriatal activity during processing of monetary rewards and losses in pathological gambling.** *Biol Psychiatry* 2012; **71**:749–757.

42. Gonzalez R, Vassileva J, Bechara A, Grbesic S, Sworowski L, Novak RM, et al. **The influence of executive functions, sensation seeking, and HIV serostatus on the risky sexual practices of substance-dependent individuals.** *J Int Neuropsychol Soc* 2005; **11**:121–131.

43. Ernst T, Chang L, Jovicich J, Ames N, Arnold S. **Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients.** *Neurology* 2002; **59**:1343–1349.

44. Ernst T, Yakupov R, Nakama H, Crocket G, Cole M, Watters M, et al. **Declined neural efficiency in cognitively stable human immunodeficiency virus patients.** *Ann Neurol* 2009; **65**:316–325.

45. Chang L, Tomasi D, Yakupov R, Lozar C, Arnold S, Caparelli E, et al. **Adaptation of the attention network in human immunodeficiency virus brain injury.** *Ann Neurol* 2004; **56**:259–272.

46. Chang L, Yakupov R, Nakama H, Stokes B, Ernst T. **Antiretroviral treatment is associated with increased attentional load-dependent brain activation in HIV patients.** *J Neuroimmune Pharmacol* 2007; **3**:95–104.

47. Ances BM, Vaida F, Yeh MJ, Liang CL, Buxton RB, Letendre S, et al. **HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging.** *J Infect Dis* 2010; **201**:336–340.

48. Hardy DJ, Hinhn CH. **Reaction time performance in adults with HIV/AIDS.** *J Clin Exp Neuropsychol* 2002; **24**:912–929.

49. Durvasula RS, Miller EN, Myers HF, Wyatt GE. **Predictors of neuropsychological performance in HIV positive women.** *J Clin Exp Neuropsychol* 2001; **23**:149–163.

50. Stern RA, Arruda JE, Somerville JA, Cohen RA, Boland RJ, Stein MD, et al. **Neurobehavioral functioning in asymptomatic HIV-1 infected women.** *J Int Neuropsychol Soc* 1998; **4**:172–178.

51. Maki PM, Cohen MH, Weber K, Little DM, Fornelli D, Rubin LH, et al. **Impairments in memory and hippocampal function in HIV-positive vs. HIV-negative women: a preliminary study.** *Neurology* 2009; **72**:1661–1668.

52. Maki PM, Rubin LH, Valcour V, Martin E, Crystal H, Young M, et al. **Cognitive function in women with HIV: findings from the Women's Interagency HIV Study.** *Neurology* 2015; **84**:231–240.

Chapter 5

Prefrontal cortical thinning in HIV infection is associated
with impaired striatal functioning

53

CrossMark

Prefrontal cortical thinning in HIV infection is associated with impaired striatal functioning

Stéfan du Plessis[1] · Matthijs Vink[2] · John A. Joska[5] · Eleni Koutsilieri[3] ·
Asif Bagadia[6] · Dan J. Stein[4,5] · Robin Emsley[1]

Abstract While cortical thinning has been associated with HIV infection, it is unclear whether this reflects a direct effect of the virus, whether it is related to disruption of subcortical function or whether it is better explained by epiphenomena, such as drug abuse or comorbid medical conditions. The present study investigated the relationship between cortical thickness and subcortical function in HIV+ patients. Specifically, we examined the relationship between prefrontal cortical thickness and striatal function. Twenty-three largely treatment naïve, non-substance abusing HIV+ participants and 19 healthy controls matched for age, gender, and educational status were included. Cortical morphometry was performed using FreeSurfer software analysis. Striatal function was measured during an fMRI stop-signal anticipation task known to engage the striatum. Any cortical regions showing significant thinning were entered as dependent variables into a single linear regression model which included subcortical function, age, CD4 count, and a measure of global cognitive performance as independent predictors. The only cortical region that was significantly reduced after correction for multiple comparisons was the right superior frontal gyrus. Striatal activity was found to independently predict superior frontal gyral cortical thickness. While cortical thinning in HIV infection is likely multifactorial, viral induced subcortical dysfunction appears to play a role.

Keywords fMRI · Cortex · Atrophy · HIV · Inhibition · Striatum

✉ Stéfan du Plessis
stefandup@sun.ac.za

[1] Department of Psychiatry, 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town 7505, South Africa

[2] Departments of Experimental and Developmental Psychology, Faculty of Social Sciences, Utrecht University, Utrecht, The Netherlands

[3] Institute for Virology and Immunobiology, University of Würzburg, Sanderring 2, 97070 Würzburg, Germany

[4] Medical Research Council (Unit on Anxiety and Stress Disorders), 2nd Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town 7505, South Africa

[5] Department of Psychiatry, University of Cape Town, J-Block, Groote Schuur Hospital, Observatory, Cape Town, South Africa

[6] Department of Radiology, 5th Floor Clinical Building, Faculty of Heath Sciences, University of Stellenbosch, Fransie van Zijl Avenue, Tygerberg, Cape Town 7505, South Africa

Introduction

HIV infection produces a typical fronto–subcortical pattern of neuropsychiatric impairment, (Navia et al. 1986), characterized by specific motor (Hardy and Hinhn 2002), cognitive (Antinori et al. 2007), and behavioral symptoms (Castellon et al. 1998). Despite the widespread availability of combination antiretroviral therapy (cART), HIV infection continues to have a negative impact on cognitive function in up to 52 % of infected individuals (Heaton et al. 2010). HIV is thought to enter the brain via infected monocytes in the early stage of illness (An et al. 1999) and to concentrate primarily in subcortical regions, such as the striatum (Wiley et al. 1998). Here, it is thought to cause synapto-dendritic damage (Moore et al. 2006)/apopotosis either by the induction of neuroinflammation (Desplats et al. 2013) or due to direct toxic effects of viral proteins,

such as tat (Bruce-Keller et al. 2003) and GP120 proteins (Agrawal et al. 2010).

Neuroimaging studies consistently report generalized cortical volume loss in patients with HIV infection (Elovaara et al. 1990; Chang et al. 2011; Pfefferbaum et al. 2012). Additionally, in keeping with the clinical features of subcortical dementia, imaging studies have reported loss of gray matter in the basal ganglia (Elovaara et al. 1990; Aylward et al. 1993; Ances et al. 2012), and subcortical volume reductions have been found even in patients receiving cART treatment (Ances et al. 2012). These volume reductions have been related to viral load (Dewey et al. 2010) as well as the AIDS dementia complex stage (Aylward et al. 1993). It is surprising, therefore, that other studies have not found striatal atrophy in HIV+ patients (Melrose et al. 2008; Heaps et al. 2010). This is all the more unexpected given that frontal striatal dysfunction was consistently found in HIV patients in a recent meta-analysis of working memory studies (Ernst et al. 2002) as well as in studies of episodic memory(Maki et al. 2009) and delay discounting paradigms (Meade et al. 2011). Striatal dysfunction has also been shown to be specifically affected in a simple motor paradigm (Ances et al. 2011), reward processing (du Plessis et al. 2015a) as well as with an inhibition task (du Plessis et al. 2015b). Furthermore, a fMRI study in HIV patients has demonstrated reduced functional connectivity between subcortical and cortical regions, in the presence of normal striatal volumes (Melrose et al. 2008). Thus, the exact relationship between structural and functional changes observed in HIV remains to be determined. Taken together, these findings suggest that it may be that structural and functional imaging techniques are sensitive to different aspects of HIV-related neuropathology.

