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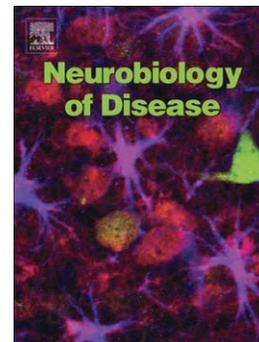
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INCREASED SUBCORTICAL NEURAL ACTIVITY AMONG HIV+ INDIVIDUALS DURING A

LEXICAL RETRIEVAL TASK

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Abstract

Background: Deficits in lexical retrieval, present in approximately 40% of HIV+ patients, are thought to reflect disruptions to frontal-striatal functions and may worsen with immunosuppression. Coupling frontal-striatal tasks such as lexical retrieval with functional neuroimaging may help delineate the pathophysiologic mechanisms underlying HIV-associated neurological dysfunction.

Objective: We examined whether HIV infection confers brain functional changes during lexical access and retrieval. It was expected that HIV+ individuals would demonstrate greater brain activity in frontal-subcortical regions despite only minimal differences between groups on neuropsychological testing. Within the HIV+ sample, we examined associations between indices of immunosuppression (recent and nadir CD4+ count) and task-related signal change in frontostriatal structures.

Method: 16 HIV+ participants and 12 HIV- controls underwent fMRI while engaged in phonemic/letter and semantic fluency tasks. Participants also completed standardized measures of verbal fluency.

Results: HIV status groups performed similarly on phonemic and semantic fluency tasks prior to being scanned. fMRI results demonstrated activation differences during the phonemic fluency task as a function of HIV status, with HIV+ individuals demonstrating significantly greater activation in BG structures than HIV- individuals. There were no significant differences in frontal brain activation between HIV status groups during the phonemic fluency task, nor were there significant brain activation differences during the semantic fluency task. Within the HIV+ group, current CD4+ count, though not nadir, was positively correlated with increased activity in the inferior frontal gyrus and basal ganglia.

Conclusion: During phonemic fluency performance, HIV+ patients recruit subcortical structures to a greater degree than HIV- controls despite similar task performances suggesting that fMRI may be sensitive to neurocompromise before overt cognitive declines can be detected. Among

HIV+ individuals, reduced activity in the frontal-subcortical structures was associated with lower

CD4+ count.

Highlights

- We examined whether HIV infection confers brain functional changes during a task of lexical retrieval/verbal fluency.

- HIV+ individuals demonstrated greater recruitment of basal ganglia structures than HIV-individuals during in-scanner letter fluency task.

- There were no HIV status group differences on out-of-scanner verbal fluency performance.

- Within the HIV+ group, current CD4+ count, though not nadir, was positively correlated with increased activity in the inferior frontal gyrus and basal ganglia.

INTRODUCTION

While the number of HIV-infected patients who present with HIV-associated dementia has declined dramatically since the introduction of highly active antiretroviral therapy (HAART), approximately 50% of patients will still continue to present with primarily mild-to-moderate degrees of neurocognitive impairment (Harezlak et al., 2011; Heaton et al., 2010; Heaton et al., 2011; Robertson et al., 2007). Characteristically, the types of cognitive problems observed in HIV+ adults reflect that of frontal-subcortical dysfunction (Grant & Heaton, 1990; Hestad et al., 1993; Paul, Cohen, & Stern, 2003; Reger, Welsh, Razani, Martin, & Boone, 2002) involving frontal-subcortical networks (Ances et al., 2006; Ernst, Chang, Jovicich, Ames, & Arnold, 2002; Lentz et al., 2009; Melrose, Tinaz, Castelo, Courtney, & Stern, 2008; Paul, Cohen, Navia, & Tashima, 2002). Additionally, smaller caudate volume has been correlated with poorer performance on cognitive tests in patients with advanced HIV disease (Ances et al., 2006; Kieburtz et al., 1996; Paul et al., 2002). Among individuals with a diagnosis of AIDS, thinning of the prefrontal cortex in particular has been associated with the severity of cognitive impairment (Thompson et al., 2005).

Cognitive tasks such as verbal fluency that require intact frontal-striatal circuitry (e.g., Henry & Crawford, 2004; Millikin, Trépanier, & Rourke, 2004; Wecker, Kramer, Hallam, & Delis, 2005) have been shown to be sensitive to HIV-associated neurocognitive impairment. Verbal fluency is an executive-mediated task that involves rapid word generation beginning with a prescribed letter (phonemic fluency) or given category (semantic fluency). With regard to neural networks, phonemic fluency is neuroanatomically associated with the left inferior frontal gyrus (IFG), whereas semantic/category fluency has been linked to the superior temporal gyrus (Prince, Tsukiura, & Cabeza, 2007; Sherman & Massman, 1999). The basal ganglia (BG), preferentially targeted by HIV infection, have also been implicated in verbal fluency tasks, with reduced caudate volume significantly predicting lower phonemic fluency performance in an

HIV+ sample (Thames et al., 2012). fMRI studies of HIV- samples have reported BG involvement (Fu, Morgan, Suckling, Williams, Andrew, Vythelingum, & McGuire, 2002; Wagner, Sebastian, Lieb, Tüscher, Tadić, 2014) when individuals perform executive-mediated language tasks (e.g., word generation), suggesting that the BG play a key role in lexical retrieval outside of HIV infection.

