

UC San Diego

UC San Diego Previously Published Works

Title

Elevated cerebrospinal fluid quinolinic acid levels are associated with region-specific cerebral volume loss in HIV infection

Permalink

<https://escholarship.org/uc/item/2qn7k7z5>

Journal

Brain, 124(5)

ISSN

0006-8950

Authors

Heyes, MP

Ellis, RJ

Ryan, L

et al.

Publication Date

2001-05-01

DOI

10.1093/brain/124.5.1033

Peer reviewed

Elevated cerebrospinal fluid quinolinic acid levels are associated with region-specific cerebral volume loss in HIV infection

Melvyn P. Heyes,¹ Ronald J. Ellis,^{2,3} Lee Ryan,⁵ Meredith E. Childers,^{2,3} Igor Grant,^{2,3} Tanya Wolfson,⁶ Sarah Archibald,^{3,4} Terry L. Jernigan⁶ and the HNRC Group*

¹Laboratory of Neurotoxicology, National Institute of Mental Health, Bethesda, Maryland, Departments of ²Neurosciences and ³Psychiatry and ⁴HIV Neurobehavioral Research Center, Brain Image Analysis Laboratory, University of California San Diego, San Diego, California and Departments of ⁵Psychology and ⁶Psychiatry, University of Arizona, Tucson, Arizona, USA

Correspondence to: Ronald Ellis, HIV Neurobehavioral Research Center (HNRC), University of California, San Diego, 150 West Washington Street, 2nd Floor, San Diego, CA 92103, USA
E-mail: roellis@ucsd.edu

Summary

Neuronal injury, dendritic loss and brain atrophy are frequent complications of infection with human immunodeficiency virus (HIV) type 1. Activated brain macrophages and microglia can release quinolinic acid, a neurotoxin and NMDA (*N*-methyl-D-aspartate) receptor agonist, which we hypothesize contributes to neuronal injury and cerebral volume loss. In the present cross-sectional study of 94 HIV-1-infected patients, elevated CSF quinolinic acid concentrations correlated with worsening brain atrophy, quantified by MRI, in regions vulnerable to excitotoxic injury (the striatum and limbic cortex) but not in regions relatively resistant to excitotoxicity (the

non-limbic cortex, thalamus and white matter). Increased CSF quinolinic acid concentrations also correlated with higher CSF HIV-1 RNA levels. In support of the specificity of these associations, blood levels of quinolinic acid were unrelated to striatal and limbic volumes, and CSF levels of β_2 -microglobulin, a non-specific and non-excitotoxic marker of immune activation, were unrelated to regional brain volume loss. These results are consistent with the hypothesis that quinolinic acid accumulation in brain tissue contributes to atrophy in vulnerable brain regions in HIV infection and that virus replication is a significant driver of local quinolinic acid biosynthesis.

Keywords: excitotoxin; neurodegeneration; magnetic resonance imaging; human immunodeficiency virus (HIV)

Abbreviations: CDC = Center for Disease Control; HIV-1 = human immunodeficiency virus type 1; HNRC = HIV Neurobehavioral Research Center, San Diego

Introduction

In vivo brain imaging studies have demonstrated enlargement of cortical sulcal and ventricular fluid spaces and volume loss in the striatum, temporal limbic cortex, white matter, anterior diencephalon and non-limbic cerebral cortex, as well

as white matter abnormalities in patients infected with the human immunodeficiency virus type 1 (HIV-1) (Post *et al.*, 1988; Chrysiopoulos *et al.*, 1990; Kieburtz *et al.*, 1990; Dooneief *et al.*, 1992; Jernigan *et al.*, 1993). Post-mortem

*The San Diego HIV Neurobehavioral Research Center Group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego and the San Diego Veterans Affairs Healthcare System, and includes: Director, Igor Grant; Co-Directors, J. Hampton Atkinson, J. Allen McCutchan; Center Manager, Thomas D. Marcotte; Naval Hospital San Diego, Mark R. Wallace; Neuromedical Component, J. Allen McCutchan, Ronald J. Ellis, Scott Letendre, Rachel Schrier; Neurobehavioral Component, Robert K. Heaton, Mariana Cherner, Julie Rippeth; Imaging Component, Terry Jernigan, John Hesselink; Neuropathology Component, Eliezer Masliah; Clinical Trials Component, J. Allen McCutchan, Ronald J. Ellis, Scott Letendre, J. Hampton Atkinson; Data Management Unit, Daniel R. Masys, Michelle Frybarger (Data Systems Manager); Statistics Unit, Ian Abramson, Reena Deutsch, Tanya Wolfson.

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, the Department of Defense or the United States Government.

studies have demonstrated dendritic and synaptic abnormalities that include substantial decreases in the complexity of dendritic arborization and loss of synapses (Masliah *et al.*, 1992a, b; Kerr *et al.*, 1998). Dendritic and synaptic damage in HIV infection are due, at least in part, to excitotoxic injury from excessive glutamate receptor stimulation, as demonstrated in the post-mortem brain (Masliah *et al.*, 1996; Epstein and Gelbard, 1999; Sardar *et al.*, 1999). Such loss of dendritic complexity as well as neurodegeneration probably contributes to brain volume loss. Furthermore, dendritic simplification has been related to the severity of cognitive disturbances (Masliah *et al.*, 1997). Volume reduction in the striatum and cerebral cortex are associated with higher intensity of immunostaining for the HIV envelope protein gp41 (Heindel *et al.*, 1994). Collectively, these observations suggest that the presence of replicating virus within the CNS results in the anatomical neuronal pathology of HIV-associated neurological disease and is involved in the development of cognitive deficits.

Neuronal injury and cell loss result from toxic mechanisms that are initiated and maintained by productive HIV infection in the CNS. It has been hypothesized that neurotoxicity is mediated directly by virus-coded toxins or indirectly by factors produced by brain cells, or by both mechanisms. Putative virus-coded toxins include gp120, gp41 and Tat protein (Brenneman *et al.*, 1988; Adamson *et al.*, 1999; Nath *et al.*, 2000). Host-coded mediators include the NMDA (*N*-methyl-D-aspartate) receptor agonist quinolinic acid, cytokines and nitric oxide (Heyes *et al.*, 1989; Tyor *et al.*, 1992; Adamson *et al.*, 1999; Chao *et al.*, 2000). The observations that HIV in the CNS is predominantly localized in monocyte/macrophages and microglia and that macrophage-tropic isolates are associated with neurological disease to a greater extent than T-cell-tropic isolates (Koyanagi *et al.*, 1987) are consistent with the hypothesis that the production of neurotoxins from macrophages and microglia is an important component of neuropathogenesis (Heyes *et al.*, 1989, 1991a, 1993; Giulian *et al.*, 1990; Pulliam *et al.*, 1991; Brew *et al.*, 1995).

