Journal of NeuroVirology, 13: 150–159, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280701194230

informa
healthcare

Effects of human immunodeficiency virus and methamphetamine on cerebral metabolites measured with magnetic resonance spectroscopy

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Human immunodeficiency virus (HIV) and methamphetamine (METH) use disorders are associated with cerebral dysfunction. To determine whether these effects were evident on in vivo neuroimaging, quantitative, single voxel magnetic resonance (MR) spectroscopy was used to assess frontal white matter, frontal gray matter, and basal ganglia in 40 HIV+/METH+, 66 HIV+/METH-, 48 HIV-/METH+, and 51 HIV-/METH- participants. HIV was associated with lower *N*-acetylaspartate (NAA) in frontal white and frontal gray matter but METH was not associated with cerebral metabolite differences in any region. Among HIV+ individuals, lower CD4 counts and higher plasma HIV viral loads were associated with lower NAA in frontal gray matter and basal ganglia. The relationship between detectable plasma HIV viral load and NAA in frontal white matter was significantly stronger in the HIV+/METH+ group, compared to HIV+/METH-. Higher detectable plasma HIV viral load was significantly associated with higher *myo*-inositol (MI) in frontal white and gray matter for HIV+/METH+, but not HIV+/METH-. For the HIV-/METH+ group, lifetime duration of METH use was associated with higher choline levels in frontal gray matter and higher MI levels in basal ganglia. Our findings are consistent with significant disruption of neuronal integrity in the frontal lobes of HIV-infected individuals. Although METH was not associated with cerebral metabolite levels, other findings suggested that METH use did affect the brain. For example, the relationship between detectable plasma HIV viral load and NAA levels was limited to HIV+/METH+ individuals. This evidence indicates when HIV is poorly suppressed, METH may modify the effects of the virus on neuronal integrity. *Journal of NeuroVirology* (2007) **13,** 150–159.

Keywords: brain magnetic resonance spectroscopy (MRS), human immunodeficiency virus (HIV), methamphetamine, N-acetylaspartate

Introduction

Substance use is a common risk factor for transmission of human immunodeficiency virus (HIV), through both intravenous drug use (i.e., needle sharing) and unsafe sexual practices. Although HIV and methamphetamine (METH) abuse/dependence have been separately associated with cerebral dysfunction, relatively few studies have addressed their joint effects. HIV and METH would be expected to exert an additive influence resulting in more severe brain

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The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

This work was supported by DA12065 from the National Institute of Drug Abuse. The HIV Neurobehavioral Research Center (HNRC) is supported by Center award MH 62512 from NIMH. The authors would like to thank Dexter Walpole for his valuable assistance in data collection and preparation.

Received 14 November 2006; accepted 18 December 2006.

injury, particularly in those regions most vulnerable to HIV or METH alone, such as frontostriatal areas.

The mechanism of injury from HIV is thought to be indirect, resulting from the release of neurotoxins by resident and migrating cells. Frontostriatal circuitries appear to be especially vulnerable to such damage (Itoh *et al*, 2000; Reyes *et al*, 1991). Similar brain regions appear to be impacted by METH (Eisch *et al*, 1996; Wilson *et al*, 1996), which can cause increased dopamine and glutamatergic transmission, leading to excitotoxic neuronal injury and death (Davidson *et al*, 2001; Langford *et al*, 2003; Marshall *et al*, 1993; Ohmori *et al*, 1996; Stephans and Yamamoto, 1994; Wilson *et al*, 1996). Thus, the combination of HIV and METH could result in greater damage in the frontostriatal system than either one alone. In fact, studies from our own group indicate both separate and additive effects of HIV and METH dependence on rates of neuropsychological impairment (Rippeth *et al*, 2004) and brain morphometry (Jernigan *et al*, 2005).

Proton magnetic resonance spectroscopy (MRS) provides a noninvasive approach to examine brain metabolites. The most reliably measured compounds using a standard 1.5-Tesla MRI (MR imaging) scanner are *N*-acetylaspartate (NAA), a putative marker of neuronal integrity (Demougeot *et al*, 2001); choline and choline-containing compounds (CHO), a measure of cell membrane degradation and lipid changes; *myo*-inositol (MI), a possible indicator of glial proliferation and/or osmolar changes; and creatine + phosphocreatine (CR), an indicator of high energy stores that is often used as a relative standard for other metabolites.

