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Increased glial metabolites predict increased working memory network activation in HIV brain injury

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Abstract

Deficits in attention and working memory are common in human immunodeficiency virus type 1 (HIV-1)-infected patients, but the pathophysiology of these deficits is poorly understood. Modern neuroimaging techniques, such as proton magnetic resonance spectroscopy (^1H MRS) and functional MRI (fMRI), can assess some of the processes underlying HIV brain injury. To evaluate the model that attentional deficits in early HIV brain disease are related to brain inflammation, ^1H MRS and fMRI were performed in 14 HIV-positive subjects [acquired immunodeficiency syndrome (AIDS) dementia complex stage 1 or less]. Increasing attentional load on three working memory tasks was assessed with fMRI, and the concentrations of brain metabolites were measured with ^1H MRS in the frontal gray and white matter, and basal ganglia. Metabolite concentrations were correlated with fMRI blood oxygenation level-dependent (BOLD) signals, using a random-effects linear regression model in SPM99. Several positive correlations were observed between the BOLD signal strength in the working memory network (posterior parietal cortex and lateral prefrontal cortex) and the concentrations of frontal white matter and basal ganglia metabolites that are predominant in glial cells (choline-containing compounds, myo-inositol, and total creatine). In contrast, BOLD signals in the working memory network were not correlated with the concentration of *N*-acetyl compounds, which are markers of neuronal viability, or with metabolite concentrations in the frontal gray matter. These findings are consistent with previous results that mild HIV brain injury is associated with increased glial activation without major involvement of neuronal abnormalities. We propose that the inflammatory glial abnormalities reduce the efficiency of neural processing, and necessitate compensatory increases in attention in patients, and associated BOLD signals, to perform a given task. The same mechanism may also contribute to cognitive dysfunction in other brain diseases that involve inflammation.

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Introduction

Despite enormous advances in the treatment of human immunodeficiency virus-1 (HIV-1) infection, the number of people who are HIV seropositive continues to rise. In 2002, approximately 40 million adults and 3 million children were living with HIV. Approximately 20–40% of these subjects develop cognitive deficits in sustained attention, mental flexibility, general motor speed, memory (Miller et al., 1990; Selnes and Miller, 1994), and especially working

memory (McArthur et al., 1993; Law et al., 1994; Martin et al., 1995; Hinkin et al., 1996; Grassi et al., 1999). These deficits may ultimately evolve into a more severe HIV-related dementia syndrome (Qureshi et al., 1998). Because it is difficult to obtain noninvasive clinical markers for the severity of brain injury in these patients, modern neuroimaging techniques can provide important biological markers for HIV brain disease. One of these techniques, proton magnetic resonance spectroscopy (^1H MRS), has been used widely to assess brain chemistry in HIV patients (Chong et al., 1993; Jarvik et al., 1993; Barker et al., 1995; Laubenberg et al., 1996; Paley et al., 1996; Tracey et al., 1996; Chang et al., 1999, 2002; Meyerhoff et al., 1999). Typical findings early in the course of HIV brain disease include

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increased metabolites that are present primarily in glial cells [myo-inositol (MI) and choline-containing compounds (CHO)], or are involved in energy metabolism [total creatine (CR)]. Patients with moderate to severe dementia show further increases in these markers, but additionally develop decreases in *N*-acetyl compounds (neuronal markers) (Chang et al., 1999). The glial marker MI shows good correlation with clinical disease severity [acquired immunodeficiency syndrome (AIDS) dementia complex stage, or ADC stage, viral loads, and CD4 counts] (Chang et al., 1999), with cognitive function (Chang et al., 1999, 2002), and may even be elevated in patients without cognitive deficits (Meyerhoff et al., 1993; Chang et al., 1999).

Therefore, MRS studies indicate that glial activation in the white matter and subcortical gray matter, rather than neuronal loss, occurs early in the course of HIV brain injury (Barker et al., 1995; Laubenberger et al., 1996; Chang et al., 1999, 2002). These findings are consistent with neuropathology reports showing that HIV encephalitis is characterized by giant cells, microglial nodules, inflammatory infiltrates, and gliosis, and occurs predominantly in the central white and deep gray matter (Budka, 1991; Sharer, 1992; Bell et al., 1998). Furthermore, neuronal loss is not a prominent feature during the early stages of dementia (Seilhean et al., 1993). Severe dementia occurs primarily in those with both cortical gray and white matter involvement and less so when the pathology is confined to the white matter (Bell et al., 1998).