Heaton et al. (2011) reported that the rates of frank impairments in verbal fluency have declined in the combination antiretroviral therapy (CART) era. Similarly, a recent study involving a large cohort of HIV+ and HIV- women found no significant differences in verbal fluency performance across HIV status groups after adjusting for relevant socio-demographic and behavioral characteristics (Maki et al., 2015). In contrast, results from meta-analytic studies have revealed that mild deficits in verbal fluency performance may still persist among individuals with HIV. A meta-analysis involving studies of HIV+ individuals conducted in the pre-HAART era (Cysique, Maruff, & Brew, 2006) found similar verbal fluency deficit effect sizes to a meta-analysis involving both pre- and post-HAART studies, suggesting that mild fluency deficits may still be present in the context of HIV infection (Iudicello et al., 2007) regardless of HAART use.

Greater phonemic compared to semantic fluency impairments have generally been observed in HIV infection, suggesting that subcortical disorders are more likely to target executive-mediated search and retrieval mechanisms. For example, a study by Woods, Carey, Tröster, & Grant (2005) found that 26% of individuals with HIV demonstrated deficits in letter fluency, whereas only 13% demonstrated impairment in semantic fluency. However, a meta-analytic study conducted by the same group found greater semantic versus phonemic fluency deficits among individuals with HIV (Iudicello et al., 2007), however, this difference dissipated when they examined only studies that included both types of verbal fluency tasks. Currently, the mechanisms by which HIV infection disrupts access to phonemic and semantic lexicons require further study.

fMRI is a technique often used in combination with neurobehavioral tasks that involves measuring real-time activation of brain systems during task performance, thus allowing for direct insight into the neural systems that are disrupted during cognition. It has been demonstrated that HIV+ individuals with normal cognitive function show greater magnitude of brain activation than controls, suggestive of nascent damage to neural substrates that could necessitate increased use of neurological reserve to sustain normal cognitive function (Ernst, Chang, Jovicich, Ames, & Arnold, 2002). Similar findings have been reported among HIV+ individuals identified as cognitively impaired on neurobehavioral assessment, as they showed increases in cerebral blood volume of grey matter structures when compared to HIV+ patients without neuropsychological impairment (Tracey et al., 1998).   Contrary findings were reported by Melrose and colleagues (2008) which found that HIV+ individuals demonstrated reduced activity of the left caudate, left dorsolateral prefrontal cortex (PFC) and bilateral prefrontal cortex relative to controls using task-based fMRI. Further, in this same study higher CD4 count among HIV+ individuals was associated with reduced activity of the left DPFC (Melrose et al., 2008). Therefore, whether HIV-infection confers hypoactivation or hyperactivation of these neural structures requires further investigation.

HIV-associated neurocognitive impairments may worsen with severe immunosuppression (as traditionally measured by CD4 count) (Childs et al., 1999; Cohen et al., 2010; Ellis et al., 2011; Jernigan et al., 2011; McArthur, Brew, & Nath, 2005; Valcour et al., 2006), and contribute to the variability in cognitive problems and neuroimaging findings reported. HIV+ individuals with or without overt cognitive dysfunction who have poorer immunological status may exert greater effort and recruit additional neurological resources when required to complete complex tasks (e.g., Hinkin, van Gorp, Mandelkern, Gee, Satz, Holston et al., 1995). Among HIV+ participants with currently undetectable plasma viral loads, lower nadir CD4 counts are associated with significantly increased rates of neuropsychological impairment, particularly in attention/working memory and executive functioning (Muñoz-Moreno

et al., 2008). Lower nadir CD4 counts are also associated with a higher prevalence of HIV-associated neurocognitive disorder (Ellis et al., 2011) including HIV-associated dementia (Valcour et al., 2006) as well as sharper declines in cognition over time (Cysique et al., 2006). Lower nadir CD4 has also been linked to structural neuroimaging abnormalities such as reduced frontal and temporal lobe volumes, smaller hippocampi and thalami, as well as lower overall white matter volumes and higher ventricular volumes (Cohen et al., 2010).

Together, these studies suggest that HIV status and immunocompromise can serve as risk factors for mild-to-moderate impairments in brain functioning, which may go undetected by traditional measures of neurobehavioral performance. Although HIV infection is thought to result in frontal-subcortical impairments, it is less clear how the function of these structures is affected by severe historical and recent immunosuppression.

Therefore, the primary aim of this study is to define and functionally assess brain structures and neural circuits that are compromised by HIV infection and immunocompromise during lexical retrieval. We expected to observe functional activation differences between HIV+ and HIV- participants under the verbal fluency paradigm. Specifically, we hypothesized that HIV+ adults would exhibit greater brain activation in frontal-subcortical regions than HIV- adults, reflecting frontal-subcortical compromise (even if statistically significant performance differences were not found on behavioral testing). We also hypothesized that lower nadir and current CD4 would be associated with increased activity in prefrontal cortices.

METHODS

Participants

The study sample consisted of 28 (16 HIV+; 12 HIV-) right-handed adults. All participants provided informed consent to study procedures, and institutional approval was granted from the University of California, Los Angeles and the West Los Angeles VA prior to data collection.

Inclusion criteria. Participants who were over the age of 18, indicated that they were able to speak and understand English, and were able to provide informed consent were eligible. HIV-1 serostatus and recent CD4+ count were confirmed by medical records. Cognitive testing and neuroimaging components of this study were conducted on two separate occasions (within a 1-month time span).