Multiple studies of HIV-infected subjects using proton MRS have demonstrated reductions in cerebral NAA, particularly in frontal lobe white matter (Lee *et al*, 2003; Stankoff *et al*, 2001; Suwanwelaa *et al*, 2000; Tarasow *et al*, 2003) and basal ganglia (Chang *et al*, 2004; Meyerhoff *et al*, 1996; Moller *et al*, 1999; Yiannoutsos *et al*, 2004). In addition, elevations in MI and CHO have been associated with HIV infection (Chang *et al*, 2002, 2003, 2004; Chong *et al*, 1993; Ernst *et al*, 2000; Lee *et al*, 2003; Lopez-Villegas *et al*, 1997; Tarasow *et al*, 2003; von Giesen *et al*, 2001). Such changes have also been related to disease stage and neurocognitive function (Chong *et al*, 1993, 1994; Jarvik *et al*, 1993; Laubenberger *et al*, 1996; Paley *et al*, 1996; Tracey *et al*, 1996; Wilkinson *et al*, 1997).

The literature evaluating the impact of METH using MRS is much more limited. Lower NAA in frontal white matter and basal ganglia (Ernst *et al*, 2000) and lower NAA/CR in frontal gray matter (Nordahl *et al*, 2002, 2005) have been reported in abstinent METH users compared to controls. METH has been associated with elevated CHO (Ernst *et al*, 2000) and CHO/CR (Nordahl *et al*, 2002, 2005) in frontal gray matter. In addition, lower CR and CR/CHO have been found in the basal ganglia of METH users (Ernst *et al*, 2000; Sekine *et al*, 2002).

Taken together, the literature suggests that both HIV and METH dependence may injure neurons, as measured by NAA, with concomitant glial proliferation and inflammation as measured by CHO and MI. To date, the focus of these studies have been on the striatum and frontal lobes, which have been hypothesized to be susceptible to these cerebral alterations.

In terms of combined HIV and METH effects, a preliminary magnetic resonance spectroscopy (MRS) study from our laboratory (Taylor *et al*, 2000) demonstrated significantly lower NAA in the frontal gray matter of a small group of HIV seropositive (HIV+) users of methamphetamine and/or cocaine ($n=7$) compared to HIV seronegative controls (HIV−; $n=5$). Smaller reductions were evident in participants with only one risk factor (i.e., HIV or stimulant use alone).

In the only other study of the combined effects of HIV and METH on cerebral metabolites using proton MRS, each factor was independently associated with changes in most metabolites (Chang *et al*, 2005). Although no statistically significant HIV × METH interactions were present, the pattern of the results was interpreted to provide evidence of an additive effect given the largest metabolite differences were found for the HIV+/METH+ group. The main findings were 6% to 9% lower NAA in frontal white matter, frontal gray matter, and basal ganglia in the HIV+/METH+ group compared to HIV−/METH− controls. Frontal white matter and basal ganglia NAA were intermediate (i.e., 4% to 6% lower) for the two groups with only one risk factor compared to HIV−/METH− controls. A similar pattern of results was found for CR in the basal ganglia. Higher MI was also present in frontal white matter of the HIV+ groups. There were no significant correlations between METH use variables and metabolite concentrations, but lower CD4 counts were correlated with higher CHO and MI in frontal white and frontal gray matter regions.

The present study was conducted with the goal of assessing the separate and joint impact of HIV and METH on the brain using proton MR spectroscopy. Neuronal integrity (as measured by NAA) was expected to be compromised most in dually affected individuals, whereas the groups with HIV or METH alone were predicted to exhibit an intermediate effect. Membrane degradation and glial proliferation (as measured by CHO and MI) were expected to be greatest in the HIV+/METH+ group and at intermediate levels in groups with HIV or METH alone. Severity of HIV illness and intensity of METH dependence were hypothesized to be related to the level of neuronal change or inflammation.

Results

Effects of HIV and METH

HIV was associated with lower NAA in the frontal white matter and frontal gray matter, with 4% to 5% lower NAA evidenced in the HIV+ groups compared to the HIV− groups, regardless of METH status. An unexpected main effect of HIV was also present for CR

Table 1 Group means and standard deviations of *N*-acetylasparate (NAA), choline-containing compounds, creatine + phosphocreatine, and *myo*-inositol by region of interest

