Published in final edited form as:

NMR Biomed. 2013 April ; 26(4): 480–488. doi:10.1002/nbm.2889.

Global Gray and White Matter Metabolic Changes following SIV-Infection in CD8-Depleted Rhesus Macaques: Proton MR Spectroscopic Imaging at 3 T

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Abstract

To test the hypotheses that global decreased neuro-axonal integrity reflected by decreased N-acetylaspartate (NAA) and increased glial activation reflected by an elevation in its marker, the *myo*-inositol (mI), present in a CD8-depleted rhesus macaque model of HIV-associated neurocognitive disorders. To this end we performed quantitative MRI and 16×16×4 multivoxel proton MR spectroscopic imaging (TE/TR=33/1400 ms) in 5 macaques *pre*- and 4-6 weeks *post*-simian immunodeficiency virus infection. Absolute NAA, creatine, choline (Cho), and *mI* concentrations, gray and white matter (GM, WM) and cerebrospinal fluid fractions were obtained. Global GM and WM concentrations were estimated from 224 voxels (at 0.125 cm³ spatial resolution over ~35% of the brain) using linear regression. *Pre*- to *post*-infection global WM NAA declined 8%: 6.6±0.4 to 6.0±0.5 mM (*p*=0.05); GM Cho declined 20%: 1.3±0.2 to 1.0±0.1 mM (*p*<0.003); global *mI* increased 11%: 5.7±0.4 to 6.5±0.5 mM (*p*<0.03). Global, GM and WM brain volume fraction changes were statistically insignificant. These metabolic changes are consistent with global WM (axonal) injury and glial activation, and suggest possible GM host immune response.

Keywords

Animal Disease Models; Brain; HIV-1; *Macaca mulatta*; Magnetic Resonance Spectroscopy; Simian immunodeficiency virus

INTRODUCTION

HIV-associated neurocognitive disorders (HAND) are a significant problem for the million infected in the US alone (1,2). Unfortunately, incomplete understanding of its pathogenesis hinders development of effective therapies. Recent evidence has shown that despite the benefits of highly-active antiretroviral therapy, HAND incidence among advanced HIV

patients has not changed and its prevalence continues to climb (3). Moreover, mounting evidence (4,5) suggests that even while the virus is maximally suppressed in cerebrospinal fluid (CSF) and blood plasma, HIV may still facilitate dementia. This is thought to be due to patients living significantly (up to 20+ years) longer with the disease (6), allowing infected monocytes, macrophages and microglia to produce a toxic cascade that ultimately results in neuronal death (7). MRI and proton MR spectroscopy (^1H -MRS) have been useful in detecting brain abnormalities (8-11), and provide critical knowledge of the dynamics of cerebral injury during HIV-infection (12-15).

Due to practical challenges to retrospective HIV studies, *e.g.*, unknown infection date and overlapping secondary conditions, animal models are often used (16). Simian immunodeficiency virus (SIV)-infected rhesus macaque shares similar pathology with HIV-infection, including development of AIDS, disease of the central nervous system and cognitive or behavioral deficits (17-20). Although several ^1H -MRS studies of SIV-infected macaque models are reported, all used low spatial resolution ($>3\text{ cm}^3$) single-voxels in few brain regions that typically suffer CSF, gray and white matter (GM, WM) partial volume (21-23). Consequently, one outstanding, therapy-relevant question remains the relative dysfunction of the *global* GM versus WM.

Indeed, prior histopathology of SIV-infected macaques has shown neuro-axonal dysfunction and death in both GM and WM regions (24,25), as well as glial activation in subcortical WM and cortical GM suggestive of developing encephalitis (26,27). Based on these findings, our goal in this paper was to test the hypothesis that decreases in global GM and WM *N*-acetylaspartate (NAA), the marker for neuronal integrity, and elevated global GM and WM *myo*-inositol (*mI*), the marker for glial activation (28,29), are already detectable in an accelerated non-human primate model of HAND. To overcome the spatial coverage restriction of single voxel ^1H -MRS, we use an approach that combines the data from high, 0.125 cm^3 , spatial resolution 3D multivoxel ^1H -MRS imaging (MRSI) with MRI tissue segmentation (30), to analyze hundreds of voxels cooperatively, thereby increasing the overall precision (30) - at the cost of sensitivity to possible regional variations. We use this approach to compare the global GM and WM levels of NAA, *mI*, creatine (Cr) and choline (Cho) - the markers of cellular energy/density and membrane turnover (28,29), in a large, 28 cm^3 (~35%) portion of 5 rhesus macaque brains before and 4 – 6 weeks after SIV infection, when they are persistently CD8+ lymphocyte-depleted (23).

EXPERIMENTAL

Non-human primates

Five (2 females, 3 males; 5.0 – 8.6 kg weight) healthy 3 – 4 year-old adult rhesus macaques (*Macaca mulatta*) were scanned under constant veterinary supervision. Each was tranquilized with 15 – 20 mg/kg intramuscular ketamine hydrochloride and intubated to ensure a patent airway during the experiment (no mechanical ventilation was needed). Intravenous injection of 0.4 mg/kg atropine was used to prevent bradycardia. Continuous infusion of 0.25 mg/kg/minute propofol was maintained via a catheter in a saphenous vein. Heart and respiratory rates, oxygen saturation and end-tidal CO_2 were monitored continuously and a water blanket used to prevent hypothermia. All animals were then

subsequently infected by intravenous injection with SIV and their CD8⁺ lymphocytes persistently depleted (>28 days post-infection) to speed up progression to AIDS and SIV encephalitis, both late-stage events similar to those of HIV-infection (31,32), with an antibody targeted against CD8 (cM-T807) at 6, 8 and 12 days *post*-inoculation. CD8⁺ depletion without SIV infection has been demonstrated to not produce metabolic changes or pathological abnormalities in the rhesus macaque brain (33). The animals were rescanned 4 or 6 weeks later to assess disease activity. The protocol was approved by the Harvard Medical School and Massachusetts General Hospital Institutional Animal Care and Utilization Committees.