There are several possible explanations for the cortical thinning reported in HIV infection. First, it could be a direct consequence of viral infection in cortical structures. Second, indirect viral mediated immune processes may be involved (Thompson et al. 2005). Third, epiphenomena such as substance abuse (Jernigan et al. 2005) and age (Ances et al. 2012) should be considered. Finally, cortical thickness reductions could be secondary to the observed subcortical dysfunction in these patients. To understand how subcortical pathology could influence cortical morphology (Draganski et al. 2008), the functional relationship of these two systems needs to be considered. Current thinking is that the cortex and basal ganglia are connected in parallel, as functionally segregated circuits (Alexander and Crutcher 1990). This is based on anatomical (Middleton and Strick 2002), functional connectivity (Zandbelt and Vink 2010) and probabilistic tracking studies (Draganski et al. 2008). Being functionally connected in this manner, it is reasonable to propose that subcortical dysfunction caused by direct viral infection could

result in reductions in cortical thickness in areas that are functionally connected.

In the present study, we investigated the relationship between cortical thickness and subcortical function in HIV+ patients compared to healthy controls. Specifically, we examined the relationship between prefrontal cortical thickness and striatal function, since HIV has been shown to affect the frontostriatal system specifically (du Plessis et al. 2013). We predicted that the striatal hypofunction we have shown in a group of HIV+ participants in a previously published analysis would be related to frontal atrophy in the same group. For the fMRI, we selected an inhibition task that we previously used to demonstrate that the striatum, particularly the putamen, is hypoactive in HIV+ participants (i.e., during successful versus unsuccessful STOPs), whereas frontal functioning was not affected during proactive inhibition (du Plessis et al. 2015b). The morphometric measures for the new analysis in the same cohort, were obtained using the standard Desikan–Killiany Atlas in FreeSurfer to generate regions of interest (Desikan et al. 2006).

These include a number of bilateral prefrontal regions. In particular, activity in the right inferior and superior frontal gyrus is normally functionally coupled with activity in the left putamen (Zandbelt and Vink 2010) during the correct versus incorrect STOPs BOLD contrast.

Methods and materials

Participants

The study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University and the Human Research Ethics Committee of the University of Cape Town, Cape Town, South Africa. Prior to enrolment, all participants provided written consent after receiving a full description of this study. Our sample was recruited from a medically stable clinic-attending population during routine HIV care and testing. A total of 23 HIV+ participants were included in the study together with 19 gender, education, and ethnicity matched healthy controls. The controls were HIV negative, as confirmed by the enzyme-linked immunosorbent assay (ELISA).

Participants were screened using the Mini International Neuropsychiatric Interview (MINI) 6.0.0/MINI-PLUS 6.0.0. (Sheehan et al. 1998). All HIV+ participants received a full physical examination and were excluded if there was: a current comorbid psychiatric/neurological or general medical condition that could confound the diagnosis of HIV-associated neurocognitive disorders (HAND); any history of substance use/abuse as assessed by the Substance Abuse and Mental Illness Symptoms Screener

(SAMISS) screening questionnaire (Pence et al. 2005); a score for smoking greater than 1 according to the Kreek–McHugh–Schluger–Kellogg (KMSK) scale (Kellogg et al. 2003); current active treatment for tuberculosis. All participants were right-handed as confirmed by the Edinburgh Handedness Inventory (Oldfield 1971).

HIV+ participants underwent detailed neuropsychological assessment within two weeks of neuroimaging and controls within one year, for characterization purposes only. The test battery assessed the following cognitive domains: abstraction/executive function, memory, learning, speed of information processing, verbal fluency, motor and sensory/perception (Grant 2008). From these scores, a composite global deficit score (GDS) was derived (Carey et al. 2004) using normative data from a larger parent study (Joska et al. 2010).

The following laboratory measures were performed in the HIV+ participants, within two weeks of neuroimaging: CD_4 count, HIV viral load, rapid plasma reagin for syphilis (RPR), and thyroid stimulating hormone (TSH) level. Urinary drug screen was performed on all participants prior to undergoing the MRI assessments. While hepatits C co-infection has been associated with increased risk and severity of cognitive impairment in HAND, participants were not screened as it is not endemic to the region (Amin et al. 2004). An experienced radiologist reviewed all of the scans for intracranial pathology that could potentially confound functional imaging measurement results.

Image acquisition

All scans were acquired on a 3T Siemens Allegra at the Combined Universities Brain Imaging Centre (CUBIC). For the study of brain morphology, a T1 ME-MPRAGE-weighted structural scan was acquired (TR = 2530 ms; $TE_1 = 1.53$ ms $TE_2 = 3.21$, ms, $TE_3 = 4.89$ ms, $TE_4 = 6.57$ ms, flip-angle: 7°, FOV: 256 mm, 128 slices, 1 mm isotropic voxel size) (van der Kouwe et al. 2008).

Structural imaging analysis

Preprocessing of structural scans

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/). As FreeSurfer is computationally intensive, reconstructions were performed utilizing custom batching scripts, on the Centre for High Performance Computing (CHPC) Rosebank, Cape Town, Sun Intel Nehalem cluster (http://www.chpc.ac.za/).

Scans were processed and analyzed using FreeSurfer stable release version 5.1. The technical details of these procedures are described in prior publications (Dale et al.

1999). Briefly, slices were resampled to a three-dimensional image with 1-mm isotropic voxels. Non-uniform intensity normalization was then performed, and images were registered to the Montreal Neurological Institute (MNI) space. A second normalization step was performed with a different algorithm in which control points were automatically identified and normalized to a standard intensity value. This was followed by an automated skull strip procedure. Gross brain anatomy was then delineated into cortical and subcortical labels. All scans were visually checked for errors in Talaraich transformation, skull stripping as well as segmentation and corrected if possible.

Structural regions of interest analysis

Initially we compared whole brain cortical volumes as well as subcortical volumes, corrected for total intracranial volume (Buckner et al. 2004) between groups to ascertain the general impact of HIV on brain volume in our sample. Then, we subdivided the frontal lobes into regions using standard FreeSurfer regions of interest (Desikan et al. 2006) for their cortical thickness measures. We specifically selected the frontal lobe regions as they are functionally connected to both right and left putamen activation during successful inhibition (Zandbelt and Vink 2010). Apart from playing an important role in executive functions, such as response inhibition (Zandbelt and Vink 2010) and working memory [i.e., rostral middle frontal gyrus (Kikinis et al. 2010)], these regions also play an important role in functions associated with motivational control, such as reward processing [i.e., rostral anterior cingulate, lateral orbitofrontal gyrus, medial orbitofrontal gyrus (Hoogendam et al. 2013)].The following frontal regions were included: bilateral rostral anterior cingulate thickness, medial orbitofrontal thickness, pars opercularis thickness, superior frontal thickness, lateral orbitofrontal thickness, and rostral middle frontal thickness. To limit the number of comparisons, we restricted our a priori analysis to these regions. The regions were compared between groups using a two sample t test. All statistical tests were corrected for multiple comparisons using the Bonferroni method.

Stop-signal anticipation fMRI task

During the fMRI experiment, participants performed the STOP-signal anticipation task (Zandbelt and Vink 2010). Not all participants recruited in the present study could be included in a combined functional and structural analysis due to poor structural and/or poor fMRI data quality (see "Demographics" section). The present set of patients, therefore, represent a new subsample of data previously reported on (du Plessis et al. 2015b).

Functional imaging analysis

Image preprocessing

Image data were modeled using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Preprocessing and first-level statistical analyses were performed as previously described (Zandbelt and Vink 2010). In brief, preprocessing involved correction for slice timing differences, realignment to correct for head motion, spatial normalization to the Montreal Neurological Institute template brain, and spatial smoothing to accommodate inter-individual differences in functional and gyral anatomy during inter-subject averaging. Head motion parameters were analyzed to ensure that the maximum motion did not exceed a pre-defined threshold (scan-to-scan >3 mm) (Van Dijk et al. 2012).

Individual fMRI analyses

The fMRI data were modeled voxel-wise, using a general linear model, in which successful STOP-signal trials and failed STOP-signal trials events were included as regressors. The fMRI data were high-pass filtered (cutoff 128 s) to remove low-frequency drifts. For each participant, we computed a contrast image of activation during successful STOP-signal trials versus failed STOP-signal trials.