	HIV−		*HIV+*		*HIV effect*		*METH effect*		*HIV × METH effect*	
	METH− (n=51)	*METH+ (n=48)*	*METH− (n=66)*	*METH+ (n=40)*	*F*	*P*	*F*	*P*	*F*	*P*
Frontal white matter										
NAA	7.94	7.96	7.62	7.65	5.21	.02	0.02	.89	0.00	.97
(SD)	(0.77)	(0.87)	(1.00)	(1.22)						
Choline	1.55	1.63	1.61	1.59	0.07	.80	0.78	.38	1.80	.18
(SD)	(0.28)	(0.26)	(0.27)	(0.30)						
Creatine	4.95	4.91	4.87	4.85	0.47	.49	0.06	.81	0.00	.96
(SD)	(0.79)	(0.60)	(0.82)	(0.74)						
myo-Inositol	4.61	4.17	4.36	4.46	0.01	.92	0.62	.43	1.55	.21
(SD)	(2.29)	(0.75)	(1.16)	(1.31)						
Frontal gray matter										
NAA	7.72	7.60	7.33	7.29	9.38	.003	0.48	.49	0.16	.69
(SD)	(0.73)	(1.00)	(0.79)	(0.65)						
Choline	1.32	1.37	1.32	1.27	1.75	.19	0.01	.94	1.63	.20
(SD)	(0.25)	(0.27)	(0.25)	(0.24)						
Creatine	5.42	5.35	5.07	5.13	6.43	.01	0.01	.92	0.30	.58
(SD)	(0.79)	(0.93)	(0.76)	(0.69)						
myo-Inositol	4.12	4.20	3.87	4.04	3.51	.06	1.25	.27	0.14	.71
(SD)	(0.66)	(0.90)	(0.74)	(0.89)						
Basal ganglia										
NAA	7.11	7.20	7.13	7.21	0.01	.92	0.34	.56	0.00	.98
(SD)	(1.07)	(0.82)	(1.06)	(1.12)						
Choline	1.60	1.64	1.67	1.71	1.93	.17	0.53	.47	0.00	.96
(SD)	(0.29)	(0.30)	(0.42)	(0.36)						
Creatine	6.46	6.54	6.80	6.71	2.05	.15	0.00	.96	0.21	.65
(SD)	(0.96)	(0.96)	(1.47)	(1.17)						
myo-Inositol	4.48	4.67	4.83	4.66	0.67	.42	0.01	.94	0.73	.39
(SD)	(0.91)	(1.39)	(1.56)	(1.32)						

Note. F values and *P* values are from 2 × 2 ANOVAs.

in the frontal gray matter with 5% lower levels in the HIV+ groups. Counter to our hypotheses, there were no statistically significant main effects for METH or the interaction of HIV and METH. Metabolite values by region and group are presented in Table 1 with results from the 2 × 2 analyses of variance (ANOVAs).

Effects of CD4 and plasma HIV

For HIV-infected subjects, lower CD4 count was significantly correlated with lower NAA in frontal gray matter ($r = .20$, $P = .04$) and basal ganglia ($r = .25$, $P = .01$). For HIV+/METH+, higher detectable plasma HIV viral load was significantly associated with lower NAA in frontal white matter ($r = -.68$, $P < .001$), with similar trends in frontal gray matter ($r = -.36$, $P = .10$) and basal ganglia ($r = -.39$, $P = .08$). In addition, higher detectable plasma HIV viral load was significantly associated with higher MI in frontal white matter ($r = .35$, $P = .007$), and frontal gray matter ($r = .46$, $P = .03$) for HIV+/METH+. None of the correlations between viral load and metabolites reached statistical significance for the HIV+/METH− group. The interaction between plasma HIV viral load and METH status predicting NAA in frontal white matter was not statistically significant in a multiple regression. However, as demonstrated in Figure 1, the correlation between HIV viral load and NAA in frontal white matter was significantly stronger in HIV+/METH+ compared to HIV+/METH− ($z = 2.72$, $P = .008$) using the test for differences between correlations for two independent groups with Fisher's *r*-to-*z* transformation (Cohen and Cohen, 1983). There were no statistically significant relationships found between CSF HIV viral load and any metabolites.

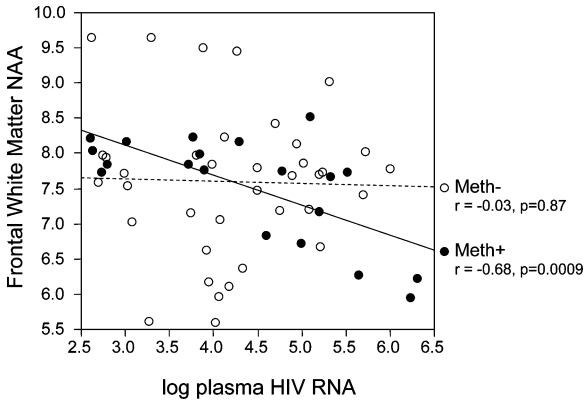

Figure 1 Relationship of frontal white matter NAA to plasma HIV concentration. (Only HIV+ persons with detectable HIV viral load were included in the analysis.)