Quinolinic acid is an endogenous neurotoxic metabolite of the tryptophan–kynurenine pathway and is produced in large quantities by activated macrophages and microglia (Heyes *et al.*, 1992c, 1996; Espey *et al.*, 1997; Kerr *et al.*, 1997; Chao *et al.*, 2000). Quinolinic acid activates the NMDA class of excitatory amino acid receptors to produce alterations in neuronal activity, neuronal damage and neurodegeneration (Stone, 1993). Some brain regions and neuronal populations are more vulnerable to the neurotoxic effect of quinolinic acid than others (Stone, 1993). For example, infusions of nanomolar amounts of quinolinic acid locally into rat brain resulted in neuronal losses that were greatest in the striatum and hippocampus and less marked in the lateral septal nuclei, substantia nigra, hypothalamus, cerebellum and non-limbic cerebral cortex (Schwarcz and Köhler, 1983). Studies of HIV-infected patients and SIV (simian immunodeficiency

virus)-infected macaques have established that concentrations of quinolinic acid are elevated in the CSF, brain tissue and blood (Heyes *et al.*, 1989, 1991a, b; Achim *et al.*, 1993; Brouwers *et al.*, 1993; Gulevich *et al.*, 1993; Sei *et al.*, 1995). Although the CSF compartment is distinct from that of brain tissue, the two compartments are nevertheless contiguous and CSF quinolinic acid levels have been shown to be highly representative of parenchymal values in terminal patients (Heyes *et al.*, 1998). Elevations in CSF quinolinic acid begin promptly after infection in association with virus entry into the CNS (Heyes *et al.*, 1992a; Smith *et al.*, 1995; Lane *et al.*, 1996) and correlate with the severity of perceptual–motor slowing in the early stages of infection (Martin *et al.*, 1992). High CSF quinolinic acid concentrations are found in patients with the AIDS (acquired immunodeficiency syndrome) dementia complex and encephalitis, and, importantly, correlate with the severity of neurological dysfunction in adults as well as measures of neurological developmental delays in children (Heyes *et al.*, 1991a, c, 1998; Achim *et al.*, 1993; Brouwers *et al.*, 1993; Gulevich *et al.*, 1993; Sei *et al.*, 1995). It is important to note that, whereas a small percentage of quinolinic acid in CSF and brain tissue can originate in blood, local tissue production is the predominant source during CNS inflammation and encephalitis (Heyes and Morrison, 1997; Heyes *et al.*, 1998). Attenuation of quinolinic acid biosynthesis by inhibition of kynurenine pathway enzymes in macrophages and microglia is associated with reduced HIV-induced neurotoxicity in susceptible neurones (Kerr *et al.*, 1997; Chao *et al.*, 2000).

We hypothesize that accumulations of quinolinic acid are involved in the pathogenesis of neuronal injury and brain atrophy in HIV-1-infected patients. The relationship of CSF quinolinic acid to measures of *in vivo* brain atrophy, however, has not been described. The rationale for the present study of the relationships between CSF quinolinic acid concentrations and regional brain atrophy is as follows. First, if quinolinic acid contributes to region-specific neuronal damage in HIV infection, then CSF quinolinic acid levels should correlate with the degree of volume loss, as measured by quantitative MRI, particularly in vulnerable structures—the striatum and limbic cortex—but may or may not correlate in brain regions that are less sensitive to the neurotoxic effects of quinolinic acid (the thalamus, non-limbic cortex and white matter). Secondly, in contrast to CSF quinolinic acid, serum quinolinic acid concentrations would not be related to volume loss in specific brain regions because the CNS is the predominant source of local quinolinic acid accumulations, and the two compartments may be metabolically independent. Thirdly, quinolinic acid production is probably stimulated both by HIV-1 replication and by non-specific ‘immune activation’ in the CNS. We evaluated the relative roles of these stimuli by examining the correlation between CSF quinolinic acid levels and levels of CSF HIV RNA and b₂-microglobulin, a non-specific marker of activation of microglia and other CNS immune cells.

Methods

Participants

Ninety-four HIV-infected men enrolled at the San Diego HIV Neurobehavioral Research Center (HNRC) were selected on the basis of availability of complete quantified neuroimaging data and contemporaneous CSF samples. A repeatedly positive ELISA (enzyme-linked immunosorbent assay) and a confirmatory test for HIV-1 antibody established HIV infection. Participants had no history of intravenous drug use, and infection occurred through sexual exposure. The group had a mean (\pm SD) age of 34 ± 7 years (range 22–50 years) and a mean education level of 14 ± 2 years (range 10–20 years). Following an explanation of all procedures, informed consent was obtained from each participant prior to his participation.

Details concerning the sources of participants and exclusion criteria have been described previously (Heaton *et al.*, 1995). Exclusion criteria included conditions that might increase the likelihood of finding neuroradiological abnormalities unrelated to HIV, such as head injury complicated by prolonged loss of consciousness; a history of a neurological disorder such as epilepsy or encephalitis, metabolic or endocrine diseases; cardiopulmonary disorders; current dependence on alcohol or other substances; or any diagnosis of psychosis prior to HIV infection. Also excluded were patients with a clinical diagnosis of opportunistic disease of the brain, such as cryptococcal meningitis, progressive multifocal leucoencephalopathy or toxoplasmosis.

Clinical evaluations and CSF assays

Medical examinations and collections of blood and CSF were performed within a period of 11 weeks. The average time between an MRI examination and lumbar puncture was 15 days; 50% of the sample was examined within 1 week of the MRI, 75% within 3 weeks and the remaining 25% between 3 and 11 weeks. Lumbar puncture was performed according to standard techniques, with 5–15 ml of CSF collected. Samples were aliquoted and stored at -70°C and subsequently assayed in batches for β_2 -microglobulin by enzyme immunoassay (Pharmacia Diagnostics, Fairfield, NJ, USA). β_2 -Microglobulin is associated with class I MHC antigens on cell membranes, and is important both for the immunological recognition of self and for mounting cellular immune responses (Flynn *et al.*, 1992; Glas *et al.*, 1992). β_2 -Microglobulin elevation in CSF increases in concert with HIV disease progression (Lukey *et al.*, 1991; Gulevich *et al.*, 1993). Unlike quinolinic acid, β_2 -microglobulin has no known neurotoxic effects. Quinolinic acid was quantified by electron-capture negative chemical ionization mass spectrometry with [$^2\text{H}_3$]quinolinic acid as internal standard (Heyes *et al.*, 1992c, 1998). Test–retest reliability of the CSF quinolinic acid measurement was determined by repeating the assay on different aliquots from the same lumbar puncture for 19 participants chosen at random from the present sample.