Exclusion criteria. Participants presenting with a history of psychosis or alcohol/illicit substance abuse or dependence within the last month were excluded in the parent study based upon results of data collected using the Structured Clinical Interview for DSM-IV, Axis I Disorders (First, Spitzer, Gibbon, & Williams, 1996). All participants underwent urine toxicology screens for detecting marijuana, cocaine, amphetamine, and opiate metabolites. None of the participants met current criteria for mood and/or anxiety disorders. Participants were also excluded if they: (1) presented with history of CNS confounds known to affect cognitive function (e.g., central nervous system opportunistic infections or neoplasm, seizures, or hepatitis C virus). For the current study, we excluded participants if they were unable to tolerate MRI procedures or presented with MRI contraindications. All HIV+ were on antiretroviral therapy. Duration of ARV use is provided in Table 1.

Procedure

fMRI procedure for the verbal fluency task. The stimulation paradigm consisted of a verbal fluency task containing both a letter-word and semantic-word condition that was modified from a prior study (Cuenod, Bookheimer, Hertz-Pannier, Zeffiro, Theodore, Le Bihan, 1995). On the day of scanning, participants were given verbal instructions for the fluency task, and the same instructions were repeated two times (before the participant entered the scanner, and just before the task began) to ensure complete understanding. All participants indicated that they understood the task requirements. We used an intermittent design involving presentation of a baseline (*rest*) condition for 20 seconds, followed by an activation (*fluency task*) condition for 20 seconds and a control condition (*count*) for 20 seconds. This cycle was repeated four times over

the course of 4 minutes and 30 seconds for both letter-word and semantic-word conditions. For the letter condition, participants were cued by auditory presentation of a letter (i.e., "F", "A", "S","C") to generate as many different words as possible beginning with that letter. Unlike the standard FAS task, participants were instructed to "think" rather than vocalize the generated words for each trial. During the baseline condition, participants were instructed to "rest." In the control condition, participants were instructed to "count beginning at 1." The baseline, activation, and control conditions of the semantic-word task were presented identically to that of the letter-word condition. During the activation condition, participants were asked to think of as many words as possible belonging to a specified semantic category (i.e., "think of boys names," "think of animals," "think of fruit," "think of furniture"). Participants from both groups reported that they were able to perform both tasks in the scanner without difficulty.

Verbal fluency performance (out-of-scanner). In order to gather actual performance data verbal fluency was assessed outside of the scanner using the Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1976). This task requires subjects to produce words beginning with a particular letter of the alphabet (i.e., 'F,' 'A,' and 'S') in three respective 60-second trials. In semantic/category fluency, participants were asked to generate words belonging to a specific category (i.e., animals) in a 60-second trial.

fMRI data acquisition. Functional imaging was performed with a 1.5 T Magnetom Sonata scanner (Siemens AG, Erlangen, Germany) using a gradient-echo, echo-planar acquisition sequence in which the repetition time was 2.5 sec, echo time was 35 msec, flip angle was 80°, image matrix was 128 x 64, field of view was 40 by 20 cm, and in-plane resolution was 3 mm. Sixteen slices, each 4 mm thick, with a 1-mm gap between slices were obtained every 2.5 sec for 35 sec while participants completed a letter or semantic verbal fluency task (conducted during separate scans). High-resolution spin-echo echo-planar scans (128 by 256 matrix; in-plane resolution, 1.5 mm; repetition time, 4000 msec; echo time, 54 msec; four excitations), obtained in the same plane as the functional scans, and were acquired

with bandwidth matched to that of the functional studies. The spatial distortions of the functional and high-resolution spin-echo echo-planar imaging scans were held in common to facilitate the subsequent spatial normalization procedure. Scans were positioned perpendicular to the AC-PC line, with the most anterior scan beginning three slices anterior to the leading edge of the cingulated sulcus.

Immunosuppression (recent and historical). Participants current CD4+, nadir CD4 and plasma viral load counts were obtained via self-report and we were able to verify current CD4 count and plasma viral load by the participant's recent medical records.

Data Analysis

Independent samples t-test and chi-square analyses were used to analyze HIV-status group differences on socio-demographic, behavioral, and clinical characteristics as well as standardized verbal fluency tests. Analyses of verbal fluency performance differences between the HIV status groups were performed with and without age as a covariate. Power analysis suggested that a sample size of 14 participants per comparison group was sufficient for the in-scanner verbal fluency task (e.g., 80% power at the .05 level of significance), considering the large size reported by Melrose et al. (2008) between HIV+ and HIV- individuals. We were underpowered for detecting medium effect sizes (as reported in Iudicello et al., 2007) for analyses of out-of-scanner verbal fluency performance.

Verbal fluency task. Total number of words generated for the phonemic and semantic fluency trials were converted to demographically-corrected T-scores (with a mean of 50 and a standard deviation of 10) using published normative data (Heaton, Grant, & Matthews, 2004). Additional outcome variables included mean cluster size for phonemic and semantic fluency trials and number of switches for phonemic and semantic fluency based upon methods outlined in Troyer, Moscovitch, and Winocur, 1997.

fMRI processing. From the original sample of thirty-two participants who underwent scanning procedures, four participants were excluded from analysis due either to extreme head motion (n = 2) or corrupted images (n = 2). fMRI data analysis was performed using FSL version 4.1 (FMRIB's Software Library: http://www.fmrib.ox.ac.uk/fsl/) using the following steps: motion correction using the Linear Registration Tool (MCFLIRT); exclusion of non-brain areas using the Brain Extraction Tool (BET); spatial smoothing with a Gaussian kernel of 5 mm full-width half maximum; mean-based intensity normalization to remove linear trends; and non-linear, high-pass, temporal filtering to exclude low frequency confounds, such as breathing (Gaussian-weighted least squares straight line fit, with sigma = 25.0 s). Time series statistical analysis was carried out using Improved Linear Model (FILM) with local autocorrelation correction.