Effects of METH use history
Longer lifetime duration of methamphetamine use was significantly correlated with higher CHO in frontal gray matter ($r = .34$, $P = .02$) and higher MI in basal ganglia ($r = .31$, $P = .04$) in the HIV−/METH+ group. There were no other statistically significant correlations present between metabolites and measures of methamphetamine use.

Effects of other substance use
The impact of lifetime dependence on alcohol or cocaine was evaluated using 2 × 2 (HIV status × lifetime dependence) ANOVA with metabolites as the independent variables. This analysis included only METH+ participants as they were the only group with large enough samples to reasonably address this issue. None of the ANOVAs yielded statistically significant effects of other drugs or their interaction with HIV.

Effects of ARV treatment
In addition, the influence of antiretroviral therapy in the HIV+ groups was examined using 2 × 2 (antiretroviral status × METH status) ANOVA with metabolites as the independent variables. None of the ANOVAs yielded statistically significant effects of antiretroviral status or its interaction with METH status.

Discussion

The results reported in the present study confirm a cerebral impact of HIV that can be measured reliably with MR spectroscopy. Specifically, a 4% to 5% reduction in the neuronal integrity marker NAA was observed in HIV+ compared to HIV− groups, consistent with the effects found in other studies. Though not hypothesized, the 5% reduction in CR in frontal gray matter is consistent with a recent report (Chang *et al*, 2005). The statistically significant correlations between lower CD4 count and NAA in the frontal gray matter and basal ganglia of HIV+ subjects suggest that severity of HIV illness predicts poorer neuronal integrity.

Counter to our hypotheses, the effects of methamphetamine and their interaction with HIV (or even additive impact) were not detected in our study despite the relatively large sample size. This apparent lack of methamphetamine effects on cerebral metabolites should not be interpreted to mean that methamphetamine dependence does not have deleterious effects on the brain. In fact, neuropsychological data from the same cohort (Rippeth *et al*, 2004) indicate that methamphetamine and HIV have independent and additive effects on the rate of cognitive impairment. Furthermore, Jernigan and colleagues (Jernigan *et al*, 2005) reported that *larger* gray matter volumes in METH users were actually associated with worse neurocognitive performance (again, opposite to the HIV effect, in which volume loss predicted worse cognitive functioning), suggesting that the processes underlying volume increases were not normal.

The lack of group differences between METH users and nonusers on metabolite measurements precluded testing for possible HIV × METH interactions in the standard manner. However, we believe there may be evidence from the relationships between plasma HIV load and metabolites in the HIV+/METH+ group that suggests an augmentation of HIV effects by METH. We found significant associations between lower NAA and higher plasma viral load, and positive associations between MI and CR and viral load in that group. These data provide tentative support for the notion that METH augments the adverse effects of HIV on brain metabolites. This may be especially true in those persons whose HIV replication has not been fully suppressed by antiretroviral therapy. Several prior reports support this notion. For example, METH is associated with higher levels of HIV RNA in plasma (Ellis *et al*, 2003), can exacerbate the neurotoxicity of the HIV-encoded protein, Tat, *in vitro* (Flora *et al*, 2003; Maragos *et al*, 2002; Theodore *et al*, 2006), and is associated with increased expression of interferon inducible genes in brains of those dying with HIV (Everall *et al*, 2005). In addition, we were able to detect a relationship between longer lifetime duration of methamphetamine use and a marker of inflammation in frontal gray matter and gliosis in basal ganglia in the HIV−/METH+ group.

A recent report by Chang and colleagues (Chang *et al*, 2005) demonstrated methamphetamine-associated NAA reductions of 3.5% in basal ganglia and 3.8% in frontal white matter. Their results also suggested an additive effect of HIV and METH. The lack of congruity between our study and theirs was quite surprising given the similarity in the methodology employed. The two MRS sequences were nearly identical, as were the regions of interest. There were some minor differences in the processing approach (e.g., different processing software), although the lack of a methamphetamine effect is unlikely due to this factor given the similarity of HIV findings.

A more detailed comparison of the two studies reveals a substantive difference in the type of participants recruited for the methamphetamine dependent groups, particularly in amount of methamphetamine exposure. In the study by Chang and colleagues, HIV−/METH+ subjects reported average lifetime methamphetamine use of 8241 g (SD = 16,850), using an average of 2.2 g per day (SD = 3.2), 6.5 days per week (SD = 0.9). The HIV+/METH+ group reported significantly lower methamphetamine use of 2167 g lifetime (SD = 2788), 0.8 g per day (SD = 0.6), 5.6 days per week (SD = 1.4). Using the mean plus two standard deviations from their study, a conservative upper limit of approximately 42,000 g can be inferred, compared to a maximum of 16,902 g in our study. The frequency of methamphetamine use also appears to be quite different, with their participants

using almost every day (6.6 days per week), compared to approximately 5 days per week in our study.