Quinolinic acid concentrations measured from the two samples yielded a Pearson r value of 0.99 ($P < 0.001$).

Imaging protocol

MRI was performed with a 1.5 tesla superconducting magnet (Signa; General Electric, Milwaukee, Wis., USA) at the UCSD/AMI Magnetic Resonance Institute. Two spatially registered images were obtained simultaneously for each section, using an asymmetrical, multiple-echo sequence [TR (repetition time) = 2000 ms, TE (echo time) = 25, 70 ms, 256×256 matrix, FOV (field of view) = 24 cm]. Section thickness was 5 mm with a 2.5 mm gap in the axial plane.

Details of the image analysis methods have been described previously (Jernigan *et al.*, 1991c, 1993). Briefly, all image analyses were conducted blind to any subject characteristics. Each pixel location within a section of the imaged brain was classified on the basis of its signal values in both original images (TE = 25 ms, TE = 70 ms) as most resembling CSF, grey matter, white matter or signal hyperintensity (tissue abnormality). Consistently identifiable landmark positions and structural boundaries were then designated on the segmented images by trained image analysts. The processed image data were transformed spatially to conform to a common anatomical coordinate system. Some regional boundaries were then defined relative to this coordinate scheme (i.e. stereotaxically). The volume for each structure was estimated by summing the voxels in all images that included the designated structure.

Quantitation of brain regional volumes

The MRI volume measures were expressed as Z scores corrected for the normal variation in volume associated with age and cranial size (Jernigan *et al.*, 1991c). Adjustments were based on analyses conducted previously with a large group of normal controls that estimated the polynomial functions relating supratentorial cranium size and age to the structural volumes. The adjusted structure volumes were expressed as deviations from the normative predicted values divided by age-adjusted standard deviations, producing measures that were independent of brain size variation and age differences, and represent the degree to which a structure volume deviates from the expected size in the normal population. Since the volume measures represent estimated Z scores, they are expressed on roughly equivalent scales, allowing direct comparisons between structures to be made. These age- and cranium size-corrected scores have been shown to be useful in detecting region-specific brain changes associated with Alzheimer's dementia, Huntington's disease, HIV encephalitis and alcoholism (Jernigan *et al.*, 1991a, b, 1993).

The total brain, excluding the cerebellum and pons, was measured in five regions: the striatum, the thalamus, the limbic cortex, the non-limbic cortex and the white matter. The thalamus consisted of a single standardized volume

Table 1 Medical characteristics and CSF and serum parameters of study participants grouped according to CDC disease stage

	All (<i>n</i> = 94)	CDC disease stage			<i>P</i> value (ANOVA)
		A (<i>n</i> = 52)	B (<i>n</i> = 25)	C (<i>n</i> = 17)	
CD4 ⁺ lymphocytes (cells/ml)	415 (35)	548 (244)	388 (244)	117 (181)	0.0001
tCSF HIV RNA (log ₁₀ copies/ml)	2.3 (1.5)	2.1 (1.5)	2.9 (1.5)	2.3 (1.3)	0.138
CSF β ₂ -microglobulin (μg/ml)	1.5 (1.0)	1.1 (0.53)	1.6 (1.1)	2.4 (1.4)	0.0001
Receiving antiretrovirals [no. (%)]	50 (53)	19 (37)	21 (84)	10 (56)	0.0005
CSF quinolinic acid (nM)	58.7 (67.9)	49 (54)	78 (97)	60 (49)	0.21
Serum quinolinic acid (nM)	1358 (939)	1251 (646)	1427 (971)	1881 (1559)	0.07

The ANOVAs compare values across the three CDC groups. Values are mean (SD) unless stated otherwise.

separated from the hypothalamic region on the basis of a stereotaxically defined coronal plane. The limbic cortex included structures that constitute the septohippocampal system (Gray and McNaughton, 1983) and was calculated as the average of two standardized volumes, the mesial temporal lobe (the uncus, amygdala, hippocampus and parahippocampal gyrus) and the anterior diencephalic region (the hypothalamus and the septal nuclei). The striatum volume comprised the combined volumes of the caudate nucleus, putamen and globus pallidus. The non-limbic cortex region comprised a single standardized volume of all cortical grey matter other than the limbic cortex as described above, including the frontal, lateral temporal, parietal and occipital lobes. The total volume of white matter included any regions of white matter hyperintensity. The volume of the supratentorial cranium was estimated by summing supratentorial voxels (including CSF, signal hyperintensities, grey matter and white matter) over all sections.

Statistical analyses

Because CSF quinolinic acid, serum quinolinic acid and CSF β₂-microglobulin values were positively skewed in this cohort (Table 1), as in others described previously, raw values were transformed on a log₁₀ scale. A Shapiro–Wilk test confirmed that the distribution of the new values did not deviate from normality. Log₁₀-transformed values were used in all analyses reported here.

To evaluate whether regional brain volume losses reflected overall disease progression or degree of immunosuppression, the relationships between volume loss and CDC (Centers for Disease Control) (1993) clinical class (A, B or C; A = least advanced, C = most advanced) were examined. CDC clinical class was analysed as a marker of disease severity with respect to a history of opportunistic diseases that reflect impaired cellular immunity. This was particularly important because regional brain volumes have been shown to decrease with advancing stage of disease (Jernigan *et al.*, 1993).

Bivariate correlation coefficients were calculated as Pearson *r* values. Multiple regression analyses were conducted to examine regional brain volume loss and the

following predictor variables: CSF quinolinic acid concentration, β₂-microglobulin concentration and disease severity (A, B or C). Correlation coefficients were also employed to assess the relationship between CSF quinolinic acid and CSF HIV RNA levels, as well as CSF quinolinic acid and CSF β₂-microglobulin concentrations. Values presented are mean ± standard deviation (SD) or standard error of the mean (SEM), as indicated.