Regions of interest analyses

As previously described (du Plessis et al. 2015b), group activation differences in the left and right putamen were determined by a region-of-interest analysis in predefined a priori regions, defined by a previous experiment (Zandbelt and Vink 2010), in which an independent sample of healthy volunteers performed the same task. These ROIs were defined using a cluster-level threshold (cluster-defining threshold $p < 0.001$, cluster probability of $p < 0.05$, family-wise error corrected for multiple comparisons). For each participant, the mean activation level (expressed as percent signal change) during the contrasts of interest specific to successful STOP-signal trials versus failed STOP-signal trials was calculated over all the voxels of each ROI.

Regression analysis

Brain regions showing significant cortical thinning were entered into a linear regression model as the dependant variable. Contralateral left putamen activation during successful versus unsuccessful stops was entered as a predictor as it has been previously shown to be primarily associated with right superior frontal lobe activity during inhibition

(Zandbelt and Vink 2010). Age, CD4 count, and GDS scores were also included in the model, as they are known to affect cortical thickness (Thompson et al. 2005; Schnack et al. 2015). We did not include subcortical volume measures, as there we no differences between patients and controls. Viral load was also not included, as there were two missing data points. Finally, we did not compare cortical activation with cortical thickness as we found no differences in cortical function between HIV+ participants and controls in our previously reported fMRI results in this cohort (du Plessis et al. 2015b).

Post hoc exploratory correlation analysis

Lastly, we wished to clarify whether the relationship between striatal activation and frontal cortical thinning was specific to these regions or whether it represents part of a more general association between frontal cortical structure and subcortical function. To this end, we performed an uncorrected correlation analyses between all of the FreeSurfer regions known to be related to response inhibition.

Results

Demographics

Twenty-five HIV+ participants and 19 matching controls were initially included in the study. Two HIV+ participants and one control were excluded from the structural analysis due to poor scan quality. Thus, 23 HIV+ participants and 18 healthy controls were included in the final structural analysis (see Table 1). A further six HIV+ participants were excluded from the regression analysis for the following reasons: Tested positive for benzodiazepines ($n = 1$); uncertainty regarding general medical condition ($n = 1$); technical problems with the response box ($n = 2$); poor scan quality on their functional scans ($n = 1$); and missing data ($n = 1$).

The groups did not differ with regards to age, gender, educational level or final GDS score (see Table 1). All subjects were from a similar socioeconomic background. All of the HIV+ participants were ambulant and healthy enough to participate in the fMRI task. No intracranial pathology was found on inspection of the MRI scans.

Imaging results

Structural regions of interest analysis

Compared to healthy volunteers, the HIV+ participants showed a smaller total cortical volumes [$t(39) = 3.14$, $p = 0.003$], suggestive of general cortical atrophy. However, no differences were found in subcortical volumes

Table 1 Demographic characteristics of the diagnostics groups

	HIV ($N = 23$)	HC ($N = 18$)	Test statistic	P^*
Gender (M/F)	4/19	3/15	$\chi^2 = 0.004$	1.000
Age (years)	31 (27–34)	30 (22–32)	$U = 127$	0.14
Education (years)	11 (10–12)	12 (11–12)	$U = 179.5$	1.000
Viral load (copies/ml)	$42{,}364 \pm 55{,}376$			
CD_4 (cells/μl)	401 ± 207			
Participants with AIDS defining CD_4 count	9			
GDS (Summary Score)	0.15 (0.05–0.3)	0.133 (0–0.36)	$U = 160$	1.000
On cART	2			

Age, viral load, and CD_4 data represent mean $\pm$ SD. Education and GDS data represent median and interquartile range between 25th and 75th centiles

F female, *M* male, *GDS* Global Deficit Score (22), *HC* healthy controls, *HIV* HIV+ participants, *AIDS* CD_4 count of 350 cells/μl used, *cART* combined antiretroviral therapy

* P values are adjusted for multiple comparisons using the Bonferroni method

between the groups [$t(39) = 1.14$, $p = 0.26$]. Specifically, we found no differences in right [$t(39) = 0.453$, $p = 0.653$] or left [$t(39) = 0.381$, $p = 0.705$] putamen volumes.

For the cortical thickness measures, the only region of interest to be significantly reduced compared with healthy controls after Bonferrroni correction was the right superior frontal gyrus [$t(39) = 3.15$, $p = 0.003$]. At an uncorrected threshold of $p = 0.05$, we found reduced cortical thickness in patients in the right medial orbitofrontal gyrus [$t(39) = 2.56$, $p = 0.01$], left pars opercularis [$t(39) = 2.07$, $p = 0.05$], left [$t(39) = 1.98$, $p = 0.05$] superior frontal gyrus as well as left [$t(39) = 2.62$, $p = 0.01$] rostral and right [$t(39) = 2.05$, $p = 0.05$] middle frontal gyrus. This suggests a trend to overall frontal cortical thickness reductions in HIV (see Table 2).

Functional regions of interest analysis

Successful inhibition

We found hypo-activation in the left [$t(32) = 2.279$, $p = 0.029$] as well as the right [$t(32) = 2.233$, $p = 0.033$] putamen in this particular subsample confirming our results from our previous analysis in the larger cohort (du Plessis et al. 2015b).

Regression analysis

Reduced right superior frontal gyral thickness in the HIV+ group was predicted by a decrease in left putamen BOLD activity ($\beta = 0.381$, $p = 0.033$), as well as by lower GDS score ($\beta = -0.531$, $p = 0.003$), more advanced age ($\beta = -0.381$, $p = 0.030$), and lower CD4 count ($\beta = 0.543$, $p = 0.004$). Together, these predictors accounted for 76.5 % of the total variance [$R^2 = 0.765$, $R^2_{adjusted} = 0.687$, $F(4,12) = 9.763$, $p = 0.001$] (Fig. 1).

To determine whether striatal activation was related to cortical thickness in the other frontal regions that did not show significant thinning, we also sought correlations between putamen activation and these other regions of interest. We did not correct for multiple comparisons in this additional exploratory analysis. Significant positive correlations were found between task-related putamen BOLD activity during inhibition and cortical thickness in 9 out of our 12 frontal lobe regions of interest for the left and 3 out of 12 for the right putamen (see Table 3).

Discussion

In this study, we investigated the relationship between prefrontal cortical thickness and striatal function in 23 HIV+ participants and 18 matched healthy controls. To the best of our knowledge, this is the first study to explore the relationship between subcortical function and cortical thickness in HIV infection. Our main finding was that decreased striatal activity was associated with reduced prefrontal cortical thickness in a region that is involved in inhibition, i.e., the superior frontal gyrus. Rather than being an isolated association with the superior frontal gyrus, our uncorrected post hoc analyses suggest that this was part of a general trend toward reduced cortical thickness being related to decreased striatal function. While we cannot infer causality from our findings, one possible explanation is that the frontal cortical thinning is a consequence of viral induced striatal hypofunction. This would be consistent with neuropathological evidence suggesting that HIV concentrates primarily in subcortical regions, such as the striatum (Wiley et al. 1998), and would also explain the typical pre-treatment clinical presentation of a subcortical dementia (Navia et al. 1986). However, an alternative explanation that should be kept in mind is that cortical

Table 2 Group differences comparing total gray matter volume, subcortical volume, and region of interest cortical thickness