Because the level of methamphetamine use in the study by Chang and colleagues (Chang *et al*, 2005) was higher than what is typically experienced in our laboratory, we sought to determine if our METH users were somehow atypical (i.e., less severe addicts), by comparing our data to those reported by other studies of methamphetamine in humans.

A review of 21 studies conducted by different research groups across the United States revealed a range of METH use from 0.3 to 1.6 g per day, 0.9 to 4.4 days used per week, and 9 to 11 years of use. The values for METH exposure in the present study are consistent with these nationally reported data. In addition, a study of METH use patterns in Los Angeles (where Chang *et al* conducted their study) revealed a typical range of daily METH use of 0.5 to 1.0 g (Simon *et al*, 2002). The METH+ participants from the Chang study, however, appear to have been much heavier and more frequent users, and this may be one reason our findings diverged.

Another possibility emerges from our recent brain morphometric observations that the effects of HIV and METH on brain structure are quite different: whereas volumes of selected gray and white matter regions show the expected shrinkage in HIV infection, METH users actually have volume *increases* in some regions, when compared to HIV−/METH− controls (Jernigan *et al*, 2005). In HIV+/METH+ it appeared that the HIV-related shrinkage typically seen in certain structures (e.g., the caudate nucleus) was balanced by METH-associated increases, resulting in a normal appearing caudate volume. The biologic processes underlying these volume increases are not understood, but might involve, in part, aberrant dendritic sprouting associated with METH that has been reported in both animal and human neuropathologic studies (Langford *et al*, 2003; Robinson and Kolb, 1997, 1999).

In summary, although this study demonstrated robust effects of HIV, we were unable to detect an effect of methamphetamine on brain metabolites, including NAA, MI, CHO, and CR, in groups of HIV-seronegative detoxified methamphetamine-dependent persons whose histories of consumption were comparable to most series in the literature. The lack of effect may on the one hand suggest that METH users with typical histories of abuse either do not experience such changes, or have recovered with the 3 to 4 months of abstinence that was typical for our METH+ group. Alternatively, the apparent lack of effects might reflect more complex neurobiological processes associated with methamphetamine dependence, including the possibility that neural sprouting in some cell populations may contribute NAA signal that cancels NAA loss due to injury to other cell populations. Among HIV+/METH+ persons our data demonstrating that higher plasma viral load correlates with metabolite changes, suggesting neuronal injury and inflammation are consistent with the possibility that HIV effects on the brain are augmented by METH in those whose viral replication was not successfully suppressed.

Method

Subjects

A total of 205 participants were included in this study conducted at the San Diego HIV Neurobehavioral Research Center (HNRC; see Appendix). Participants were recruited through extensive outreach to community based organizations and treatment programs focused on HIV or methamphetamine. METH+ participants were recruited while undergoing treatment in residential programs that required abstinence. The study was approved by the Human Research Protections Program at the University of California, San Diego. After a complete description of the study to the subjects, written informed consent was obtained. Individuals were stratified by HIV serostatus (HIV+ or HIV−) and methamphetamine dependence (METH+ or METH−) as follows: 40 HIV+/METH+; 66 HIV+/METH−, 48 HIV−/METH+; and 51 HIV−/METH−.

Group characteristics are presented in Table 2. The groups were comparable on age, however the two METH+ groups had significantly fewer years of education, and the two HIV+ groups contained a greater proportion of males than the HIV− groups. The HIV−/METH+ group was comprised of somewhat fewer ethnic minorities, although this difference was not statistically significant. As expected, the HIV+ groups had significantly lower CD4 cell counts than their HIV− counterparts. In addition, the medical status of the two HIV+ groups was comparable based on their CD4 cell counts, percent with AIDS diagnoses, percent on antiretroviral therapy, and HIV viral load in plasma and cerebrospinal fluid (CSF). Although the duration of abstinence for the HIV−/METH+ group did not differ significantly from the HIV+/METH+, their estimated lifetime methamphetamine use was significantly greater.