Results

Clinical characteristics and measurements of quinolinic acid and β₂-microglobulin in CSF and serum

The distribution of participants in the CDC clinical categories was as follows: Category A, 55% (*n* = 52); Category B, 26% (*n* = 25); Category C, 19% (*n* = 17). Thirty participants (32%) met the 1993 criteria for AIDS (i.e. individuals in Category C or with <200 CD4 T cells/μl). Table 1 provides data on CD4 T-cell counts, antiretroviral treatment status, CSF and serum quinolinic acid concentrations and CSF β₂-microglobulin concentrations for the participants at the time of collection, grouped according to CDC disease stage.

In the cohort as a whole, participants had substantially elevated CSF quinolinic acid levels, with a range of 5.7–485.0 nM (mean ± SD, 58.7 ± 67.9 nM) at the time of their MRI scan. A previous study found that CSF quinolinic acid levels among HIV seronegative subjects of comparable ages ranged from 11.6 to 40.3 nM (18.4 ± 3.4 nM) (Heyes *et al.*, 1989) and another found that seronegative subjects' CSF quinolinic acid levels ranged from 15 to 35 nM (22.1 ± 2.1 nM) (Heyes *et al.*, 1998). CSF quinolinic acid concentrations did not differ significantly among participants in CDC stages A, B and C. Serum quinolinic acid levels in the present cohort of HIV⁺ subjects (mean ± SD, 1358 ± 939 nM) exceeded those previously found in HIV⁻ controls of similar age (416 ± 122 nM) (Halperin and Heyes, 1992). However, among HIV⁺ subjects, the three CDC groups did not differ significantly from one another. The correlation between CSF and serum quinolinic acid concentra-

Table 2 Regional brain volume losses measured on MRI

	All (n = 94)	CDC disease stage			P value (ANOVA)
		A (n = 52)	B (n = 25)	C (n = 17)	
Striatum	-0.74 (0.85)	-0.67 (0.75)	-0.51 (0.84)	-1.25 (0.99)	0.02
Limbic cortex	-0.38 (1.16)	-0.26 (1.10)	-0.79 (0.97)	-0.13 (1.46)	0.11
Non-limbic cortex	-0.38 (1.27)	-0.26 (1.07)	-0.42 (1.38)	-0.66 (1.63)	0.52
Thalamus	-0.83 (1.17)	-0.85 (.91)	-0.71 (1.44)	-0.97 (1.44)	0.76
Subcortical white matter	-0.04 (1.32)	0.15 (1.08)	0.03 (1.48)	-0.68 (1.56)	0.07

Values are Z scores with an expected mean value in the control population of 0 and SD of 1. The ANOVAs compare values across the three CDC groups.

Table 3 Pearson correlation coefficients (r) examining the relationships of regional MR brain volumes with levels of CSF quinolinic acid (QUIN), serum QUIN and CSF β_2 -microglobulin

Brain region	CSF QUIN		Serum QUIN*		CSF β_2 -microglobulin	
	r	P	r	P	r	P
Striatum	-0.22	0.03	-0.16	0.16	0.14	0.19
Limbic cortex	-0.24	0.02	-0.03	0.82	-0.02	0.69
Non-limbic cortex	0.14	0.19	-0.07	0.57	0.01	0.90
Thalamus	-0.11	0.30	-0.12	0.32	0.01	0.90
White matter	-0.06	0.54	-0.02	0.86	0.11	0.14

*Values not available for 22 subjects.

tions in the present group was +0.42 ($P < 0.001$). The mean CSF β_2 -microglobulin concentration was elevated compared with normal values (Gulevich *et al.*, 1993) and increased progressively according to CDC stage. The mean level of CSF HIV RNA in this group was $2.3 \pm 1.5 \log_{10}$ copies per ml; 25% of samples had undetectable CSF HIV RNA levels.

MRI analyses and prediction of regional brain volumes

Table 2 lists regional brain volumes according to the subjects' CDC disease stage classification. Regional volumes in the present cohort as a whole were decreased relative to previously studied normative samples (Jernigan *et al.*, 1993; Heindel *et al.*, 1994). As expected, more pronounced volume loss was observed in the striatum and white matter of subjects with advanced clinical disease (CDC class C).

Correlations between MRI volumes, CSF and serum quinolinic acid concentrations and CSF β_2 -microglobulin concentrations are listed in Table 3. CSF quinolinic acid levels were correlated with the degree of volume loss in both the striatum ($r = -0.22$, $P = 0.03$) and the limbic cortex ($r = -0.24$, $P = 0.02$) (Fig. 1). Notably, in a multiple regression analysis in which the volume of the striatum was the outcome variable and CSF quinolinic acid and CDC classification (AIDS/CDC C versus non-AIDS/CDC A + B) were predictor variables, CSF quinolinic acid levels (partial $F = 5.5$, $P = 0.021$) and CDC classification (partial $F = 5.1$, $P = 0.026$) made independent contributions to predicting

the volume of the striatum. In a parallel analysis for limbic volume, CSF quinolinic acid (partial $F = 5.7$, $P = 0.02$) but not CDC classification (partial $F = 1.2$, $P = 0.27$) contributed significantly to the model. Tests for interactions were performed by splitting CSF quinolinic acid levels at the median, creating high and low CSF quinolinic acid categories and performing an ANOVA examining three predictors: CSF quinolinic acid (categorical), CDC class and the interaction term CSF quinolinic acid \times CDC class. In both cases, the interaction terms were not significant. Thus, correlations between CSF quinolinic acid and striatal and limbic atrophy could not be explained simply by disease stage. CSF quinolinic acid levels were not significantly related to volumes in the non-limbic cortex, thalamus or white matter. Serum quinolinic acid concentrations did not correlate significantly with volume loss in any brain region.

CSF HIV RNA levels were not significantly related to volume loss in any of the brain regions. A significant correlation was found, however, between HIV RNA CSF concentrations and CSF quinolinic acid levels ($r = 0.37$, $P < 0.002$).

Levels of CSF β_2 -microglobulin, a marker of CNS immune activation, were not related to volume loss in any region. There was a significant correlation between CSF quinolinic acid levels and CSF β_2 -microglobulin ($r = 0.39$, $P < 0.0001$). Multiple regression analysis showed that β_2 -microglobulin and CSF viral RNA made independent contributions to the prediction of CSF quinolinic acid levels ($F = 6.9$, $P < 0.01$ and $F = 6.4$, $P < 0.01$, respectively).