FreeSurfer region of interest	HIV	Controls	Std. error difference	P
Subcortical gray matter volume (mm^3)	1185.34	1228.38	37.89	0.26
Total gray matter volume (mm^3)	4060.03	4345.17	90.88	0.003**
Total intra-cranial volume (mm^3)	1,387,129.69	1,357,170.04	40,345.38	0.46
Left rostral anterior cingulate thickness (mm)	2.74	2.85	0.10	0.32
Right rostral anterior cingulate thickness (mm)	2.56	2.63	0.07	0.34
Left medial orbitofrontal thickness (mm)	2.51	2.53	0.08	0.81
Right medial orbitofrontal thickness (mm)	2.34	2.49	0.06	0.01**
Left pars opercularis thickness (mm)	2.46	2.60	0.07	0.04*
Right pars opercularis thickness (mm)	2.55	2.68	0.07	0.09
Left superior frontal thickness (mm)	2.77	2.90	0.07	0.05*
Right superior frontal thickness (mm)	2.69	2.87	0.06	0.003**
Left lateral orbitofrontal thickness (mm)	2.59	2.70	0.07	0.13
Right lateral orbitofrontal thickness (mm)	2.54	2.54	0.07	0.98
Left rostral middle frontal thickness (mm)	2.23	2.36	0.05	0.01**
Right rostral middle frontal thickness (mm)	2.25	2.35	0.05	0.05*

** Significant at the 0.01 level

* Significant at the 0.05 level
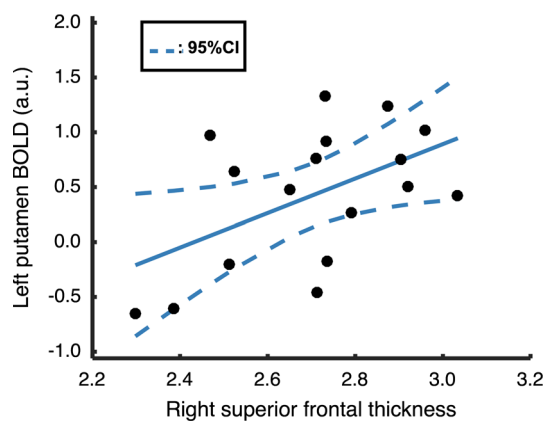
Fig. 1 Scatterplot showing a significant linear relationship between subcortical function loss and right superior frontal cortical thinning in HIV+ participants. *CI* confidence interval

thinning is a consequence of a more global insult of HIV on the brain, and that striatal hypofunction is secondary to this process. Our study confirms previous findings that a decreased CD4 count is associated with reductions in cortical thickness (Stout et al. 1998; Thompson et al. 2005). Also, as with previous functional imaging (Ances et al. 2010), we were able to show that our findings were independent of age-related changes. However, as our sample is relatively young, future work in older populations should further investigate the effects of age on brain function and morphology in HIV infection. Taken together, our results suggest that cortical thinning in HIV infection is likely multifactorial, and that in addition to factors such as

immune status, age, and substance abuse, striatal dysfunction may be an important factor to consider.

Our finding of generalized cortical volume reduction in HIV-infected individuals confirms previous studies (Elovaara et al. 1990; Aylward et al. 1993; Thompson et al. 2005; Ances et al. 2010; Dewey et al. 2010). On the other hand, our failure to find evidence of subcortical volume reduction is not consistent with earlier studies reporting subcortical atrophy in HIV (Elovaara et al. 1990; Aylward et al. 1993; Dewey et al. 2010; Ances et al. 2012). However, our results are similar to more recent studies reporting no striatal volume loss (Melrose et al. 2008; Heaps et al. 2012). At face value, these results seem to contradict the long held classification of HIV as primarily a fronto–subcortical neurodegenerative process (Navia et al. 1986). One possible explanation is that the more recent studies included patients identified at an earlier stage of illness. Also, volume reductions due to neuronal damage could possibly be masked by active glial proliferation in these regions, as the striatum is thought to be a site of active viral replication (Wiley et al. 1998).

Our study is limited by a small sample size, which limits our ability to detect more subtle relationships between HIV-inducted striatal function and brain morphology. Also, we only used one functional paradigm, therefore limiting our ability to determine whether functional loss in other cortico–striatal circuits is also related to changes in brain morphology. As our sample included participants who were relatively cognitively intact, we could not effectively investigate the potential interaction between cognitive

Table 3 Correlations between regions of interest and subcortical BOLD activity during reactive inhibition

	Right putamen BOLD	P	Left putamen BOLD	P
Left rostral anterior cingulate thickness	0.398	0.102	0.599[a]	0.009
Right rostral anterior cingulate thickness	−0.064	0.800	−0.023	0.929
Left medial orbitofrontal thickness	0.045	0.860	0.355	0.148
Right medial orbitofrontal thickness	0.377	0.123	0.583[b]	0.011
Left pars opercularis thickness	0.490[b]	0.039	0.541[b]	0.020
Right pars opercularis thickness	0.295	0.234	0.469[b]	0.050
Left superior frontal thickness	0.334	0.176	0.523[b]	0.026
Right superior frontal thickness	0.321	0.194	0.527[b]	0.024
Left lateral orbitofrontal thickness	0.411	0.090	0.641[a]	0.004
Right lateral orbitofrontal thickness	0.517[b]	0.028	0.717[a]	0.001
Left rostra lmiddle frontal thickness	0.492[b]	0.038	0.579[b]	0.012
Right rostral middle frontal thickness	0.306	0.216	0.462	0.054

[a] Correlation is significant at the 0.01 level (two-tailed)

[b] Correlation is significant at the 0.05 level (two-tailed)

impairment, subcortical dysfunction, and cortical atrophy. We did, however, find that, when controlling for cognitive performance, the significant relationship between subcortical dysfunction and frontal cortical thickness persisted. Our sample was carefully selected to control for recognized confounding variables. Although representative of the local clinic-going population, we excluded factors such as severe comorbid depression, the elderly, and a history of substance use/abuse. Although this resulted in a relatively homogenous sample, it also limits the generalizability of our findings. Further studies should investigate the role of such comorbidities.

In conclusion, we have shown that striatal function is related to frontal cortical thickness in HIV infection, even in the presence of normal striatal volumes. This effect was found to be independent of factors such as age, cognitive impairment, and CD4 count.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

References

Agrawal L, Louboutin J-P, Marusich E et al (2010) Dopaminergic neurotoxicity of HIV-1 gp120: reactive oxygen species as signaling intermediates. Brain Res 1306:116–130. doi:10.1016/j.brainres.2009.09.113

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271

Amin J, Kaye M, Skidmore S et al (2004) HIV and hepatitis C coinfection within the CAESAR study. HIV Med 5:174–179. doi:10.1111/j.1468-1293.2004.00207.x

An SF, Groves M, Gray F, Scaravilli F (1999) Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. J Neuropathol Exp Neurol 58:1156–1162

Ances BM, Vaida F, Yeh MJ et al (2010) HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging. J Infect Dis 201:336–340. doi:10.1086/649899

Ances BM, Vaida F, Cherner M et al (2011) HIV and chronic methamphetamine dependence affect cerebral blood flow. J Neuroimmune Pharmacol 6:409–419. doi:10.1007/s11481-011-9270-y

Ances BM, Ortega M, Vaida F et al (2012) Independent effects of HIV, aging, and HAART on brain volumetric measures. J Acquir Immune Defic Syndr 59:469–477. doi:10.1097/QAI.0b013e318249db17

Antinori A, Arendt G, Becker JT et al (2007) Updated research nosology for HIV associated neurocognitive disorders. Neurology 69:1789–1799

Aylward EH, Henderer JD, Mcarthur JC et al (1993) Reduced basal ganglia volume in HIV-1-associated dementia: results from quantitative neuroimaging. Neurology 43:2099–2104

Bruce-Keller AJ, Chauhan A, Dimayuga FO et al (2003) Synaptic transport of human immunodeficiency virus-Tat protein causes neurotoxicity and gliosis in rat brain. J Neurosci 23:8417–8422

Buckner RL, Head D, Parker J et al (2004) A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage 23:724–738. doi:10.1016/j.neuroimage.2004.06.018