The Structured Clinical Interview for DSM–IV (SCID; First *et al*, 1996) was used to assess current and lifetime history of alcohol and other substance abuse and dependence. Participants included in the METH+ groups met DSM–IV criteria for methamphetamine dependence during their lifetime. They also met criteria for methamphetamine abuse or dependence within 24 months of the examination, and were required to be abstinent for at least 10 days prior to the evaluation. Participants from all groups were excluded if they met DSM–IV criteria for alcohol dependence within a year of the evaluation or dependence on another substance within 5 years of evaluation. Participants were also excluded if they met criteria for long term dependence on alcohol or another substance (other than marijuana) during

Table 2 Means and standard deviations for demographic characteristics, and measures of illness severity

	HIV−/METH− (n=51)	*HIV−/Meth+ (n=48)*	*HIV+/METH− (n=66)*	*HIV+/METH+ (n=40)*	*P value*
Age (years)	36.4 (10.6)	37.4 (7.8)	39.0 (8.6)	36.9 (6.6)	.42
Education (years)	13.5 (2.3)	12.0 (2.3)	13.6 (2.1)	12.1 (2.6)	.0001[a]
% Female	27.5	31.3	16.7	5.0	.01[b]
% Non-white	33.3	16.7	37.9	32.5	.10
CD4 count (/ml)	896.0 (287.6)	918.8 (228.7)	432.0 (236.5)	425.6 (331.9)	<.0001[b]
Nadir CD4 count (/ml)	—	—	251.7 (201.7)	237.4 (199.2)	.73
% AIDS	—	—	40.9	52.5	.31
% on antiretroviral medications	—	—	65.2	57.5	.43
Median plasma viral load (log_{10} copies/ml)	—	—	3.04 (2.60–6.00)	2.62 (2.60–6.30)	.66
Median CSF viral load (log_{10} copies/m)[d]	—	—	1.70 (1.70–4.65)	2.60 (1.70–4.69)	.65
% detectable plasma viral load	—	—	59.4	52.5	.49
% detectable CSF viral load[d]	—	—	48.8	42.9	.60
Median days abstinent	—	92 (21–365)	—	122 (21–976)	.61
Median lifetime METH use (g)	—	3395 (40–16902)	—	1891 (8–14070)	.01[c]
Median daily METH use (g)	—	0.75 (0.06–2.66)	—	0.68 (0.06–2.25)	.40
Median duration of METH use (years)	—	11.57 (1.58–25.97)	—	8.30 (0.23–31.23)	.01[c]

Note. Medians and ranges are presented for measures with non-normal distributions. [a]METH+ < METH−; [b]HIV+ < HIV−; [c]HIV−/METH+ >HIV+/METH+; [d]HIV+/METH− $n=43$, HIV+/METH+ $n=35$.

their lifetime (e.g., 5 years of continuous cocaine dependence) or abuse of a substance other than methamphetamine within 1 year of the evaluation. Participants were not excluded for history of alcohol or marijuana abuse or past marijuana dependence, given the frequency of these comorbid diagnoses in methamphetamine dependent individuals. The four participant groups were compared on differences between rates of lifetime dependence on alcohol, marijuana, cocaine, opiates, and hallucinogens. Compared to the METH− groups, the two METH+ groups evidenced more lifetime dependence on alcohol ($P < .0001$) and cocaine ($P = .004$). The two METH+ groups did not, however, differ from each other on rates of other substance dependence. There were no significant ($P > .10$) differences between the groups on the rest of the substances examined.

Participants were excluded if they had a history of kidney, lung, heart, or autoimmune disorder whose systemic manifestations might independently influence brain metabolite concentrations. Patients with HIV-associated dementia, based on criteria established by the American Academy of Neurology, were excluded. Participants were also excluded for head injury with loss of consciousness greater than 30 min, penetrating skull wound, brain surgery, seizure disorder, and cerebral palsy. HIV+ participants had no history of central nervous system (CNS) opportunistic infection based on self-report, which was confirmed with magnetic resonance imaging (MRI).

Each participant received a comprehensive medical examination. The assessment included a neuromedical history, review of systems, neurological and general physical examinations, brain MRI, blood sampling, and in most cases lumbar puncture for CSF analysis. A neuroradiologist evaluated brain MRIs to rule out neurological complications and opportunistic brain disease that may complicate interpretation of spectroscopic data. No participant had to be excluded on this basis.

MRS

Spectra were collected using a clinical, General Electric (Fremont, California) 1.5-Tesla scanner at the VA San Diego Healthcare System. Regions of interest (ROIs) included right frontal lobe white matter ($20 \times 20 \times 20$ mm), midline frontal lobe gray matter ($20 \times 20 \times 20$ mm), and a basal ganglia region that included the head of the right caudate nucleus ($15 \times 15 \times 15$ mm) as depicted in Figure 2. Point-resolved spectroscopy (PRESS) with an echo time of 35 ms and repetition time of 3000 ms provided reliable measures of NAA, choline-containing compounds, creatine + phosphocreatine, and *myo*-inositol. Sixty-four acquisitions were averaged for each frontal lobe ROI and 96 acquisitions were averaged for the basal ganglia ROI. Water suppression was achieved by three successive radiofrequency pulses of fixed length followed by crusher gradients (CHESS) and optimized transmitter gain. Spectra were autoshimmed.