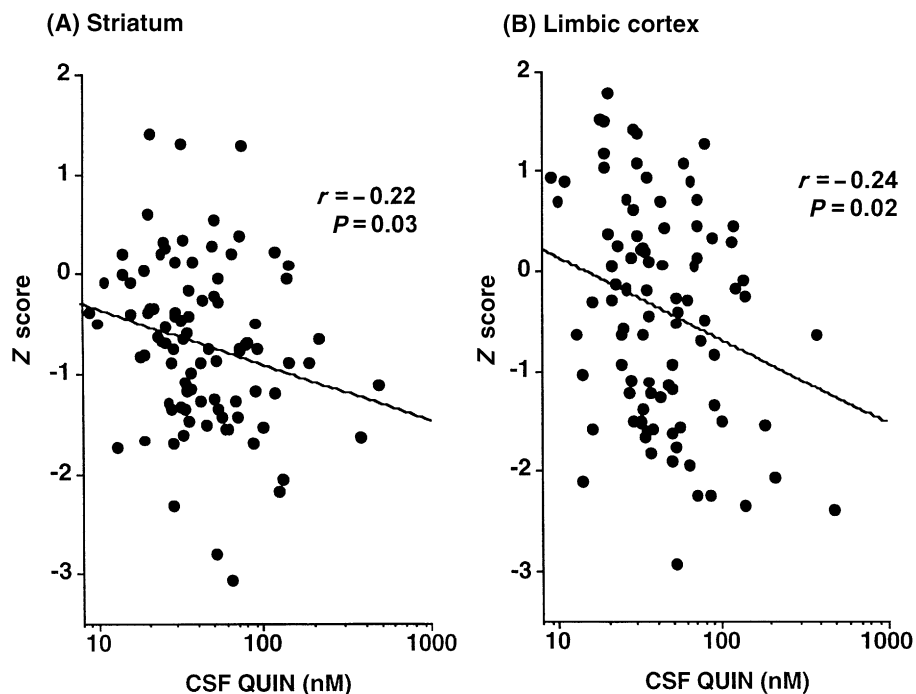


Fig. 1 Scatter plots depicting the relationships between CSF quinolinic acid (QUIN) concentration (log-transformed values) and the MRI volumetric measurement of the striatum (A) and the limbic cortex (B) for 94 HIV⁺ individuals.

Discussion

MRI-assessed brain atrophy in HIV-infected patients is linked to productive HIV infection of the CNS, to both neuronal injury and neurodegeneration and to cognitive deficits (Masliah *et al.*, 1992a, b, 1997; Heindel *et al.*, 1994; Kerr *et al.*, 1998). There is currently considerable interest in the potential role of virus-coded and host-coded neurotoxins released from activated macrophages and microglia as mediators between the presence of HIV in the CNS and the occurrence of anatomical and functional neurological disease. Certain candidate mechanisms have been identified on the basis of *in vitro* neurotoxicity, although their presence within the CNS and associations with quantitative measures of functional and anatomical disease await confirmation. While the cause of CNS disease is probably complex, with several factors contributing to differing degrees at different stages of disease, the present study focused on a hypothesis-driven approach to evaluate the contribution of one potential candidate. The results showed that increased CSF quinolinic acid levels were associated with regional volume loss in brain structures that are particularly sensitive to the excitotoxic effects of quinolinic acid, namely the striatum and limbic cortex (Table 2). Because CSF quinolinic acid levels were associated with atrophy independent of CDC stage, and because serum quinolinic acid concentrations did not correlate with regional cerebral volume measures, it is unlikely that CSF quinolinic acid concentrations and brain atrophy simply reflect disease stage or a generalized systemic process. Furthermore, regional cerebral volume measures were not correlated with CSF concentrations of β_2 -

microglobulin. Because β_2 -microglobulin is a marker of microglial activation (Morris and Esiri, 1998; Lidman *et al.*, 1999) that does not interact with glutamate receptors, these findings suggest that the volume loss was not simply a consequence of non-specific immune activation, but rather was associated with excitotoxicity due to quinolinic acid.

In order for quinolinic acid to be a significant contributor to brain atrophy in HIV-infected patients, the following must be established: (i) that quinolinic acid levels are elevated prior to or coincident with the onset of atrophy in proportion to the severity of atrophy rather than occurring after or as a consequence of atrophy; (ii) that there is a link between productive HIV-1 infection and quinolinic acid production; (iii) that quinolinic acid concentrations are elevated within susceptible brain regions; (iv) that quinolinic acid concentrations at the synapse and at NMDA receptors achieve neuroactive levels; and (v) that elevated CSF quinolinic acid levels correlate with elevated quinolinic acid levels at the synapse.

A history of previously elevated CSF quinolinic acid levels in the present cohort of patients was not available. However, in other early stage HIV-infected patients, highly significant correlations have been observed between CSF quinolinic acid levels at a given time point with values measured 6, 12 or 18 months later (Heyes *et al.*, 1992a). We have now replicated this finding with a separate cohort from the HNRC (R. J. Ellis and M. P. Heyes, unpublished observation). Thus, a single measure of CSF quinolinic acid levels is an index of values over a prolonged period of time. The association between CSF quinolinic acid levels and the severity of

atrophy during the early stages of neurological disease indicates that elevations in CSF quinolinic acid levels occur either prior to or contemporaneously with the development of brain atrophy. The elevations in CSF quinolinic acid in the present study cannot, however, be attributed to or be a consequence of brain atrophy itself because patients with other conditions causing regional brain atrophy, such as Huntington's disease and Alzheimer's disease, do not have elevated CSF or brain parenchymal quinolinic acid concentrations (Heyes *et al.*, 1991b, 1992c; Krieger *et al.*, 1994; Mouradian *et al.*, 1989).

The correlation between CSF quinolinic acid levels and CSF HIV RNA levels supports the hypothesis that viral replication is a significant driving force in local quinolinic acid production. The observation that cultures of HIV-infected macrophages and microglia release large quantities of quinolinic acid, particularly with macrophage-tropic HIV strains and wild-type virus isolated from demented patients (Brew *et al.*, 1995; Kerr *et al.*, 1997; Chao *et al.*, 2000), is consistent with this hypothesis. The correlation between CSF quinolinic acid levels and CSF β_2 -microglobulin indicates that non-specific CNS immune activation is an additional stimulus. Such non-specific immune activation may occur as a result of HIV-1 replication as well as CNS damage (Blight *et al.*, 1995). In accordance with these interpretations, large accumulations of quinolinic acid in brain tissue have been reported in HIV-infected patients with encephalitis caused either by HIV or other inflammatory processes (Sei *et al.*, 1995). Interestingly, MRI atrophy did not correlate with either CSF viral RNA or β_2 -microglobulin concentrations (Table 3). Therefore, the correlation between CSF quinolinic acid levels and the degree of brain atrophy may reflect the position of quinolinic acid as a more proximal mediator of neuronal injury than HIV replication or non-specific immune activation. Furthermore, the specificity of the relationships between CSF quinolinic acid and brain atrophy in particularly vulnerable regions is emphasized by the finding that CSF quinolinic acid levels were not predictive of damage to the non-limbic cortex, thalamus or white matter, regions that have relatively low sensitivity to quinolinic acid neurotoxicity.