Voxel-wise general linear model (GLM) analyses of the two verbal fluency conditions (i.e., letter and semantic) were modeled as explanatory variables in the first level analysis. Z (Gaussianized T/F) statistic images were thresholded using clusters determined by $Z > 2.3$, with an adjusted corrected cluster significance threshold of p = .05, corrected for multiple comparisons. Each scan was registered to a high-resolution T1 structural image using the Linear Registration Tool (FLIRT) and co-registered to MNI 152 (Montreal Neurological Institute) standard space. Contrasts at the first level compared parameter estimates of the hemodynamic response to the letter and word conditions of each verbal fluency task (letter and semantic) to rest.

The second-level, between-group analysis used FMRIB's Local Analysis of Mixed Effects (FLAME). Contrasts at this level compared group differences (e.g., HIV status) in activation between the letter/word task and rest controlling for age. For analyses, logarithmic 10 (Log 10) transformations were applied to participants' nadir CD4 count. Each participant's log 10 nadir CD4 and current CD4 was de-meaned (relative to group) and used as a covariate of interest in the HIV group comparison (Of note, we did not enter plasma viral load as a covariate,

as 87% of our sample had undetectable viral load). This multilevel approach, using Bayesian inference, allows for the assessment of the full uncertainty of signal change by inputting summary statistics from voxel-wise analysis at lower levels to the subsequent level where new summary statistics are generated. This method also incorporates the unknown random and fixed variance components within each level in the model.

To determine the functional significance of BOLD activity for the in-scanner verbal fluency task, we extracted parameter estimates of peak voxels within regions that demonstrated significant activation across all participants and correlated these with out-of-scanner task performance. Following this, we conducted a post-hoc analysis using frontal-subcortical regions of interest within the HIV group. ROI masks of the inferior frontal gyrus and basal ganglia were created using the FSL atlas. Percent signal change for a region was calculated using featquery and this was correlated with current and nadir CD4. The effect sizes associated with the average percent signal change in the inferior frontal gyrus and BG were 0.30 and 0.20, respectively.

Results

Demographics

Please see Table 1 for sample demographics of comparison groups.

Table 1

Descriptive Statistics of Participants and Key Variables

Variable	HIV+ (N = 16)	HIV- (N = 12)	p-value
Age (years), mean (*SD*)	50.64 (11.6)	48.80 (21.7)	.22
Males, *n* (%)	11 (68.8)	8 (66.7)	.88
Education (years), mean (*SD*)	13.94 (1.9)	13.40 (1.7)	.69
*WTAR (standard score)	101.59 (16.71)	99.50 (18.07)	.75
Ethnicity, *n* (%)			.35
African American	9 (56.2)	5 (41.7)	.46
Non-Hispanic White	4 (25.0)	1 (8.3)	.25
Hispanic	3 (18.8)	3 (25.0)	.69
Asian/Pacific Islander	0 (0.0)	2 (16.7)	.08
Other	0 (0.0)	1 (8.3)	.24
CD4 count, median (interquartile range)	300.0 (167.5)	--	--
Nadir CD4 count, median (interquartile range)	123.0 (397.5)	--	--
**Undetectable viral load, *n* (%)	14 (87.5)	--	--
Length of time since HIV diagnosis	12.0 (3.5)	--	
Length of antiretroviral therapy			--
% 0–11 months	0	--	
% 1–3 years	5	--	--

% over 3 years	95	--	--
Past substance abuse/dependence %(n)			
Alcohol	18.7% (3)	16.6% (2)	.94
Marijuana	25% (4)	16% (2)	.56
Cocaine	0	0	--
Amphetamine	0	0	--
Opiates	0	0	--
BDI score	7.6 (2.3)	5.4 (1.2)	.35
Current drug use (past month) %(n)			
Alcohol	25% (4)	25% (3)	1.0
# of drinks per week	2.6 (1.3)	1.5 (1.9)	.94
Tobacco	12.5% (2)	25% (3)	.39
# of times per week	15 (1.0)	21 (1.2)	.74
Marijuana	12.5% (2)	0	.20
# of times per week	1	0	--
Cocaine	0	0	--
Amphetamine	0	0	--
Opiates	0	0	--
COWAT FAS total, mean (*SD*)	44.83 (10.6)	47.51 (8.9)	.85
COWAT Animals *T*-score, mean (*SD*)	45.06 (10.2)	46.50 (11.1)	.31

Note. COWAT = Controlled Oral Word Association Test (Benton & Hamsher, 1976). *WTAR = Wechsler Test of Adult Reading. **Undetectable viral load defined as <20 copies/mL

There were no significant differences between HIV+ and HIV- status groups on demographic variables or past drug use variables (all *p*'s > .05 see table 1). Among HIV+ participants, there was a statistically significant correlation between recent CD4 count and nadir CD4 count, r_{16} = .48, *p* = .05.