Carey CL, Woods SP, Gonzalez R et al (2004) Predictive validity of global deficit scores in detecting neuropsychological impairment in HIV infection. J Clin Exp Neuropsychol 26:307–319. doi:10.1080/13803390490510031

Castellon SA, Hinkin CH, Wood S, Yarema KT (1998) Apathy, depression, and cognitive performance in HIV-1 infection. J Neuropsychiatry Clin Neurosci 10:320–329

Chang L, Andres M, Sadino J et al (2011) Impact of apolipoprotein E ε4 and HIV on cognition and brain atrophy: antagonistic pleiotropy and premature brain aging. Neuroimage 58:1017–1027. doi:10.1016/j.neuroimage.2011.07.010

Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9:179–194. doi:10.1006/nimg.1998.0395

Desikan RS, Ségonne F, Fischl B et al (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31:968–980. doi:10.1016/j.neuroimage.2006.01.021

Desplats P, Dumaop W, Smith D et al (2013) Molecular and pathologic insights from latent HIV-1 infection in the human brain. Neurology 80:1415–1423. doi:10.1212/WNL.0b013e31828c2e9e

Dewey J, Hana G, Russell T et al (2010) Reliability and validity of MRI-based automated volumetry software relative to auto-assisted manual measurement of subcortical structures in HIV-infected patients from a multisite study. Neuroimage 51:1334–1344. doi:10.1016/j.neuroimage.2010.03.033

Draganski B, Kherif F, Klöppel S et al (2008) Evidence for Segregated and integrative connectivity patterns in the human basal ganglia. J Neurosci 28:7143–7152. doi:10.1523/JNEUROSCI.1486-08.2008

du Plessis S, Vink M, Joska JA et al (2013) HIV infection and the fronto-striatal system: a systematic review and meta-analysis of fMRI studies. AIDS 28:803–811. doi:10.1097/QAD.0000000000000151

du Plessis S, Vink M, Joska JA et al (2015a) HIV infection results in ventral-striatal reward system hypo-activation during cue processing. AIDS 29:1335–1343. doi:10.1097/QAD.0000000000000680

du Plessis S, Vink M, Joska JA et al (2015b) HIV infection is associated with impaired striatal function during inhibition with normal cortical functioning on functional MRI. J Int Neuropsychol Soc 21:722–731. doi:10.1017/S1355617715000971

Elovaara I, Poutiainen E, Raininko R et al (1990) Mild brain atrophy in early HIV infection: the lack of association with cognitive deficits and HIV-specific intrathecal immune response. J Neurol Sci 99:121–136

Ernst T, Chang L, Jovicich J et al (2002) Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. Neurology 59:1343–1349

Grant I (2008) Neurocognitive disturbances in HIV. Int Rev Psychiatry 20:33–47. doi:10.1080/09540260701877894

Hardy DJ, Hinhn CH (2002) Reaction time performance in adults with HIV/AIDS. J Clin Exp Neuropsychol 24:912–929

Heaps J, Niehoff J, Lane E et al (2010) Brain imaging in behavioral medicine and clinical neuroscience. Springer, New York

Heaps JM, Joska J, Hoare J et al (2012) Neuroimaging markers of human immunodeficiency virus infection in South Africa. J Neurovirol 18:151–156. doi:10.1007/s13365-012-0090-5

Heaton RK, Clifford DB, Franklin DR Jr et al (2010) HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy. Neurology 75:2087–2095

Hoogendam JM, Kahn RS, Hillegers MHJ et al (2013) Different developmental trajectories for anticipation and receipt of reward during adolescence. Dev Cogn Neurosci 6:113–124. doi:10.1016/j.dcn.2013.08.004

Jernigan TL, Gamst AC, Archibald SL et al (2005) Effects of methamphetamine dependence and HIV infection on cerebral morphology. Am J Psychiatry 162:1461–1472. doi:10.1176/appi.ajp.162.8.1461

Joska JA, Westgarth-Taylor J, Myer L et al (2010) Characterization of HIV-associated neurocognitive disorders among individuals starting antiretroviral therapy in south Africa. AIDS Behav 15:1197–1203. doi:10.1007/s10461-010-9744-6

Kellogg SH, McHugh PF, Bell K et al (2003) The Kreek–McHugh–Schluger–Kellogg scale: a new, rapid method for quantifying substance abuse and its possible applications. Drug Alcohol Depend 69:137–150

Kikinis Z, Fallon JH, Niznikiewicz M et al (2010) Gray matter volume reduction in rostral middle frontal gyrus in patients with chronic schizophrenia. Schizophr Res 123:153–159. doi:10.1016/j.schres.2010.07.027

Maki PM, Cohen MH, Weber K et al (2009) Impairments in memory and hippocampal function in HIV-positive vs HIV-negative women: a preliminary study. Neurology 72:1661–1668. doi:10.1212/WNL.0b013e3181a55f65

Meade CS, Lowen SB, MacLean RR et al (2011) fMRI brain activation during a delay discounting task in HIV-positive adults with and without cocaine dependence. Psychiatry Res Neuroimaging 192:167–175. doi:10.1016/j.pscychresns.2010.12.011

Melrose RJ, Tinaz S, Castelo JMB et al (2008) Compromised fronto-striatal functioning in HIV: an fMRI investigation of semantic event sequencing. Behav Brain Res 188:337–347. doi:10.1016/j.bbr.2007.11.021

Middleton FA, Strick PL (2002) Basal-ganglia "projections" to the prefrontal cortex of the primate. Cereb Cortex 12:926–935

Moore DJ, Masliah E, Rippeth JD et al (2006) Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. AIDS 20:879–887. doi:10.1097/01.aids.0000218552.69834.00

Navia BA, Jordan BD, Price RW (1986) The AIDS dementia complex: I. Clinical features. Ann Neurol 19:517–524. doi:10.1002/ana.410190602

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113

Pence BW, Gaynes BN, Whetten K et al (2005) Validation of a brief screening instrument for substance abuse and mental illness in HIV-positive patients. J Acquir Immune Defic Syndr 40:434–444

Pfefferbaum A, Rosenbloom MJ, Sassoon SA et al (2012) Regional brain structural dysmorphology in human immunodeficiency virus infection: effects of acquired immune deficiency syndrome, alcoholism, and age. Biol Psychiatry 72:361–370. doi:10.1016/j.biopsych.2012.02.018

Schnack HG, van Haren NEM, Brouwer RM et al (2015) Changes in thickness and surface area of the human cortex and their relationship with intelligence. Cereb Cortex 25:1608–1617. doi:10.1093/cercor/bht357

Sheehan DV, Lecrubier Y, Sheehan KH et al (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the

development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59 Suppl 20:22–33 (quiz 34–57)

Stout JC, Ellis RJ, Jernigan TL et al (1998) Progressive cerebral volume loss in human immunodeficiency virus infection: a longitudinal volumetric magnetic resonance imaging study. HIV Neurobehavioral Research Center Group. Arch Neurol 55:161–168

Thompson PM, Dutton RA, Hayashi KM et al (2005) Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4+ T lymphocyte decline. Proc Natl Acad Sci USA 102:15647–15652. doi:10.1073/pnas.0502548102

van der Kouwe AJW, Benner T, Salat DH, Fischl B (2008) Brain morphometry with multiecho MPRAGE. Neuroimage 40:559–569. doi:10.1016/j.neuroimage.2007.12.025

Van Dijk KRA, Sabuncu MR, Buckner RL (2012) The influence of head motion on intrinsic functional connectivity MRI. Neuroimage 59:431–438. doi:10.1016/j.neuroimage.2011.07.044

Wiley CA, Soontornniyomkij V, Radhakrishnan L et al (1998) Distribution of brain HIV load in AIDS. Brain Pathol 8:277–284

Zandbelt BB, Vink M (2010) On the role of the striatum in response inhibition. PLoS One 5:e13848. doi:10.1371/journal.pone.0013848

Chapter 6

Discussion

63

Discussion

In this section, we discuss the four neuro-imaging studies investigating the early impact of HIV infection on the fronto-striatal system included in this thesis. The four studies confirmed the impact of HIV on the fronto-striatal system. Our meta-analysis demonstrated a consistent impact on this system in fMRI working memory and visual attention tasks. Furthermore, our studies demonstrated an additional impact on two key sub-networks of this system. Finally, the relationship between striatal hypofunction and prefrontal cortical atrophy highlight the importance of the early impact of HIV on this system.