In post-mortem studies of HIV-infected subjects, elevated quinolinic acid concentrations are seen in CSF, basal ganglia, cerebral cortex and cortical white matter (Achim *et al.*, 1993; Heyes *et al.*, 1998). These increased concentrations were, on average, more than 100-fold greater than the concentrations found in neurologically normal controls and patients with Huntington's disease or Alzheimer's disease (Mouradian *et al.*, 1989; Heyes *et al.*, 1991b, 1992c; Krieger *et al.*, 1994). The concentrations in HIV-infected patients averaged $>20.0 \mu\text{M}$ (20.0 pmol/g) in brain tissue and $3.79 \mu\text{M}$ in CSF. Three independent studies of susceptible neurones in culture have found quinolinic acid-induced neurotoxicity in the range of 0.1–100 μM . Specifically, Schwarcz and colleagues reported dendritic injury and neurodegeneration when organotypic corticostriatal neurones were exposed to 0.1–1.0 μM quinolinic acid for 7 weeks (Whetsell and

Schwarcz, 1989). Giulian and colleagues reported the death of rat hippocampus neurones following exposure to a concentration of quinolinic acid of 100 nM for 24 h (Giulian *et al.*, 1990). Brew and colleagues found degeneration of human cortical neurones in response to 0.5–1.2 μM quinolinic acid (Kerr *et al.*, 1997, 1998). Clearly, the quinolinic acid levels reached in post-mortem brain and CSF of HIV-infected patients exceed this neurotoxic threshold by a substantial margin.

Quinolinic acid causes neurotoxicity via activation of NMDA receptors localized on the neuronal cell membrane and accessed via the extracellular fluid space (Stone, 1993). It was not possible to measure quinolinic acid concentrations directly in the brain tissue or extracellular fluid space of the patients in the present study. However, CSF is anatomically contiguous with the extracellular fluid space and CSF quinolinic acid concentrations are proportional to those in the extracellular fluid space of the brain (Beagles *et al.*, 1998). In this context, highly significant correlations and a gradient in concentrations are found between quinolinic acid levels in brain tissue homogenates, the extracellular fluid and CSF in HIV-infected patients and in animal models of CNS-localized inflammation (Blight *et al.*, 1995; Heyes *et al.*, 1998). These observations demonstrate that locally synthesized quinolinic acid enters the extracellular fluid and eventually the CSF in a quantitative relationship (Beagles *et al.*, 1998). We interpret the elevations in CSF quinolinic acid in the present study as indicative of production and accumulation of quinolinic acid in brain tissue and entry into the extracellular fluid space, although the anatomical localization and magnitude of such increases could not be determined. Whereas the concentrations of quinolinic acid in CSF in the present study averaged $<100 \text{ nM}$ (Table 1) and were lower than those found in terminal disease (Heyes *et al.*, 1998), it has been shown previously that CSF quinolinic acid measurements underestimate parenchymal values in HIV and SIV infection by up to one order of magnitude (Heyes *et al.*, 1998). Also, local accumulations of quinolinic acid in the brain reflect local synthesis, probably by activated macrophages and microglia, where substantially larger concentrations than those predicted by CSF measures occur (Beagles *et al.*, 1998; Heyes *et al.*, 1998). An additional factor that facilitates excitotoxicity is the duration of exposure (Whetsell and Schwarcz, 1989; Heyes *et al.*, 1998; Kerr *et al.*, 1998). The available evidence is that increases in CNS quinolinic acid levels in HIV-1 infection begin promptly after infection and are sustained throughout the course of disease (Heyes *et al.*, 1991a, 1992a, c; Martin *et al.*, 1992; Brouwers *et al.*, 1993; Smith *et al.*, 1995; Lane *et al.*, 1996).

Three factors are of relevance with respect to atrophy of the basal ganglia and limbic cortex. First, these two regions are particularly sensitive to the excitotoxic effects of quinolinic acid (Stone, 1993). Other regions are less sensitive, and atrophy in areas such as the thalamus and cerebral cortex may also be related to additional neurotoxic mediators. Measurement of such factors was beyond the scope of the

present study, but their relationships to brain atrophy and neuropsychological deficits are worthy of investigation. Secondly, the basal ganglia are a preponderant target for HIV infiltration and volume loss as assessed by MRI (Heindel *et al.*, 1994; Stout *et al.*, 1998). Thirdly, dendritic and synaptic damage in HIV infection is due, at least in part, to excitotoxic injury from excessive glutamate receptor stimulation, as demonstrated in the post-mortem brain (Masliah *et al.*, 1996; Epstein and Gelbard, 1999; Sardar *et al.*, 1999). Because of our hypothesis that replicating HIV in brain parenchyma provides a significant driving force for local quinolinic acid production and accumulation, we predicted that the basal ganglia and limbic cortex would have particularly large elevations in local quinolinic acid levels in the early stages of disease.

Our findings have potential clinical implications. Elevated concentrations of CSF quinolinic acid may cause regional brain atrophy and neuronal damage to structures that mediate critical cognitive functions. Strategies that reduce CNS quinolinic acid biosynthesis and concentrations or decrease quinolinic acid-induced neurotoxicity in both early and late stages of the disease may attenuate neurological disease progression rates and restore or preserve cognitive capacity in HIV-1-infected individuals. Because quinolinic acid toxicity is mediated by the activation of NMDA-type postsynaptic receptors (Stone, 1993), therapeutic interventions could aim to reduce quinolinic acid accumulations in the brain by inhibiting anabolic enzymes (Blight *et al.*, 1995), blocking interactions between quinolinic acid and glutamate receptors or inhibiting events downstream in the neurotoxic cascade, including calcium influx (Dreyer *et al.*, 1990), glutathione depletion and oxidative damage. We also propose that measurements of CSF quinolinic acid might be a useful index of whether individual patients are likely to benefit from these targeted interventions.