Out-of-Scanner Verbal Fluency Performance

There were no significant performance differences between HIV-status groups in out-of-scanner phonemic, *t* (26) = -0.18, *p* = .85, or semantic, *t*(26) = 1.01, *p* = .31, fluency performance. The effect sizes related to group differences were small for both phonemic (d = .27) and semantic (d = .13) fluency tasks. In order to detect statistically significant group differences, we would need a sample of approximately 360-1240 individuals. Results did not change when age was included as a covariate.

fMRI findings (HIV+ and HIV- groups)

In contrasting word-rest conditions in the lexical retrieval task, there was increased activation in the supplementary motor cortex (SMA), inferior frontal gyrus (IFG), cingulate gyrus, lateral occipital gyrus, and basal ganglia (BG) (see Table 2 and Figure 1) according to MNI coordinates using FSL atlas. We found significant correlations between out-of-scanner FAS performance measures and parameter estimate values in supplementary motor cortex (SMA), inferior frontal gyrus, caudate and putamen. Specifically, FAS total words generated was significantly correlated with activity in the caudate, (r_{28} = .48, p = .009) putamen (r_{28} = .51, p = .008), and inferior frontal gyrus (r_{28} =.46, p = .01). Number of phonemic switches was significantly correlated with SMA (r_{28} = .56, p = .006), caudate (r_{28} = .43, p = .01), putamen (r_{28} = .48, p = .009) and inferior frontal gyrus (r_{28} = .55, p = .006). Number of semantic switches was significantly correlated with activity of the SMA (r_{28} = .61, p = .003) and inferior frontal gyrus (r_{28}

= .53, p = .007). We did not find associations between total words generated from the semantic

fluency trial or mean cluster size for phonemic and semantic fluency trials (p's > .05).

Figure 1. Patterns of activation during letter retrieval (words – rest).

Significant activations during phonemic fluency according to FSL random-effects analysis for the whole subject sample adjusted by age. Results are multiple-comparisons corrected with cluster-level significance level of *p* < .05.
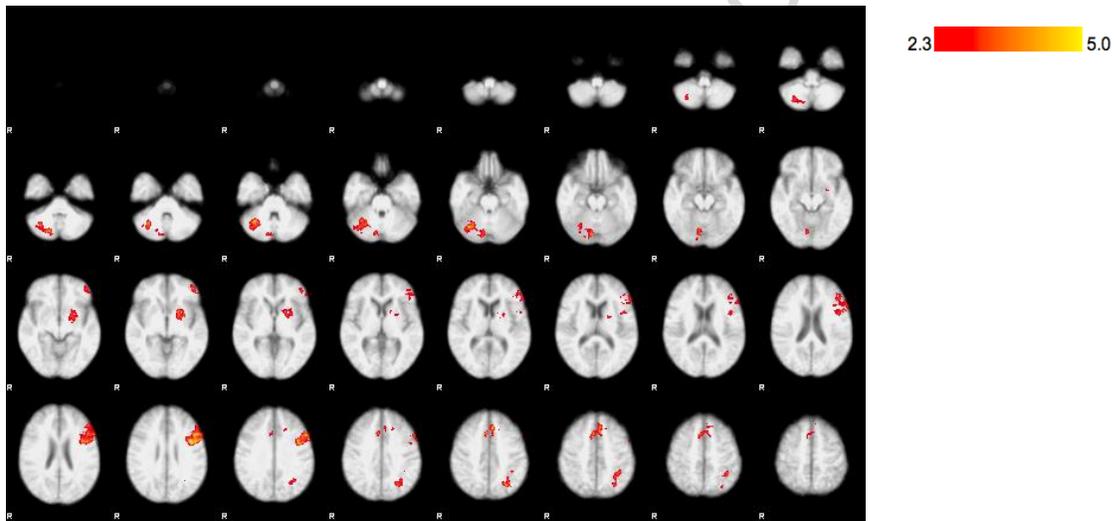

Table 2*. Regions Activated During Phonemic Fluency Task (Task-Rest) for the Whole Sample*

	MNI Coordinates						
	Brodmann area **(a)**	**x** **(b)**	**y** **(c)**	**z** **(d)**	**k** **(e)**	**z max** **(f)**	**sig HIV group difference**
Supplementary Motor Cortex	6	-2	8	60	1085	5.00	NS
Precentral gyrus/Inferior frontal gyrus, pars opercularis	4/44	-40	8	28	1588	4.54	NS
Insula	13/14	40	18	-4	646	4.13	NS
Putamen	--	18	8	-6	369	3.85	p< .05
Caudate	--	-12	16	8	369	3.85	p<.05
Paracingulate gyrus	32	-6	28	40	426	3.85	NS

Lateral Occipital Cortex, superior	19	-26	-66	38	350	3.40	NS

*Significant HIV group differences shown in column "g".