In the first study, we investigated the potential impact of HIV on brain function by performing the first quantitative meta-analysis on all suitable published fMRI data [48]. As predicted, the meta-analysis demonstrated consistent functional differences specific to the fronto-striatal system during tasks of working memory and/or visual attention. This result confirmed that functional differences in HIV are present even in the absence of severe cognitive impairment. This suggests that fMRI could be sensitive to the earliest impact of HIV on brain function.

Our findings further add to the existing literature by showing that the functional differences seen in visual attention/working memory in HIV infection are concentrated in the prefrontal cortex and the dorsal striatum. This implicates specific involvement of the fronto-striatal system, which supports the original clinical classification of HIV as being a fronto-subcortical dementia [14]. It is also in keeping with the post mortem evidence of HIV RNA pooling in the striatal regions [17]. Although visual attention and working memory function forms an

64

important part of the workings of the fronto-striatal system, the analysis methods used in the studies included in the meta-analysis were mostly undirected whole brain methods. This method of analysis does not specifically focus on the striatum and its corresponding cortical regions. Similarly, important major fronto-striatal sub-networks such as the inhibitory network and the reward system also remain unexplored in a systematic, regionally specific way. This approach does not, therefore, allow for the unambiguous clarification of the impact of HIV on striatal function.

Furthermore, despite the clear evidence of HIV infection consistently affecting the striatal regions, it is still uncertain from the literature whether this is a result of the direct impact of HIV or from other confounding factors. That is, most studies included subjects with a substantial drug abuse history, which have been shown to also affect the fronto-striatal system [33]. Another important confounding factor in the fMRI literature is cART, having also been associated with effects in the prefrontal regions [43]. Finally, fMRI studies tended to include older participants, and therefore the confounding effects of age remain unclear. The systematic review and meta-analysis therefore confirmed our initial literature review, highlighting the importance of further exploration of these subtle changes in the major sub-networks of the fronto-striatal system while controlling for important confounding factors.

Building on the gaps identified by the meta-analysis, the second publication included in the current thesis [54], investigated the potential effect of HIV on the inhibitory network, while controlling for previously mentioned important confounding factors. As hypothesized, HIV infection did indeed have an impact on striatal function during reactive inhibition in a sample of untreated, clinically stable HIV+ participants. This confirmed previous fMRI findings of an

65

effect on the motor system during simple motor tasks such as finger tapping in HIV infection, and extends these findings by demonstrating an effect of HIV on striatal function during complex motor function. Based on our finding the cortex to be unaffected during proactive inhibition, the early impact of the virus on the fronto-striatal system could be reflected in our finding of striatal hypo-function.

These results indicate that HIV itself does indeed impact an important sub-system of the fronto-striatal network crucial in executive functioning in early HIV infection before the onset of clinically measureable symptoms. This fronto-striatal function however, does not involve regions associated with goal-directed activity per se. The potential effect of HIV on the ventral regions of the striatum and corresponding regions in the cortex crucial in motivational processing still remain unexplored.

In order to address questions regarding the motivational processing, the third article included in the current thesis [55], investigated the potential impact on the fronto-ventral-striatal network through a monetary incentive delay task based on that of Knutson et al. [51]. This was the first study to demonstrate the impact of HIV on the ventral striatum, specifically during reward cue-processing. Our results therefore suggest that HIV not only has an impact on the fronto- striatal systems involved in executive functioning, but also has a direct impact on the function of the ventral–striatal reward system. As with our previous experiment, we found no evidence of an impact of HIV on the cortex.

The impact on the function of the ventral–striatal reward system is also present in early untreated illness, with no overt clinically measureable symptoms. Such an effect could have

important consequences specifically in patient motivation, treatment adherence and mood related symptoms. Importantly, this indicates that HIV has an impact on these regions even in the absence of clinically significant mood symptoms and substance abuse. As these patients are clinically stable, however, the relative impact of this striatal hypofunction is uncertain. It remains to be shown if the impact of HIV on the striatum is related to other well known neuro-imaging findings, such as prefrontal cortical atrophy [23].

As such, having explored the consistency as well as extent of the early impact of HIV on the fronto-striatal system in our first three publications [48,54,55], the fourth investigated the relationship between striatal dysfunction and cortical atrophy in HIV. While cortical atrophy is a well-established finding in HIV+ patients, its origins and correlates with striatal function have not been investigated [23,56,57]. In our final publication previous findings of frontal cortical atrophy were confirmed. Furthermore we demonstrated a positive relationship between striatal functional impairment and cortical atrophy. Importantly, this relationship was independent of other factors such as age, CD4 count and normal cognitive function. This implicates impairment of subcortical function as a contributor to brain structural changes seen in HIV infection. It is, therefore, important to consider subcortical function loss in early infection as a factor influencing treatment outcomes. These results also serve to cross-validate findings of prefrontal cortical thinning. They indicate that prefrontal cortical thinning is not only related to factors indirectly associated with HIV infection, but to the impact of the virus itself.

This study encountered a number of limitations. In terms of its generalizability, the study sample was carefully selected to control for recognized confounding variables. This limited

our sample size, which reduced our ability to detect the subtler effects in HIV-inducted striatal function and brain morphology. Despite being representative of the local clinic-going population, our excluding factors included severe comorbid depression, the elderly, and a history of substance use/abuse, which resulted in a relatively homogenous sample, further limiting generalizability. Considering these limitations, it is recommended that subsequent studies should investigate the role of other comorbidities in larger samples, such as clinical depression, drug abuse and normal aging. Large prospective studies are required to measure the effects of cART on these viral related changes as well as the brain itself.

The findings of the present thesis have several significant implications. Firstly, these results confirm that the minor categories of cognitive impairment are indeed valid. Even though the participants included in our study were found to be relatively cognitively normal, our results demonstrate reliable functional differences in important sub-networks of the fronto-striatal system early on. This is likely due to the direct impact of HIV itself, given that our sample was reasonably free of common co-morbidities. Secondly, it supports early treatment interventions, as these changes could potentially be reversible. An important caveat, is that cART could have a secondary influence on brain function and structure itself, as the present study did not include a cART treatment group. Thirdly, HIV appears to affect brain regions involved in reward based decision making. This should be considered in treatment adherence strategies, as subtle cognitive/behavioural changes could also account for failure to adhere to daily treatment regimens. Lastly, prefrontal cortical morphometric changes could serve as important, readily available clinical tools for early diagnosis and management of the effects of HIV on the brain.

References

1   UNAIDS. *UNAIDS report on the global AIDS epidemic*. Geneva: Joint United Nations Programme on HIV/AIDS (UNAIDS); 2012.

2   An SF, Groves M, Gray F, Scaravilli F. Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *J Neuropathol Exp Neurol* 1999; **58**:1156–1162.

3   Grant I. Neurocognitive disturbances in HIV. *Int Rev Psychiatry* 2008; **20**:33–47.

4   Castellon SA, Hinkin CH, Wood S, Yarema KT. Apathy, depression, and cognitive performance in HIV-1 infection. *J Neuropsychiatry Clin Neurosci* 1998; **10**:320–329.

5   Paul R, Flanigan T, Tashima K, Cohen R, Lawrence J, Alt E, *et al.* Apathy correlates with cognitive function but not CD4 status in patients with human immunodeficiency virus. *Journal of Neuropsychiatry and Clinical Neurosciences* 2005; **17**:114.