Spectral analysis was performed as reported previously (Schweinsburg *et al*, 2001), using LCModel version 5.2-1 (Provencher, 1993). LCModel analyzes *in vivo* spectra as a linear combination of a basis set of complete model spectra of metabolites *in vitro* (Provencher, 1993). Free-induction decays (FIDs) were zero-filled to double the points and filtered with a finite discrete convolution to account for field inhomogeneities and eddy currents. FIDs were automatically zero- and first-order phase corrected. A representative spectrum is presented in Figure 3. All MR spectra were evaluated for quality before being entered into statistical analyses based on visual

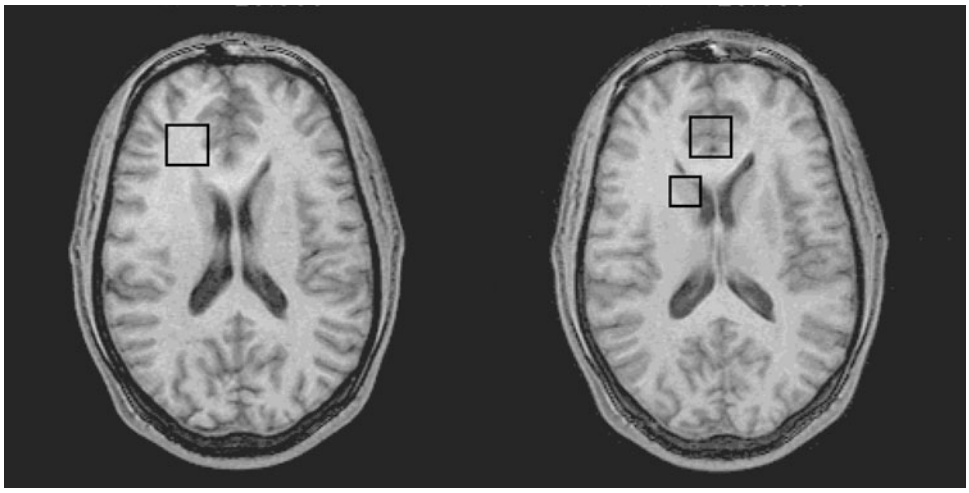

Figure 2 Representative localization of frontal white matter and frontal gray matter, and basal ganglia regions of interest.

inspection and output from LC Model. Specifically, for each metabolite in the spectrum, the data were excluded if the Cramer-Rao lower bound estimate was greater than 20. Also, the data for any particular spectrum were excluded if the peak width (full width at half maximum) was greater than 0.1 ppm. These criteria resulted in the exclusion of 2.3% of the data points for frontal white matter, 0.1% for frontal gray matter, and 10.5% in the basal ganglia. The most commonly excluded metabolite measure was MI, given its proximity to the peak for water, especially in the basal ganglia region which is the least homogeneous ROI (i.e., includes the most CSF).

Absolute concentrations were obtained by scaling the *in vivo* spectrum to the unsuppressed water peak (Soher *et al*, 1996). Metabolites were corrected for CSF content in the ROI using the algorithm employed by Schweinsburg and colleagues (Schweinsburg *et al*, 2000). This method uses a fast spin echo sequence to measure CSF in the ROI using a heavily T2-weighted imaging sequence (TE = 512 ms, TR = 9999 ms, ET = 16, FOV = 24 cm, slice thickness = 5 mm