HIV-associated brain injury has also been evaluated with blood oxygenation level-dependent (BOLD)-functional MRI (fMRI), a direct and noninvasive technique to study active brain function. HIV patients with mild dementia demonstrate increased brain activation on working memory tasks compared to control subjects, especially in the lateral prefrontal cortices (LPFC) and supplementary motor area (SMA) (Chang et al., 2001). This finding suggests increased usage of brain reserve as a compensatory response to HIV-associated brain injury. This assumption is likely correct since HIV patients with normal cognitive function, verified by neuropsychological tests, also demonstrated increased brain activation, although to a lesser degree than those with cognitive abnormalities and predominantly in the LPFC (Ernst et al., 2002). Because glial activation and inflammatory changes are neuropathological hallmarks of HIV-associated brain injury, we hypothesized that increased inflammatory markers on MRS would predict increased brain activation on fMRI, especially in the prefrontal region where the pathology is often most severe (Ketzler et al., 1990; Everall et al., 1991).

Materials and methods

Subjects

Fourteen HIV-1-positive men (age 37.8 ± 7.1 years) were scanned with ^1H MRS and fMRI while they were

performing three n-back tasks (see below) with increasing attentional load. These subjects comprised a subgroup of patients whose fMRI findings have been reported previously (Chang et al., 2001; Ernst et al., 2002). Prior to the study, each patient underwent a screening evaluation, which included an HIV dementia scale (Power and Johnson, 1995), AIDS dementia staging (Aronow et al., 1988; The Dana Consortium on Therapy for HIV Dementia and Related Cognitive Disorders, 1996), complete blood count, routine chemistry, thyroid panel, syphilis serology, CD4 count, and plasma viral load. All subjects were also evaluated with a battery of neuropsychological tests that were sensitive to early signs of HIV dementia (as described in Chang et al., 2002), as well as the California Computerized Assessment Package (CalCAP) (Miller, 1990). Major exclusion criteria were CD4 > 500/mL, positive urine toxicology screen or history of illicit drug and alcohol dependence, chronic medical or neuropsychiatric illnesses other than HIV-1 infection, structural abnormalities on MRI including opportunistic brain lesions, or head trauma with loss of consciousness (>30 min). Prior to the study, each subject signed a written consent form approved by our Institutional Review Board.

Structural MRI and ^1H MRS

All MR studies were performed on a 1.5-T scanner (Signa, General Electric, Milwaukee, WI). Structural MRI included a sagittal T1-weighted spin-echo scan (TE/TR 11/500) and a fast axial inversion recovery scan (TE/TI/TR 32/120/4000 ms). ^1H -MR spectra were acquired from the right frontal white matter (see Fig. 1), midfrontal gray matter, and the basal ganglia, using an optimized PRESS sequence (Bottomley, 1987; Ernst and Chang, 1996), with TE/TR = 30/3000 ms, 128 averages, and voxel sizes of 3–5 cc. The T2 decay of the water signal within each voxel was measured at 10 different echo times, and the water signals from brain parenchyma and cerebrospinal fluid (CSF) were extracted (Ernst et al., 1993). The concentrations of *N*-acetyl compounds (NA), CR, CHO, and MI, corrected for the partial volume of CSF within each voxel, were then determined by using the water signal from brain tissue as a concentration reference (Kreis et al., 1993). Spectral processing included low frequency filtering of the free induction decay (Kreis et al., 1991), apodization (0.5 Hz Gauss broadening), zero filling, Fourier transformation, manual zero-order phase correction, automatic DC baseline correction, and fitting of the NA, CR, CHO and MI peak areas (Kreis et al., 1993). The resulting metabolite concentrations have interindividual variations of about 10%, and an intra-subject variability of 3 to 8% (Ernst and Chang, 1996).