Acknowledgements

The HIV Neurobehavioral Research Center (HNRC) is supported by Center award P50-MH 45294 from the National Institutes of Mental Health (NIMH). M.P.H. received support from the intramural programme at NIMH while this research was conducted.

References

- Achim CL, Heyes MP, Wiley CA. Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains. *J Clin Invest* 1993; 91: 2769–75.
- Adamson DC, Kopnisky KL, Dawson TM, Dawson VL. Mechanisms and structural determinants of HIV-1 coat protein, gp41-induced neurotoxicity. *J Neurosci* 1999; 19: 64–71.
- Beagles KE, Morrison PF, Heyes MP. Quinolinic acid in vivo synthesis rates, extracellular concentrations, and intercompartmental distributions in normal and immune-activated brain as determined by multiple-isotope microdialysis. *J Neurochem* 1998; 70: 281–91.
- Blight AR, Cohen TI, Saito K, Heyes MP. Quinolinic acid accumulation and functional deficits following experimental spinal cord injury. *Brain* 1995; 118: 735–52.
- Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, et al. Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 1988; 335: 639–42.
- Brew BJ, Corbeil J, Pemberton L, Evans L, Saito K, Penny R, et al. Quinolinic acid production is related to macrophage tropic isolates of HIV-1. *J Neurovirol* 1995; 1: 369–74.
- Brouwers P, Heyes MP, Moss HA, Wolters PL, Poplack DG, Markey SP, et al. Quinolinic acid in the cerebrospinal fluid of children with symptomatic human immunodeficiency virus type 1 disease: relationships to clinical status and therapeutic response. *J Infect Dis* 1993; 168: 1380–6.
- Chao CC, Hu S, Gekker G, Lokensgard JR, Heyes MP, Peterson PK. U50,488 protection against HIV-1-related neurotoxicity: involvement of quinolinic acid suppression. *Neuropharmacology* 2000; 39: 150–60.
- Chrysikopoulos HS, Press GA, Grafe MR, Hesselink JR, Wiley CA. Encephalitis caused by human immunodeficiency virus: CT and MR imaging manifestations with clinical and pathologic correlation. *Radiology* 1990; 175: 185–91.
- Dooneief G, Bello J, Todak G, Mun IK, Marder K, Malouf R, et al. A prospective controlled study of magnetic resonance imaging of the brain in gay men and parenteral drug users with human immunodeficiency virus infection. *Arch Neurol* 1992; 49: 38–43.
- Dreyer EB, Kaiser PK, Offermann JT, Lipton SA. HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science* 1990; 248: 364–7.
- Epstein LG, Gelbard HA. HIV-1-induced neuronal injury in the developing brain. [Review]. *J Leuk Biol* 1999; 65: 453–7.
- Espey MG, Chernyshev ON, Reinhard JF Jr, Namboodiri MA, Colton CA. Activated human microglia produce the excitotoxin quinolinic acid. *Neuroreport* 1997; 20: 431–4.
- Flynn JL, Goldstein MM, Triebold KJ, Koller B, Bloom BR. Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. *Proc Natl Acad Sci USA* 1992; 89: 12013–7.
- Giulian D, Vaca K, Noonan CA. Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* 1990; 250: 1593–6.
- Glas R, Franksson L, Ohlén C, Höglund P, Koller B, Ljunggren HG, et al. Major histocompatibility complex class I-specific and -restricted killing of beta 2-microglobulin-deficient cells by CD8+ cytotoxic T lymphocytes. *Proc Natl Acad Sci USA* 1992; 89: 11381–5.
- Gray JA, McNaughton N. Comparison between the behavioural effects of septal and hippocampal lesions: a review. [Review]. *Neurosci Biobehav Rev* 1983; 7: 119–88.
- Gulevich SJ, McCutchan JA, Thal LJ, Kirson D, Durand D, Wallace M, et al. Effect of antiretroviral therapy on the cerebrospinal fluid of patients seropositive for the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1993; 6: 1002–7.