6   Hardy DJ, Hinhn CH. Reaction time performance in adults with HIV/AIDS. *J Clin Exp Neuropsychol* 2002; **24**:912–929.

7   Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, *et al.* HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy. *Neurology* 2010; **75**:2087–2095.

8   Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, *et al.* HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990-1998. *Neurology* 2001; **56**:257–260.

9   Ances BM, Vaida F, Yeh MJ, Liang CL, Buxton RB, Letendre S, *et al.* HIV Infection and Aging Independently Affect Brain Function as Measured by Functional Magnetic Resonance Imaging. *J Infect Dis* 2010; **201**:336–340.

10   Holt JL, Kraft-Terry SD, Chang L. Neuroimaging studies of the aging HIV-1-infected brain. *J Neurovirol* 2012; **18**:291–302.

11   Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, *et al.* Updated research nosology for HIV associated neurocognitive disorders. *Neurology* 2007; **69**:1789–1799.

12   Bonnet F, Amieva H, Marquant F, Bernard C, Bruyand M, Dauchy F-A, *et al.* Cognitive disorders in HIV-infected patients: are they HIV-related? *AIDS* 2013; **27**:391–400.

13   Marcotte TD, Ghate M, Deutsch R, Letendre S, Meyer R, Godbole S, *et al.* Earlier Initiation of ART Results in Better Neurocognitive Functioning. 19th Conference on Retroviruses and Opportunistic Infections. 2012.http://www.retroconference.org/2012b/Abstracts/44698.htm (accessed 24 May2013).

14   Navia BA, Jordan BD, Price RW. The AIDS dementia complex: I. Clinical features. *Ann Neurol* 1986; **19**:517–524.

15   Mirsattari SM, Power C, Nath A. Parkinsonism with HIV infection. *Mov Disord* 1998; **13**:684–689.

16   Scheller C, Sopper S, Jenuwein M, Neuen-Jacob E, Tatschner T, Grünblatt E, *et al.* Early impairment in dopaminergic neurotransmission in brains of SIV-infected rhesus monkeys due to microglia activation. *J Neurochem* 2005; **95**:377–387.

17   Kumar AM, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction. *J Neurovirol* 2007; **13**:210–224.

18   Mink JW. The basal ganglia: focused selection and inhibition of competing motor programs. *Prog Neurobiol* 1996; **50**:381–425.

19   Parent A, Hazrati L-N. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Research Reviews* 1995; **20**:91–127.

20   Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science* 1997; **275**:1593–1599.

21   Aron AR, Poldrack RA. Cortical and subcortical contributions to Stop signal response inhibition: role of the subthalamic nucleus. *J Neurosci* 2006; **26**:2424–2433.

22   Ernst T, Chang L, Jovicich J, Ames N, Arnold S. Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. *Neurology* 2002; **59**:1343–1349.

23   Elovaara I, Poutiainen E, Raininko R, Valanne L, Virta A, Valle SL, *et al.* Mild brain atrophy in early HIV infection: the lack of association with cognitive deficits and HIV-specific intrathecal immune response. *J Neurol Sci* 1990; **99**:121–136.

24   Heaps JM, Sithinamsuwan P, Paul R, Lerdlum S, Pothisri M, Clifford D, *et al.* Association between brain volumes and HAND in cART-naïve HIV+ individuals from Thailand. *J Neurovirol* 2015; **21**:105–112.

25   Melrose RJ, Tinaz S, Castelo JMB, Courtney MG, Stern CE. Compromised fronto-striatal functioning in HIV: An fMRI investigation of semantic event sequencing. *Behavioural Brain Research* 2008; **188**:337–347.

26   Chang L, Speck O, Miller EN, Braun J, Jovicich J, Koch C, *et al.* Neural correlates of attention and working memory deficits in HIV patients. *Neurology* 2001; **57**:1001–1007.

27   Ernst T, Chang L, Arnold S. Increased glial metabolites predict increased working memory network activation in HIV brain injury. *NeuroImage* 2003; **19**:1686–1693.

28   Chang L, Tomasi D, Yakupov R, Lozar C, Arnold S, Caparelli E, *et al.* Adaptation of the attention network in human immunodeficiency virus brain injury. *Ann Neurol* 2004;

56:259–272.

29   Castelo JMB, Sherman SJ, Courtney MG, Melrose RJ, Stern CE. Altered hippocampal-prefrontal activation in HIV patients during episodic memory encoding. *Neurology* 2006; **66**:1688–1695.

30   Maki PM, Cohen MH, Weber K, Little DM, Fornelli D, Rubin LH, *et al.* Impairments in memory and hippocampal function in HIV-positive vs HIV-negative women: a preliminary study. *Neurology* 2009; **72**:1661–1668.

31   Juengst SB, Aizenstein HJ, Figurski J, Lopez OL, Becker JT. Alterations in the hemodynamic response function in cognitively impaired HIV/AIDS subjects. *Journal of neuroscience methods* 2007; **163**:208–212.

32   Ances BM, Roc AC, Korczykowski M, Wolf RL, Kolson DL. Combination antiretroviral therapy modulates the blood oxygen level–dependent amplitude in human immunodeficiency virus–seropositive patients. *J Neurovirol* 2008; **14**:418–424.

33   Ances BM, Vaida F, Cherner M, Yeh MJ, Liang CL, Gardner C, *et al.* HIV and Chronic Methamphetamine Dependence Affect Cerebral Blood Flow. *J Neuroimmune Pharmacol* 2011; **6**:409–419.

34   Ances B, Vaida F, Ellis R, Buxton R. Test–retest stability of calibrated BOLD-fMRI in HIV– and HIV+ subjects. *NeuroImage* 2011; **54**:2156–2162.

35   Thomas JB, Brier MR, Snyder AZ, Vaida FF, Ances BM. Pathways to neurodegeneration: effects of HIV and aging on resting-state functional connectivity. *Neurology* 2013; **80**:1186–1193.

36   Ortega M, Brier MR, Ances BM. Effects of HIV and combination antiretroviral therapy on cortico-striatal functional connectivity. *AIDS* 2015; **29**:703–712.

37   Qiu W, Yan B, Li J, Tong L, Wang L, Shi D. A Resting-State fMRI Study of patients with HIV infection Based on Regional Homogeneity Method. Shanghai:; 2011. pp. 997–1000.

38   Schweinsburg BC, Scott JC, Schweinsburg AD, Jacobus J, Theilmann RJ, Frank LR, *et al.* Altered prefronto-striato-parietal network response to mental rotation in HIV. *J Neurovirol* 2012; **18**:74–79.

39   Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* 1990; **13**:266–271.

40   Wiley CA, Soontornniyomkij V, Radhakrishnan L, Masliah E, Mellors J, Hermann SA, *et al.* Distribution of brain HIV load in AIDS. *Brain Pathol* 1998; **8**:277–284.

41   Schultz W. Reward Processing in Primate Orbitofrontal Cortex and Basal Ganglia. *Cerebral Cortex* 2000; **10**:272–283.

42   van Hell HH, Vink M, Ossewaarde L, Jager G, Kahn RS, Ramsey NF. Chronic effects of cannabis use on the human reward system: an fMRI study. *Eur Neuropsychopharmacol*

2010; **20**:153–163.

43   Chang L, Yakupov R, Nakama H, Stokes B, Ernst T. Antiretroviral Treatment is Associated with Increased Attentional Load-Dependent Brain Activation in HIV Patients. *J Neuroimmune Pharmacol* 2007; **3**:95–104.

44   Marin RS, Biedrzycki RC, Firinciogullari S. Reliability and validity of the Apathy Evaluation Scale. *Psychiatry Res* 1991; **38**:143–162.

45   Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *Open Med* 2009; **3**:e123–130.