Functional MRI

The subjects performed each of the three n-back tasks of increasing difficulty (0-back, 1-back, and 2-back) twice as described previously (Ernst et al., 2002). All 14 subjects

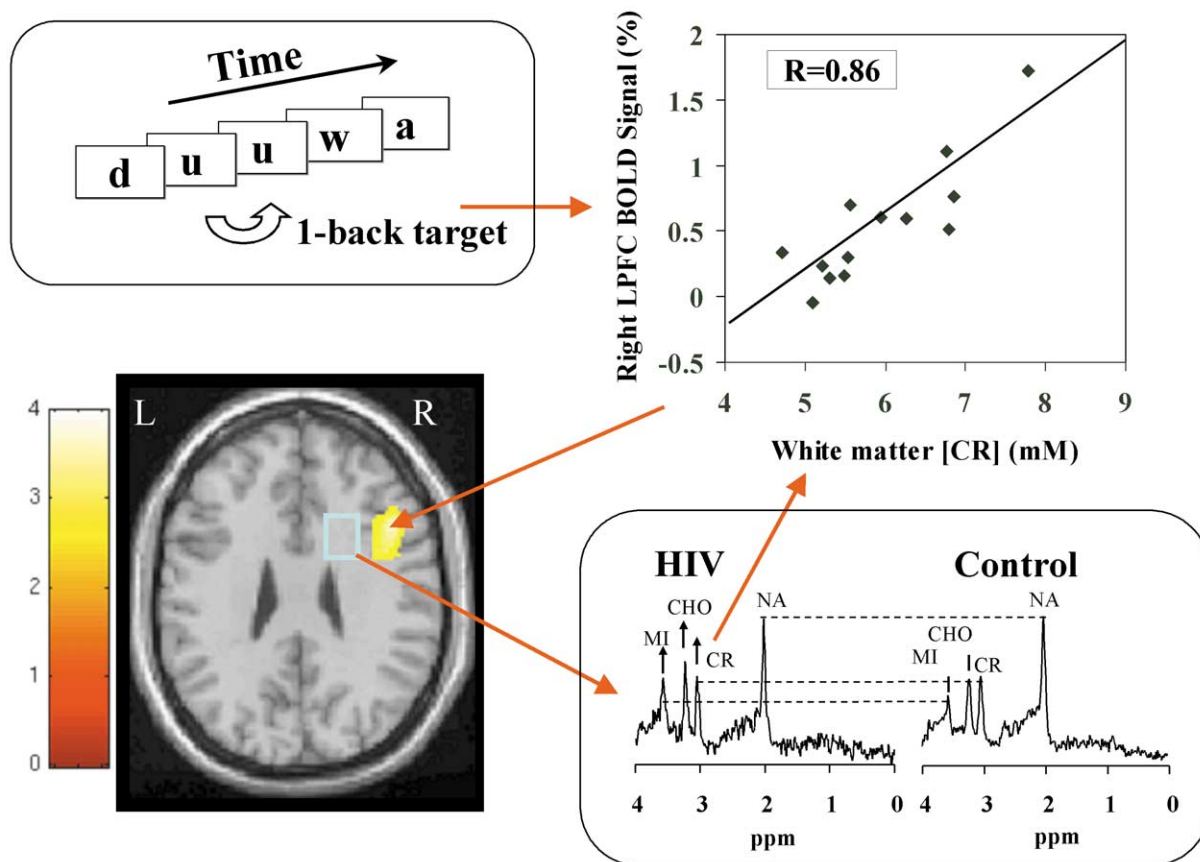


Fig. 1. Summary of the combined fMRI- ^1H MRS study design. Localized ^1H MRS was performed in the right frontal white matter (shown as a blue box on the axial MRI scan), frontal gray matter, and the basal ganglia. The insert in the right bottom corner shows a proton spectrum of an HIV patient with ADC stage 1 and a control subject. Functional MRI was performed while subjects were performing n-back working memory tasks. The 1-back paradigm is shown as an example (top left insert): random alphabetical letters were presented sequentially (rate: 1/s), and subjects were trained to press a response button whenever the current letter was the same as the one before. The activation strength on fMRI (BOLD signal) was then correlated with metabolite concentrations in each subject, and statistical parametric maps were calculated that reflect the significance of correlations for each voxel. The graph (right top corner) shows the correlation between right-frontal white matter total creatine concentration (CR) and fMRI signal strength in the LPFC region marked in yellow on the structural MRI (Talairach coordinate +46/+14/+34). The r and P values in the graph were determined post hoc, using a simple linear regression between BOLD signal and [CR]. fMRI, functional magnetic resonance imaging; ^1H MRS, proton magnetic resonance spectroscopy; HIV, human immunodeficiency virus; BOLD, blood oxygen level dependent; LPFC; lateral prefrontal cortices; NA, *N*-acetyl compounds; CHO, choline-containing compounds; MI, myo-inositol.