Figure 2. Activation differences as a function of HIV status (HIV+ > HIV-), phonemic condition only.
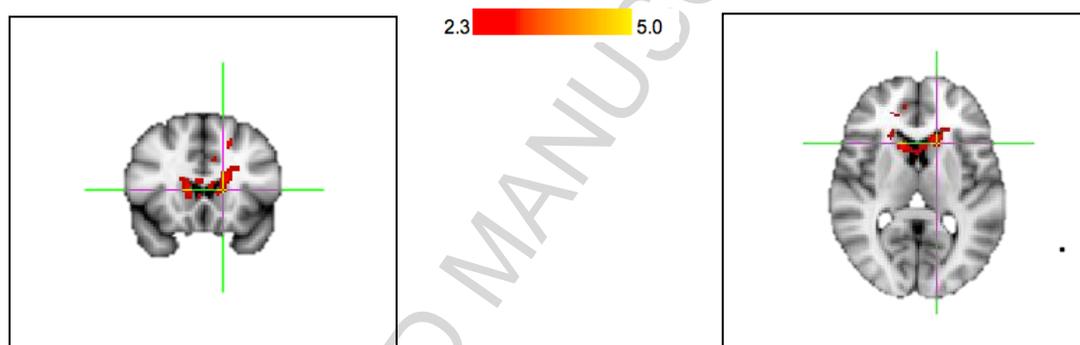
Note. MNI_152 coordinates: x = 18, y = 8, z = -6 (left putamen); x = -12, y = 16, z = 8 (left caudate).

fMRI activation differences during the phonemic (but not semantic) fluency task were found as a function of HIV status, with HIV+ individuals demonstrating greater bilateral activation in BG structures (i.e., caudate, putamen) compared to HIV- individuals (*z*-threshold 2.3; *p* < .05) (See Figure 2).

Immunosuppression (recent & historical)

Figure 3 provides the fMRI images for the HIV+ participants based on recent and lifetime immunological functioning. Recent CD4+ count was positively associated with greater percent signal change in the left IFG (r_{16} = .52, *p* = .03) and left BG (r_{16} = .72, *p* < .01) during the phonemic fluency task, but not with average percent signal change across all brain regions (r_{16} = .23, *p* = .60). There were no significant correlations with the semantic fluency task. There were also no statistically significant associations between nadir CD4 count and percent signal change in IFG and BG. There was no significant association between recent or nadir CD4 count and out-of-scanner performance on FAS (p > .10).