46   Eickhoff SB, Laird AR, Grefkes C, Wang LE, Zilles K, Fox PT. Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: A random-effects approach based on empirical estimates of spatial uncertainty. *Hum Brain Mapp* 2009; **30**:2907–2926.

47   Eickhoff SB, Bzdok D, Laird AR, Kurth F, Fox PT. Activation likelihood estimation meta-analysis revisited. *NeuroImage* 2012; **59**:2349–2361.

48   Plessis du S, Vink M, Joska JA, Koutsilieri E, Stein DJ, Emsley R. HIV infection and the fronto-striatal system: a systematic review and meta-analysis of fMRI studies. *AIDS* 2013; **28**:803–811.

49   Logan GD, Cowan WB, Davis KA. On the ability to inhibit simple and choice reaction time responses: A model and a method. *Journal of Experimental Psychology: Human Perception and Performance* 1984; **10**:276–291.

50   Zandbelt BB, Vink M. On the role of the striatum in response inhibition. *PLoS ONE* 2010; **5**:e13848.

51   Knutson B, Adams CM, Fong GW, Hommer D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci* 2001; **21**:RC159.

52   Vink M, Kleerekooper I, van den Wildenberg WPM, Kahn RS. Impact of aging on frontostriatal reward processing. *Hum Brain Mapp* 2015; **36**:2305–2317.

53   Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage* 1999; **9**:179–194.

54   Plessis du S, Vink M, Joska JA, Koutsilieri E, Bagadia A, Stein DJ, *et al.* HIV Infection Is Associated with Impaired Striatal Function during Inhibition with Normal Cortical Functioning on Functional MRI. *Journal of the International Neuropsychological Society* 2015; **21**:722–731.

55   Plessis du S, Vink M, Joska JA, Koutsilieri E, Bagadia A, Stein DJ, *et al.* HIV infection results in ventral-striatal reward system hypo-activation during cue processing. *AIDS* 2015; **29**:1335–1343.

56   Chang L, Andres M, Sadino J, Jiang CS, Nakama H, Miller E, *et al.* Impact of apolipoprotein E ε4 and HIV on cognition and brain atrophy: Antagonistic pleiotropy and premature brain aging. *NeuroImage* 2011; **58**:1017–1027.

57   Pfefferbaum A, Rosenbloom MJ, Sassoon SA, Kemper CA, Deresinski S, Rohlfing T, *et al.* Regional brain structural dysmorphology in human immunodeficiency virus infection: effects of acquired immune deficiency syndrome, alcoholism, and age. *Biol Psychiatry* 2012; **72**:361–370.

Appendix

Publications associated with the present thesis by the first author

Articles included in the body of the thesis

Article 1:
Plessis, du, S., Vink, M., Joska, J. A., Koutsilieri, E., Stein, D. J., & Emsley, R. (2013). HIV infection and the fronto-striatal system: a systematic review and meta-analysis of fMRI studies. AIDS (London, England), 28(6), 803–811. http://doi.org/10.1097/QAD.0000000000000151

Article 2:
Plessis, du, S., Vink, M., Joska, J. A., Koutsilieri, E., Bagadia, A., Stein, D. J., & Emsley, R. (2015). HIV Infection Is Associated with Impaired Striatal Function during Inhibition with Normal Cortical Functioning on Functional MRI. Journal of the International Neuropsychological Society, 21(9), 722–731. http://doi.org/10.1017/S1355617715000971

Article 3:
Plessis, du, S., Vink, M., Joska, J. A., Koutsilieri, E., Bagadia, A., Stein, D. J., & Emsley, R. (2015). HIV infection results in ventral-striatal reward system hypo-activation during cue processing. AIDS (London, England), 29(11), 1335–1343. http://doi.org/10.1097/QAD.0000000000000680

Article 4:
Plessis, S., Vink, M., Joska, J. A., Koutsilieri, E., Bagadia, A., Stein, D. J., & Emsley, R. (2016). Prefrontal cortical thinning in HIV infection is associated with impaired striatal functioning. Journal of Neural Transmission, 1–9. http://doi.org/10.1007/s00702-016-1571-0

Associated articles co-authored

Raemaekers, M., **Plessis, du, S.**, Ramsey, N. F., Weusten, J. M. H., & Vink, M. (2012). Test-retest variability underlying fMRI measurements. NeuroImage, 60(1), 717–727. http://doi.org/10.1016/j.neuroimage.2011.11.061

Horn, A., Scheller, C., **Plessis, S.**, Arendt, G., Nolting, T., Joska, J., et al. (2013). Increases in CSF dopamine in HIV patients are due to the dopamine transporter 10/10-repeat allele which is more frequent in HIV-infected individuals. Journal of Neural Transmission (Vienna, Austria : 1996), 120(10), 1411–1419. http://doi.org/10.1007/s00702-013-1086-x

Koutsilieri, E., Riederer, P., **Plessis, du, S.**, & Scheller, C. (2014). A short review on the relation between the dopamine transporter 10/10-repeat allele and ADHD: implications for HIV infection. Attention Deficit and Hyperactivity Disorders, 6(3), 203–209. http://doi.org/10.1007/s12402-014-0134-1

Vink, M., Kaldewaij, R., Zandbelt, B. B., Pas, P., & **Plessis, du, S.** (2015). The role of stop-signal probability and expectation in proactive inhibition. The European Journal of Neuroscience, 41(8), 1086–1094. http://doi.org/10.1111/ejn.12879

Fouche, J.-P., **Plessis, du, S.**, Hattingh, C., Roos, A., Lochner, C., Soriano-Mas, C., et al. (2016). Cortical thickness in obsessive-compulsive disorder: multisite mega-analysis of 780 brain scans from six centres. The British Journal of Psychiatry : the Journal of Mental Science, 1–19. http://doi.org/10.1192/bjp.bp.115.164020

General co-authorships:

Lochner, C., Fouche, J.-P., **Plessis, du, S.**, Spottiswoode, B., Seedat, S., Fineberg, N., et al. (2012). Evidence for fractional anisotropy and mean diffusivity white matter abnormalities in the internal capsule and cingulum in patients with obsessive-compulsive disorder. Journal of Psychiatry & Neuroscience : JPN, 37(3), 193–199. http://doi.org/10.1503/jpn.110059

Spottiswoode, B. S., **Plessis, du, S.**, Gretschel, A., & Lotz, J. W. (2012). Functional MRI in pre-surgical planning: Case study and cautionary notes. South African Journal of Radiology, 16(3). http://doi.org/10.7196/sajr.709

Spottiswoode, B. S., van den Heever, D. J., Chang, Y., Engelhardt, S., **Plessis, du, S.**, Nicolls, F., et al. (2013). Preoperative Three-Dimensional Model Creation of Magnetic Resonance Brain Images as a Tool to Assist Neurosurgical Planning. Stereotactic and Functional Neurosurgery, 91(3), 162–169. http://doi.org/10.1159/000345264

Emsley, R., Chiliza, B., Asmal, L., **Plessis, du, S**., Phahladira, L., van Niekerk, E., et al. (2014). Re: Less is more. Schizophrenia Research, 160(1-3), 224–225. http://doi.org/10.1016/j.schres.2014.10.014

Emsley, R., Chiliza, B., Asmal, L., **Plessis, du, S.**, Phahladira, L., van Niekerk, E., et al. (2014). A randomized, controlled trial of omega-3 fatty acids plus an antioxidant for relapse prevention after antipsychotic discontinuation in first-episode schizophrenia. Schizophrenia Research, 158(1-3), 230–235. http://doi.org/10.1016/j.schres.2014.06.004

Emsley, R., Asmal, L., Chiliza, B., **Plessis, du, S.**, Carr, J., Kidd, M., et al. (2015). Changes in brain regions associated with food-intake regulation, body mass and metabolic profiles during acute antipsychotic treatment in first-episode schizophrenia. Psychiatry Research, 233(2), 186–193. http://doi.org/10.1016/j.pscychresns.2015.06.014