performed the 0-back task, and all but one subjects ($n = 13$) performed the 1-back and 2-back tasks. The paradigms were generated on an HP workstation and displayed to subjects in the scanner via a 20-in. LCD monitor, and a mirror mounted on the head coil. Functional MRI was performed using single-shot gradient-echo echoplanar imaging (TE 60 ms, TR 2500 ms, 16 axial slices, resolution $3.125 \times 3.125 \times 8 \text{ mm}^3$). fMRI processing was performed with the Statistical Parametric Mapping program (SPM99b) (Friston et al., 1995), and included motion correction with quality control (all scans had translations $< 0.8 \text{ mm}$ or rotations < 0.8 degrees), transformation into Talairach space, and spatial smoothing (10 mm Gaussian). To determine the relationship between brain activation on fMRI and metabolite concentrations, a random-effects linear regression fMRI model was specified in SPM, in which the BOLD signal strength across subjects was modeled as being linearly dependent on the

metabolite concentrations of interest. From these analyses, statistical parametric maps (SPMs) were calculated for activation during the working memory tasks that is linearly related with each metabolite concentration.

Since the n-back tasks activate a working memory network that includes the posterior parietal cortex (PPC) and LPFC bilaterally, the SMA, and the caudate, only correlations in these six regions of the working memory network were considered. Cluster-level P values ≤ 0.05 (corrected) were used to define statistical significance of correlations. The cluster analyses were performed at a voxel-level P value of 0.0025 ($T = 3.50$). Primary analyses included only MRS variables that are known sensitive markers of early HIV brain disease, i.e., the concentrations of CR, CHO, and MI in the frontal white matter. For variables that showed statistically significant correlations, each subject's BOLD activation strength (% signal change) was determined at the