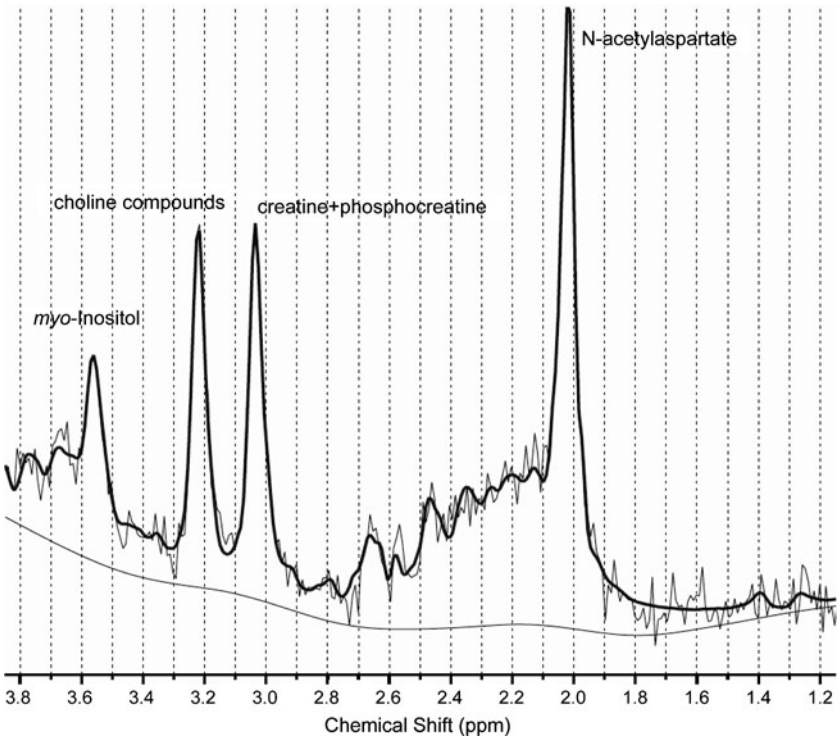

Figure 3 Representative proton MRS spectrum from a frontal white matter region of interest. The bold line represents the LCModel fit for the underlying raw data and the smooth line is the fitted baseline.

interleaved). High-intensity signal represents bulk water and can be used to determine fraction CSF (fCSF) in the spectroscopic voxel. The pure CSF signal is determined semi-automatically using a histogram obtained from the lateral ventricles. The calculation of fCSF in any ROI then becomes a fraction of the integrated intensity relative to that of pure CSF signal adjusted for volume of the prescribed ROI. Based on these methods, the groups showed comparable amounts of CSF in the frontal white matter ($F(3,\ 204) = 1.96,\ P = .12$), frontal gray matter ($F(3,\ 204) = 0.15,\ P = .93$), and basal ganglia ($F(3,\ 201) = 1.45,\ P = .23$) regions of interest. LCModel metabolite concentrations were then linearly interpolated based on the proportion of brain tissue within the ROI (McLean *et al*, 2000). This correction controls for potential between group differences in the proportion of CSF in each region of interest.

Statistical analyses were performed using JMP (SAS Institute, Inc., Cary, NC) and SPSS (SPSS, Chicago, IL). Metabolite concentrations in each region of interest were used as separate dependent variables in a series of 2×2 (HIV status $\times$ methamphetamine status) ANOVAs. Pearson Product-Moment correlations were used to evaluate the relationship between medical variables and metabolite concentrations.

Appendix

The San Diego HIV Neurobehavioral Research Center (HNRC) group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego, and the Veterans Affairs San Diego Healthcare System, and includes Director: Igor Grant, M.D.; Co-Directors: J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., and J. Allen McCutchan, M.D.; Center Manager: Thomas D. Marcotte, Ph.D.; Naval Hospital San Diego: Braden R. Hale, M.D., M.P.H. (P.I.); Neuromedical Component: Ronald J. Ellis, M.D., Ph.D. (P.I.), J. Allen McCutchan, M.D., Scott Letendre, M.D., Edmund Capparelli, Pharm.D., Rachel Schrier, Ph.D.; Neurobehavioral Component: Robert K. Heaton, Ph.D. (P.I.), Mariana Cherner, Ph.D., Steven Paul Woods, Psy.D.; Neuroimaging Component: Terry Jernigan, Ph.D. (P.I.), Christine Fennema-Notestine, Ph.D., Sarah L., Archibald, M.A., John Hesselink, M.D., Jacopo Annese, Ph.D., Michael J. Taylor, Ph.D., Brian Schweinsburg, Ph.D.; Neurobiology Component: Eliezer Masliah, M.D. (P.I.), Ian Everall, FRCPsych., FRCPath., Ph.D., T. Dianne Langford, Ph.D.; Neurovirology Component: Douglas Richman, M.D. (P.I.), David M. Smith, M.D.; International Component: J. Allen McCutchan, M.D. (P.I.); Developmental Component: Ian Everall, FRCPsych., FRCPath., Ph.D (P.I.), Stuart Lipton, M.D., Ph.D.; Clinical Trials Component: J. Allen McCutchan, M.D., J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., Scott Letendre, M.D.; Participant Accrual and Retention Unit: J. Hampton Atkinson, M.D. (P.I.), Rodney von Jaeger, M.P.H.; Data Management Unit: Anthony C. Gamst, Ph.D. (P.I.), Clint Cushman, B.A. (Data Systems Manager), Michelle Frybarger, B.A., Daniel R. Masys, M.D. (Senior Consultant); Statistics Unit: Ian Abramson, Ph.D. (P.I.), Christopher Ake, Ph.D., Deborah Lazzaretto, M.S.

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