Figure 3. Region of Interest analysis (left inferior gyrus and left basal ganglia).
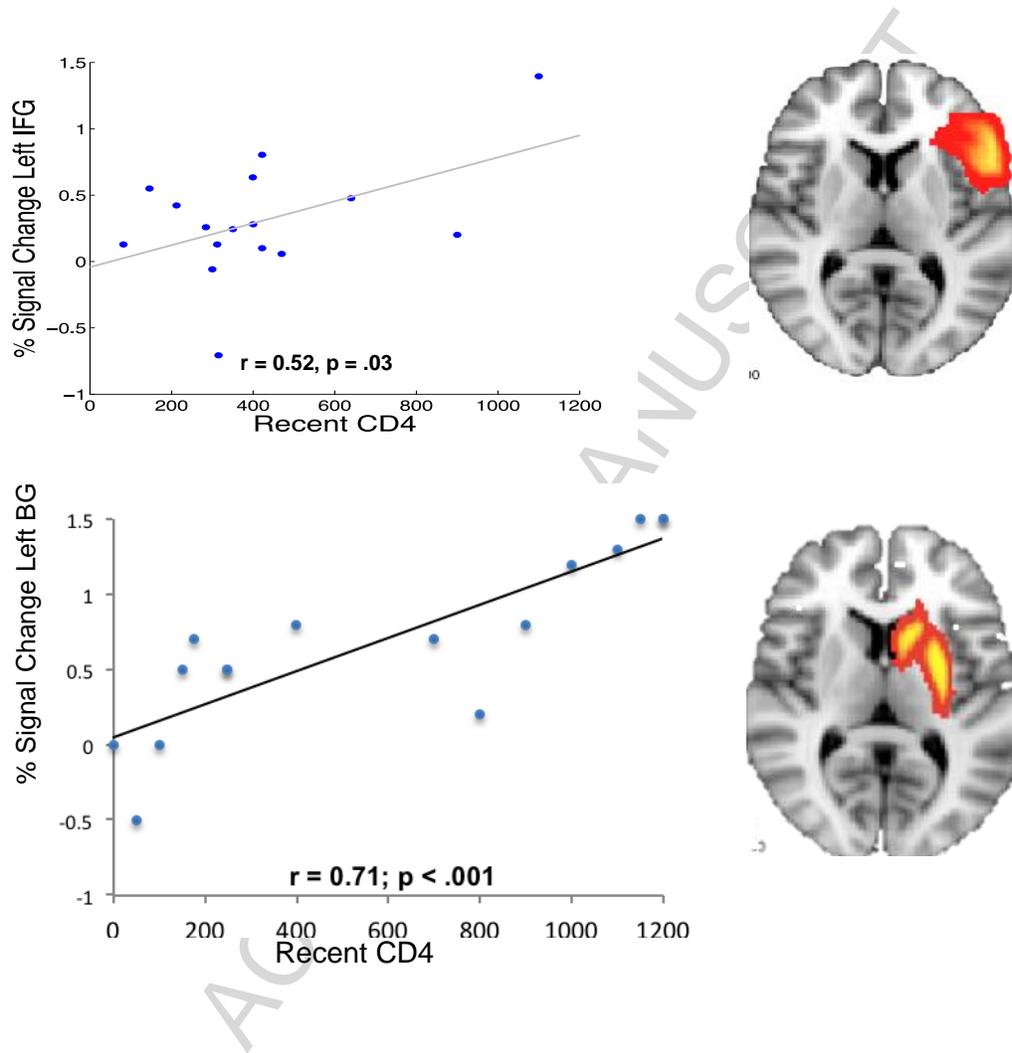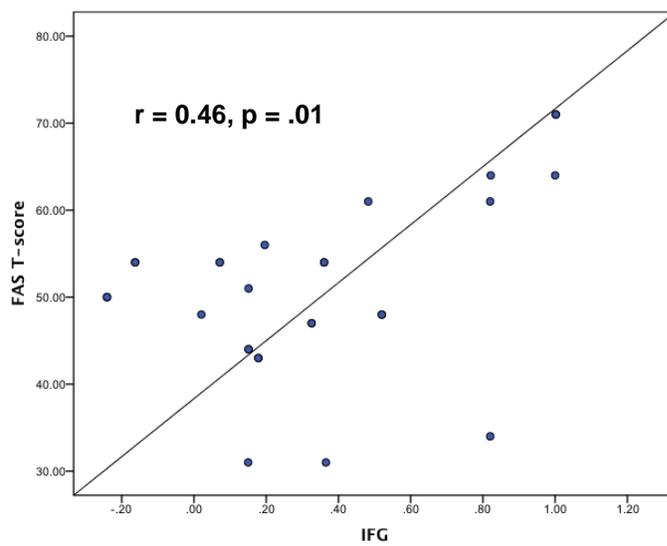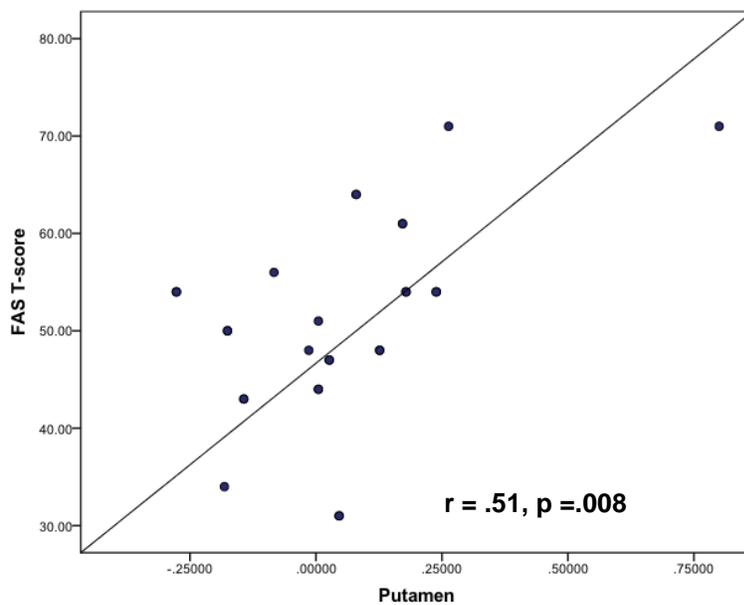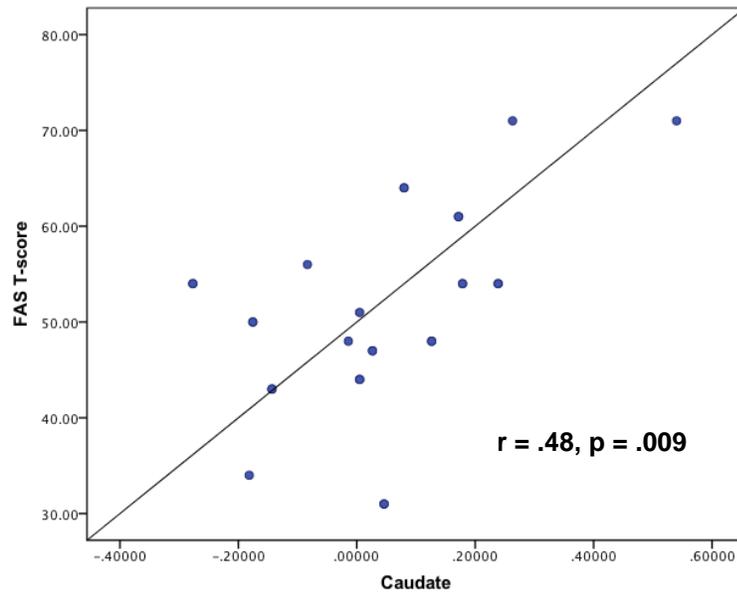

Figure 4. Correlations between FAS total words generated and IFG, caudate and putamen

activation

Discussion

In this study, we used BOLD fMRI to examine neural activation during verbal fluency performance among HIV+ and HIV- adults. Our results showed that there were no significant differences between HIV+ and HIV- groups on out-of-scanner verbal fluency tests (and both groups performed within the normal range), which have been associated in the previous literature with frontal-striatal functions (e.g., Henry & Crawford, 2004; Millikin, Trépanier, &

Rourke, 2004; Wecker, Kramer, Hallam, & Delis, 2005). In contrast, fMRI task activation differences were found as a function of HIV status in BG structures (i.e., caudate, putamen) compared to HIV- individuals ($p < .05$), with increased activity in the HIV+ group. Therefore, our findings support both the specificity of HIV-associated cognitive disorders (i.e., associated with changes in lexical but not semantic verbal fluency) and suggest that changes in BG function may contribute to verbal fluency performance, consistent with the previous neuroimaging literature.