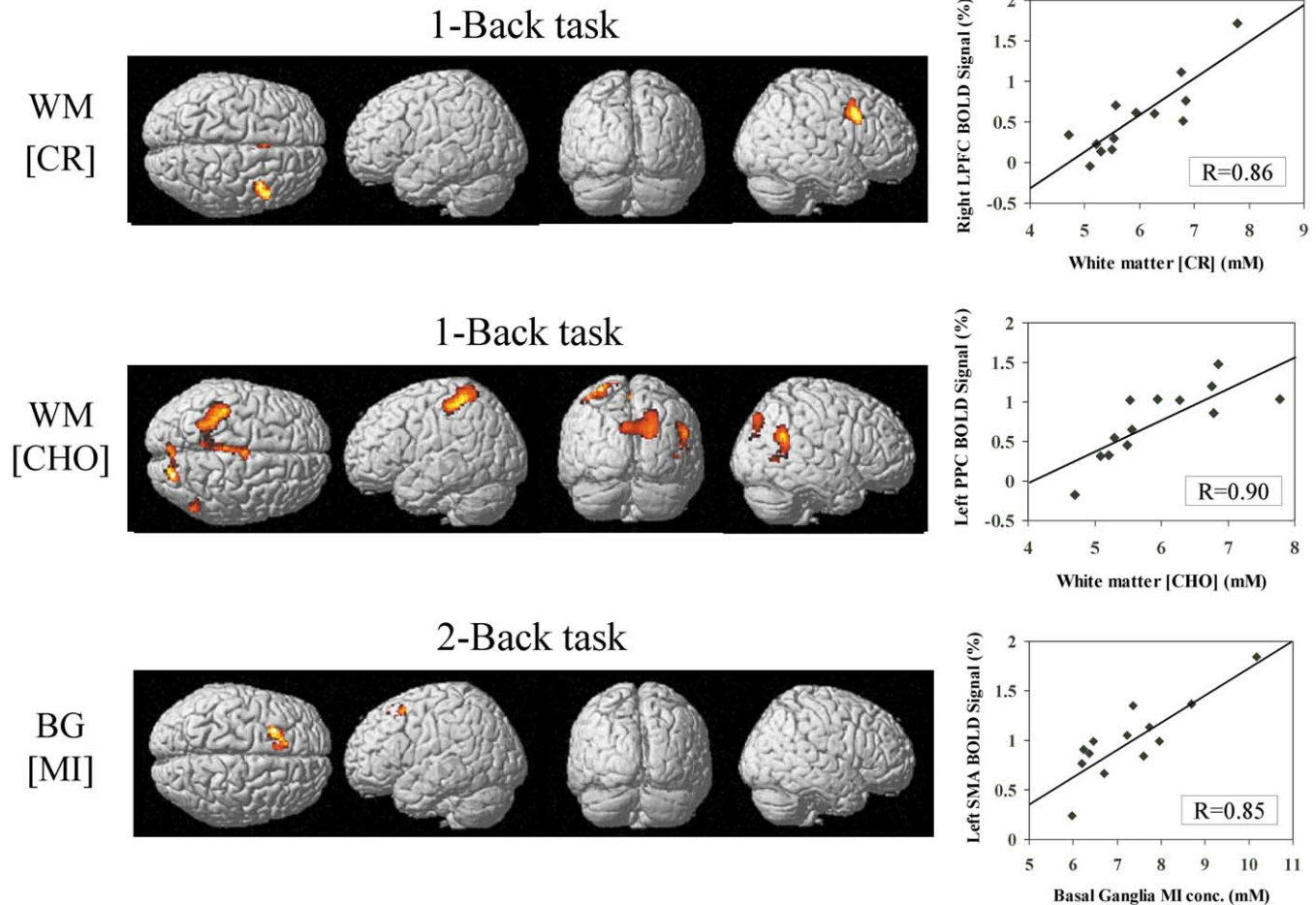


Fig. 2. Surface renderings of SPM maps showing significant correlations of the BOLD signal strength during the 1-back task with the [CR] (top row) and [CHO] (middle row) concentrations in the frontal white matter (voxel-level $t < 3.5$), as well as correlations of the BOLD signal during the 2-back task with the basal ganglia [MI] (bottom row). The graphs to the right exemplify correlations between these metabolite concentrations with the BOLD signal in the right LPFC (WM CR; top), left PPC (WM CHO; middle), and left SMA (BG MI; bottom); these correlations were significant on the SPM cluster analysis. WM, frontal white matter; BG, basal ganglia; SPM, statistical parametric map; BOLD, blood oxygen level dependent; CR, creatine; CHO, choline-containing compounds; MI, myo-inositol.

maximum of each cluster, using the “plot” function in SPM, and post hoc linear regression analyses were performed to obtain correlation coefficients. Additionally, secondary analyses were performed in HIV patients using those variables that were not hypothesized to have significant correlations with BOLD activation, i.e., the NA concentration in the frontal white matter, and all metabolites in the frontal gray matter and basal ganglia.

Results

The patients in this study had the following clinical characteristics (mean \pm standard deviation): CD4 count: 279 ± 166 /mL; nadir CD4: 237 ± 172 /mL; Log_{10} plasma HIV RNA: 3.92 ± 1.30 copies/mL; Log_{10} CSF HIV RNA: 2.92 ± 1.17 copies/mL; HIV-dementia scale: 13.3 ± 3.2 (maximum 16). Seven of the patients had no cognitive

deficits (ADC = 0), four had minor cognitive motor disorder (MCMD or ADC = 0.5), and three had mild dementia (ADC = 1). Thirteen of the subjects had been treated with stable highly active antiretroviral therapy (HAART) for at least 2 months prior to the study, whereas one patient had never been treated with antiretroviral medications. Task performance during fMRI was nearly perfect, with 100% accuracy for the 0-back and 1-back tasks, and 91% accuracy for the 2-back task.

The patterns of brain activation across the three working memory tasks resembled those previously observed in HIV patients (Chang et al., 2001; Ernst et al., 2002), and involved the LPFC, PPC, caudate, and the SMA. On the SPM analyses, several significant correlations between the BOLD signal strength and the primary metabolites of interest were observed. Specifically, [CR] in the right frontal white matter was positively correlated with right LPFC activation (BOLD signal) during the 1-back task [$P = 0.02$ cluster

level, corrected; $r = 0.86$; Talairach coordinate (+42/+8/+42); see Figs. 1 and 2]. Similarly, the right frontal [CHO] was positively correlated with the left PPC activation during the 0-back task [$P = 0.001$ cluster level, corrected; $r = 0.89$; (-28/-58/+68)] and with PPC activation bilaterally during the 1-back task (left: $P = 0.001$; $r = 0.90$; (-42/-36/+60); right: $P = 0.02$; $r = 0.90$; (+48/-54/+24). No significant correlations between metabolite concentrations in the frontal white matter and fMRI activation were observed in the HIV patients for the 2-back task. The [MI] in the frontal white matter also did not correlate with BOLD activation on any task. None of the inverse correlations between the primary MRS variables in the right frontal white matter and BOLD activation in the primary areas of interest reached statistical significance.