- Halperin JJ, Heyes MP. Neuroactive kynurenines in Lyme borreliosis. *Neurology* 1992; 42: 43–50.
- Heaton RK, Grant I, Butters N, White DA, Kirson D, Atkinson JH, et al. The HNRC 500—neuropsychology of HIV infection at different disease stages. HIV Neurobehavioral Research Center. *J Int Neuropsychol Soc* 1995; 1: 231–51.
- Heindel WC, Jernigan TL, Archibald SL, Achim CL, Masliah E, Wiley CA. The relationship of quantitative brain magnetic resonance imaging measures to neuropathologic indexes of human immunodeficiency virus infection. *Arch Neurol* 1994; 51: 1129–35.
- Heyes MP, Morrison PF. Quantification of local de novo synthesis versus blood contributions to quinolinic acid concentrations in brain and systemic tissues. *J Neurochem* 1997; 68: 280–8.
- Heyes MP, Rubinow D, Lane C, Markey SP. Cerebrospinal fluid quinolinic acid concentrations are increased in acquired immune deficiency syndrome. *Ann Neurol* 1989; 26: 275–7.
- Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, Sidtis JJ, et al. Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. *Ann Neurol* 1991a; 29: 202–9.
- Heyes MP, Swartz KJ, Markey SP, Beal MF. Regional brain and cerebrospinal fluid quinolinic acid concentrations in Huntington's disease. *Neurosci Lett* 1991b; 122: 265–9.
- Heyes MP, Brew BJ, Saito K, Quearry BJ, Price RW, Lee K, et al. Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and beta 2-microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients. *J Neuroimmunol* 1992a; 40: 71–80.
- Heyes MP, Jordan EK, Lee K, Saito K, Frank JA, Snoy PJ, et al. Relationship of neurologic status in macaques infected with the simian immunodeficiency virus to cerebrospinal fluid quinolinic acid and kynurenic acid. *Brain Res* 1992b; 570: 237–50.
- Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain* 1992c; 115: 1249–73.
- Heyes MP, Saito K, Major EO, Milstien S, Markey SP, Vickers JH. A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. *Brain* 1993; 116: 1425–50.
- Heyes MP, Achim CL, Wiley CA, Major EO, Saito K, Markey SP. Human microglia convert L-tryptophan into the neurotoxin quinolinic acid. *Biochem J* 1996; 320: 595–7.
- Heyes MP, Saito K, Lackner A, Wiley CA, Achim CL, Markey SP. Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques. *FASEB J* 1998; 12: 881–96.
- Jernigan TL, Butters N, DiTraglia G, Schafer K, Smith T, Irwin M, et al. Reduced cerebral grey matter observed in alcoholics using magnetic resonance imaging. *Alcohol Clin Exp Res* 1991a; 15: 418–27.
- Jernigan TL, Salmon DP, Butters N, Hesselink JR. Cerebral structure on MRI, Part II: specific changes in Alzheimer's and Huntington's diseases. *Biol Psychiatry* 1991b; 29: 68–81.
- Jernigan TL, Archibald SL, Berhow MT, Sowell ER, Foster DS, Hesselink JR. Cerebral structure on MRI, Part I: localization of age-related changes. *Biol Psychiatry* 1991c; 29: 55–67.
- Jernigan TL, Archibald S, Hesselink JR, Atkinson JH, Velin RA, McCutchan JA, et al. Magnetic resonance imaging morphometric analysis of cerebral volume loss in human immunodeficiency virus infection. The HNRC Group. *Arch Neurol* 1993; 50: 250–5.
- Kerr SJ, Armati PJ, Pemberton LA, Smythe G, Tattam B, Brew BJ. Kynurenine pathway inhibition reduces neurotoxicity of HIV-1-infected macrophages. *Neurology* 1997; 49: 1671–81.
- Kerr SJ, Armati PJ, Guillemin GJ, Brew BJ. Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex. *AIDS* 1998; 12: 355–63.
- Kiebertz KD, Ketonen L, Zettlmaier AE, Kido D, Caine ED, Simon JH. Magnetic resonance imaging findings in HIV cognitive impairment. *Arch Neurol* 1990; 47: 643–5.
- Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, Chen IS. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science* 1987; 236: 819–22.
- Krieger C, Hansen S, Heyes MP. Amyotrophic lateral sclerosis: quinolinic acid levels in cerebrospinal fluid and spinal cord. *Neurodegeneration* 1994; 2: 237–41.
- Lane JH, Sasseville VG, Smith MO, Vogel P, Pauley DR, Heyes MP, et al. Neuroinvasion by simian immunodeficiency virus coincides with increased numbers of perivascular macrophages/microglia and intrathecal immune activation. *J Neurovirol* 1996; 2: 423–32.
- Lidman O, Olsson T, Piehl F. Expression of nonclassical MHC class I (RT1-U) in certain neuronal populations of the central nervous system. *Eur J Neurosci* 1999; 11: 4468–72.
- Lucey DR, McGuire SA, Clerici M, Hall K, Benton J, Butzin CA, et al. Comparison of spinal fluid beta 2-microglobulin levels with CD4+ T cell count, in vitro T helper cell function, and spinal fluid IgG parameters in 163 neurologically normal adults infected with the human immunodeficiency virus type 1. *J Infect Dis* 1991; 163: 971–5.
- Martin A, Heyes MP, Salazar AM, Kampen DL, Williams J, Law WA, et al. Progressive slowing of reaction time and increasing cerebrospinal fluid concentrations of quinolinic acid in HIV-infected individuals. *J Neuropsychiatry Clin Neurosci* 1992; 4: 270–9.
- Masliah E, Achim CL, Ge N, DeTeresa R, Terry RD, Wiley CA. Spectrum of human immunodeficiency virus-associated neocortical damage. *Ann Neurol* 1992a; 32: 321–9.
- Masliah E, Ge N, Morey M, DeTeresa R, Terry RD, Wiley CA. Cortical dendritic pathology in human immunodeficiency virus encephalitis. *Lab Invest* 1992b; 66: 285–91.
- Masliah E, Ge N, Mucke L. Pathogenesis of HIV-1 associated neurodegeneration. [Review]. *Crit Rev Neurobiol* 1996; 10: 57–67.
- Masliah E, Heaton RK, Marcotte TD, Ellis RJ, Wiley CA, Mallory M, et al. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. *Ann Neurol* 1997; 42: 963–72.

- Morris CS, Esiri MM. The expression of cytokines and their receptors in normal and mildly reactive human brain. *J Neuroimmunol* 1998; 92: 85–97.
- Mouradian MM, Heyes MP, Pan J-B, Heuser IJ, Markey SP, Chase TN. No changes in central quinolinic acid levels in Alzheimer's disease. *Neurosci Lett* 1989; 105: 233–8.
- Nath A, Haughey NJ, Jones M, Anderson C, Bell JE, Geiger JD. Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: protection by memantine. *Ann Neurol* 2000; 47: 186–94.
- Post MJ, Tate LG, Quencer RM, Hensley GT, Berger JR, Sheremata WA, et al. CT, MR, and pathology in HIV encephalitis and meningitis. *AJR Am J Roentgenol* 1988; 151: 373–80.
- Pulliam L, Herndier BG, Tang NM, McGrath MS. Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J Clin Invest* 1991; 87: 503–12.
- Sardar AM, Hutson PH, Reynolds GP. Deficits of NMDA receptors and glutamate uptake sites in the frontal cortex in AIDS. *Neuroreport* 1999; 10: 3513–5.
- Schwarcz R, Köhler C. Differential vulnerability of central neurons of the rat to quinolinic acid. *Neurosci Lett* 1983; 38: 85–90.
- Sei S, Saito K, Stewart SK, Crowley JS, Brouwers P, Kleiner DE, et al. Increased human immunodeficiency virus (HIV) type 1 DNA content and quinolinic acid concentration in brain tissues from patients with HIV encephalopathy. *J Infect Dis* 1995; 172: 638–47.
- Smith MO, Heyes MP, Lackner AA. Early intrathecal events in rhesus macaques (*Macaca mulatta*) infected with pathogenic or nonpathogenic molecular clones of simian immunodeficiency virus. *Lab Invest* 1995; 72: 547–58.
- Stone TW. Neuropharmacology of quinolinic and kynurenic acids. [Review]. *Pharmacol Rev* 1993; 45: 309–79.
- Stout JC, Ellis RJ, Jernigan TL, Archibald SL, Abramson I, Wolfson T, et al. Progressive cerebral volume loss in human immunodeficiency virus infection: a longitudinal volumetric magnetic resonance imaging study. *Arch Neurol* 1998; 55: 161–8.
- Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, et al. Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* 1992; 31: 349–60.
- Whetsell WO Jr, Schwarcz R. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. *Neurosci Lett* 1989; 97: 271–5.

Received September 11, 2000. Revised January 16, 2001.

Accepted January 22, 2001