Our findings of increased activation among the HIV group were somewhat inconsistent with those found by Melrose et al., 2008, who reported greater percent signal change in the left caudate among the control group. However, we believe that this difference may be due to fMRI task differences between the two studies. Briefly, the task used in Melrose et al., 2008 involved a semantic event-sequencing task (using pictures) where participants had to plan ahead and sequence events chronologically. Both groups activated areas of the PFC, BG as well as parietal cortex, occipital lobe, thalamus and posterior hippocampus. And, while greater percent signal change was found in the caudate among controls, greater basal ganglia-parietal connectivity was found in the HIV group and thought to represent a compensatory mechanism for successful task completion. In considering the working memory demands for successfully performing the semantic sequencing task (which require recruitment of BG-parietal networks), it may be that the BG becomes more heavily recruited among HIV+ participants to perform in an equal fashion to controls. Our methodology included a letter fluency task that preferentially activates frontal-striatal networks, and analysis of task-related activation showed virtually no significant parietal involvement, rather regions primarily involved included SMA, inferior frontal gyrus, BG and occipital cortex. It has been previously reported that the BG play a more subtle role in the generation of words, with regard to executive language functions, which is consistent with the anatomic position of basal ganglia structures in multiple frontal–basal-ganglia–thalamic loops (Middleton & Strick, 2000). Thus, when considering the neural networks required for

successful task completion of the letter fluency task, our finding of HIV+ individuals recruiting greater BG during task performance is consistent with our knowledge of functional compensation. To further support this notion, performance on phonemic fluency performance (i.e., total words generated and number of switches) was positively associated with increased activity in the inferior frontal gyrus and basal ganglia, suggesting that these structures are both important for successful phonemic fluency performance.

CD4+ count was positively associated with percent signal change in IFG and BG during the phonemic fluency task, but not with average percent signal change across all brain regions, providing further evidence that frontostriatal systems are preferentially targeted in HIV-infection. Also of note is that our group of HIV+ individuals performed within normal limits on the lexical fluency task despite the observed functional brain changes, suggesting that CD4+ count may predict subclinical difficulties, which are detected by the degree of frontostriatal recruitment. In other words, our findings suggest that in order to perform similarly to controls, HIV+ individuals place heavier recruitment on subcortical structures.

We did not detect group differences in neural activity under conditions of semantic fluency. It may be that our HIV+ group failed to demonstrate activation differences under the semantic fluency condition because of the preferential involvement of frontal-striatal circuitry, whereas semantic fluency has been associated with specific activation of the left temporal gyrus in the processing of semantic verbal fluency tasks (Baldo, Schwartz, Wilkins, Dronkers, 2006; Gold, Balota, Kirchhoff, Buckner, 2005).

Further analysis in our HIV+ sample revealed that those with lower CD4 counts demonstrated reduced IFG and BG activation during the phonemic fluency task. This is generally consistent with findings by Melrose et al. (2008), who suggest that despite adequate immune functioning, the presence of HIV-infection confers functional brain changes in frontal systems. It has been suggested that BG hypermetabolism may occur early in HIV-associated brain disease that becomes hypometabolic with disease progression, as has been

demonstrated in earlier HIV investigations (Castelo, Courtney, Melrose, & Stern, 2007; Hinkin et al., 1995; van Gorp, Mandelkern, Gee et al., 1992; Rottenberg et al., 1997). We did not find that nadir CD4 was associated with percent signal change in our preselected structures. This unanticipated finding may be due to limitations in relying upon self-reported nadir CD4 (in contrast to self-reported recent CD4, which was confirmed by medical records).

Our sample size ($N = 28$) was small, even for an fMRI study, which is a limitation to the current study and this study would benefit from replication among a larger sample. Nevertheless, using a relatively stringent statistical threshold, we detected significant differences between groups, suggesting a large effect of neural activation. It is possible that we did not observe overt significant differences across groups on the verbal fluency tasks due to our small sample size, as most neuropsychological studies of verbal fluency demonstrate small effect sizes (Iudicello, Woods, Deutsch, Grant, & HIV Neurobehavioral Research Program [HNRP] Group 2012).. Our sample was also comprised of mostly males, and therefore our findings may not be generalizable to female cohorts.

Despite these limitations, our findings highlight a neuroanatomical dissociation in regions activated between HIV+ and HIV- adults while undergoing a word retrieval task, which theoretically utilize specific neural systems. Based upon these findings, it appears that when required perform a cognitively demanding task (i.e., phonemic fluency), HIV+ adults showed increased activity (relative to HIV- adult controls) in the precise neural mechanisms involved in task performance in order to perform similarly to controls on the task. Further, poorer immune status was associated reduced recruitment of these structures, which may highlight a dysfunction in the mechanisms that are required for performing frontal-striatally mediated tasks. Although not statistically significant, increased CD4 count was associated with higher scores on the FAS task, further supporting the contention that healthier immune functioning is associated with better performance. Future research is necessary to confirm the mechanisms by which

immune functioning influences brain response and to determine how this may change throughout the course of HIV-disease.

Our results highlight the importance of detecting subtle cognitive abnormalities, which may go unnoticed when using standard neurobehavioral assessment. A prior study conducted by our group (Thames et al., 2011) directly tested the cognitive reserve hypothesis among HIV+ older adults and found that after matching participants on neuropsychological performance, individuals with higher levels of cognitive reserve demonstrated greater atrophy of the basal ganglia. These results suggested that higher levels of cognitive reserve may be able to shoulder greater disease burden before overt signs of disease emerge. The current findings further support the contention that normal performance on neuropsychological assessment does not always reflect intact brain function. Considering that BOLD fMRI is an indirect measure of neural function, this method can shed light onto which neural systems become preferentially targeted in HIV. Future studies should employ longitudinal designs to examine the predictive value of fMRI in detecting downstream observable cognitive impairments.

Acknowledgements

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