Several significant correlations were found on the secondary analyses: the basal ganglia [CHO] was positively correlated with activation during the 1-back task in the left and right PPC [left: $P = 0.002$; $r = 0.84$; (-26/-54/+42); right: $P = 0.01$; $r = 0.84$; (+36/-54/+36)] as well as in the left LPFC [$P = 0.004$; $r = 0.94$; (-42/-12/+44)]. In addition, [MI] in the basal ganglia was positively correlated with the left SMA activation during the 2-back task [$P = 0.03$; $r = 0.85$; (-8/-18/+62), Fig. 2 bottom]. No other correlations were found on the secondary analyses; in particular, no positive or negative correlations were observed between BOLD signals in the areas of interest and any of the frontal gray matter metabolites, or any of the NA concentrations.

Discussion

The goal of this study was to determine what metabolic factors might underlie functional abnormalities in patients with mild HIV brain disease. Our discussion is based on a three-step model of HIV brain disease. In the first step, HIV infection causes brain inflammation and brain injury, either directly due to the neurotoxic viral proteins (gp120 and tat) or indirectly due to the deleterious effects of cytokines (TNF-alpha, IL-1-beta, IL-6) and chemokines (monocyte chemoattractant proteins, RANTES, macrophage inflammatory proteins) that are released by HIV-infected monocytes (microglia or macrophages) and activated astrocytes (Kaul et al., 2001). Histopathological and neuroimaging studies of HIV brain disease demonstrate that these inflammatory and glial abnormalities are most pronounced in the frontal white matter and deep gray matter, while the cortex is relatively spared (Bell et al., 1998; Chang et al., 2002). In the second step, brain inflammation and injury lead to metabolic abnormalities, some of which can be detected with modern neuroimaging techniques. For example, HIV-infected patients commonly show glial activation and proliferation, which can be monitored with glial neuroimaging markers such as CHO or MI on ^1H MRS. Furthermore, HIV-associated brain inflammation may lead to abnormal energy

metabolism; this has been demonstrated with positron emission tomography (PET) (Rottenberg et al., 1996) and ^{31}P MRS (Bottomley et al., 1990; Deicken et al., 1991; Meyerhoff et al., 1995; Patton et al., 2001). In the third and final step of the model, the cellular and metabolic abnormalities impair neural processing. To compensate for these impairments, affected patients require increased attention (or neural processing) to maintain normal cognitive function, which can be detected with BOLD fMRI. Ultimately, as cellular and metabolic abnormalities become more severe, compensatory mechanisms are overburdened and patients develop cognitive deficits.

This model is supported by the major findings of this study. First, HIV patients showed significant positive correlations of glial markers (CHO, MI, and CR) in the frontal white matter or basal ganglia with BOLD signal strength in the working memory network. In contrast, BOLD signal strength was not related to the neuronal marker NA or any of the metabolite concentrations in the frontal gray matter. Therefore, our study suggests that working memory abnormalities in HIV patients are driven by glial abnormalities in the white matter and basal ganglia, in agreement with our model.

The metabolites CHO, MI, as well as CR primarily represent the glial population, since their concentrations are much higher in glial cells than in neuronal cells (Brand et al., 1993; Urenjak et al., 1993). Elevations of both [MI] and [CHO] are correlated with important clinical assessments of HIV brain disease, such as ADC stage (Laubenberger et al., 1996; Chang et al., 1999; von Giesen et al., 2000), CD4 count (Chong et al., 1993; Chang et al., 1999, 2002), plasma viral load (Chang et al., 2002), CSF viral load (Chang et al., 1999), and even neuropsychological tests (Chang et al., 2002). The elevated [MI] and [CHO] are thought to reflect inflammatory glial activation.

Recent studies demonstrate that glial cells (astrocytes, microglia, and oligodendrocytes) interact extensively with neuronal elements through signals such as ion fluxes, neurotransmitters, cell adhesion molecules, and specialized molecules released from synaptic and nonsynaptic regions of the neuron (Fields and Stevens-Graham, 2002). These signaling processes are means by which glial cells can affect neuronal excitability and thereby influence their activity, including formation and rebuilding of synapses, and modulation of synaptic strength, both during physiological conditions as well as pathological disturbances (Hansson and Ronnback, 2003). For example, tumor necrosis factor-alpha (TNF-alpha), a protein produced by glia, enhances synaptic efficacy by increasing surface expression of AMPA receptors (Beattie et al., 2002). Since elevated TNF-alpha appears to play a major role in HIV-associated neuronal injury (De et al., 2002) and maintenance of HIV replication (Kast, 2002–2003), the increased BOLD signals in HIV-positive subjects may be related to the modulation of synaptic strength by the activated glia, as shown by elevated [MI] and [CHO].

Creatine and phosphocreatine serve additional functions in brain energy metabolism, both via the creatine kinase (Ames, 2000). First, phosphocreatine (PCr) may provide a buffer for rapid transient increases in energy demand, for instance, due to onset of neuronal firing (Smith et al., 2002). The second, lesser known and more controversial role of the creatine kinase/PCr system is related to transport of high-energy phosphate from the mitochondria to distant ATPases (Yoshizaki et al., 1990). Therefore, an upregulated energy state and increased flux in the high-energy phosphate metabolism, due to CNS injury and the resulting glial activation, may necessitate higher concentrations of the major shuttles Cr and PCr. However, ^1H MRS studies in HIV brain disease have found variable levels of [CR], depending on brain regions and disease severity (Chang et al., 1999; Chang et al., 2002). HIV patients naive to antiretrovirals had slightly increased [CR] in the frontal regions during the asymptomatic or minor-cognitive motor disorder stages, but significant elevation of [CR] in the frontal lobe and markedly decreased [CR] in the basal ganglia during moderate to severe dementia (Chang et al., 2002). Earlier ^{31}P MRS studies also found reduced PCr concentrations in patients with more severe HIV dementia (Bottomley et al., 1990; Meyerhoff et al., 1995). Therefore, the positive correlations between the frontal white matter [CR] and BOLD activation in the prefrontal cortex of our HIV patients with relatively mild dementia are consistent with prior MRS and fMRI studies, and support our hypothesis that inflammatory changes in the brain may necessitate increased attentional modulation during working memory tasks.

One of the limitations of our study is the lack of an HIV-negative control group. Therefore, it is possible that the observed relationships between BOLD signals and glial metabolite concentrations are not related to HIV-serostatus, but may also present in control subjects. Demonstration of such a relationship probably would require a substantially larger sample size since the variability of MRS and fMRI variables is smaller in a healthy population. However, the lack of a control group does not diminish the major implication of this study, that working memory deficits in HIV-positive subjects are likely to be driven by glial abnormalities in the white matter and basal ganglia.

Few published studies have evaluated the relationship between brain activation and biological markers of brain injury in neuropsychiatric disorders, despite the great potential of such studies to improve the understanding of neural mechanisms underlying these diseases. One recent study of schizophrenia patients found a specific relationship between prefrontal NA/CR and brain activation on working memory. Lower NA/CR in the dorsolateral prefrontal cortex on ^1H MRS predicted reduced prefrontal activity, using [^{15}O] water PET, in patients who performed a working memory (2-back) task (Bertolino et al., 2000). Decreased [NA] or increased [CR], or both, could lead to decreased NA/CR, and may be associated with poorer performance on the working memory task. An important difference between

the schizophrenia study and the current study is that our HIV patients had 91% performance accuracy on the 2-back task, whereas the schizophrenic patients had only 50% accuracy, probably as a result of more severe disease. Therefore, decreased brain activation in the schizophrenic patients may have been related to decreased performance due to neuronal loss (decreased NA), which was not observed in our HIV patients. In contrast, our HIV patients had increased “resting” glial markers that predict increased neuronal activation during “cognitive performance.”

In summary, our study demonstrates that increased concentrations of the glial markers CHO, MI, and CR in the frontal white matter and basal ganglia of HIV patients are associated with increased BOLD activation during working memory tasks. These findings suggest that working memory deficits in HIV patients are modulated by inflammation in the white matter and basal ganglia. We propose that a similar mechanism may also contribute to cognitive dysfunction in other brain diseases that involve inflammation.

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