MRI

All experiments were done in a 3 T whole-body MR imager (Magnetom TIM Trio, Siemens AG, Erlangen, Germany), running software version VB13P. It was equipped with the manufacturer's circularly-polarized transmit-receive knee-coil capable of delivering a peak 2 KHz (45.2 μ T) radio-frequency (RF) **B**₁ field with ~1 kW of power. To guide the ¹H-MRS volume-of-interest (VOI) and for tissue segmentation, sagittal and axial T2-weighted turbo spin echo (TSE) MRI: TE/TR=16/7430 ms, 140×140 mm² field-of-view (FOV), 512×512 matrix, were acquired. To cover the entire macaque head, 24 sagittal images 2.0 mm thick each and 40 axial 1.2 mm thick slices were obtained, as shown in Fig. 1.

Multivoxel 3D ¹H-MRSI

A 4.0 cm anterior-posterior (AP) ×3.5 cm left-right (LR) ×2.0 cm inferior-superior (IS) =28 cm³ VOI was image-guided, onto the corpus callosum and angled along the genu-splenium line of each animal to maximize the number of brain voxels within it while avoiding air-filled sinuses and skull lipids, as shown in Fig. 1. The manufacturer's automatic procedure adjusted the first- and second-order shims to 26±1 Hz full-width-at-half-maximum (FWHM) VOI water line. The VOI was excited using *TE*=33 ms PRESS with two, 1 cm-thick, second-order Hadamard-encoded slabs (two 0.5 cm thick slices each), interleaved along the IS direction every 720 ms for an effective *TR* of 1440 ms for each slab (and slice), as shown in Fig. 1b. This results in optimal signal-to-noise-ratio (SNR) and spatial coverage (34), as well as allows a strong, 9 mT/m Hadamard slice-selection gradient to keep the maximal 1.6 ppm chemical shift displacement between NAA and *mI* to 0.5 mm, 10% of each slice's thickness (35). The four slices' planes were encoded with 16×16 2D-Chemical shift imaging over an 8×8 cm² (LR×AP) FOV to yield nominal (0.5 cm)³=0.125 cm³ voxels [1.12×1.12×0.75≈0.15 cm³ given the FWHM of the 2D point spread function (36)]. The VOI was defined in their planes by the two 9 ms 4.9 kHz bandwidth 180° RF pulses, under 3.3 and 2.9 mT/m. The localization grid produced 8×7 voxels per slice, for a total of 224 in the VOI, as shown in Fig. 1. The MRS signal was acquired for 256 ms at ±1 kHz bandwidth. Each 16×16×2 ¹H-MRSI scan took ~12.5 minutes and the 4 averages ~50 minutes.

Brain Volumetry

The TSE images were segmented with our FireVoxel package (37). It first corrects all images for non-uniform intensities due to the coil's RF inhomogeneities, using the common histogram devolution technique of Sled *et al.* (38). Next, a WM signal intensity, I_{WM} , is

selected in a periventricular “seed” region. Following automatic detection of all pixels at or above 50% (but below 172.5% to exclude the CSF) of I_{WM} , a tissue-mask is constructed per slice in three steps: morphological erosion, recursive region growth retaining pixels connected to the seed; and morphological inflation to reverse the effect of erosion. Pixels of intensity above 172.5% of I_{WM} are classified as CSF, as shown in Fig. 2a. Following a similar process, all pixels above 120% (but below 172.5%) of I_{WM} are classified as part of the GM mask; and pixels under 120% (but over 50% to exclude air cavities) classified as WM, as shown in Fig. 2b and 2c. The masks were co-registered with the ^1H -MRSI grid using in-house software (MATLAB, The Mathworks Inc., Natick, MA) estimating their volume in every j -th voxel in the k -th animal (V_{jk}^{GM} , V_{jk}^{WM} , V_{jk}^{CSF}).

Metabolic quantification

The ^1H -MRSI data was processed offline using in-house software written in IDL (Research Systems Inc., Boulder, CO). The data was voxel-shifted to align the NAA grid with the VOI, then Fourier transformed in the time, AP and LR dimensions and Hadamard reconstructed along the IS direction with no spectral or spatial filters. The spectra were each automatically frequency aligned and phased in reference to the NAA peak. Relative levels of the i -NAA, Cr, Cho (choline + phosphorylethanolamine), mI metabolite in the $j=1\dots224$ VOI voxel of the $k=1\dots5$ animal were estimated from their peak areas, S_{ijk} , using the SITools-FITT parametric spectral modeling software using Cho, Cr, mI , NAA, glutamate and glutamine full model functions (39). The S_{ijk} -s were scaled into absolute amounts, Q_{ijk} , relative to a 0.5 L sphere of $C_j^{vitro}=12.5, 10.0, 3.0$ and 7.5 mM NAA, Cr, Cho and mI in water at physiological ionic strength to load the coil and similar VOI size and position in order to sample the $\mathbf{B}_1$ profile as closely as possible:

$$Q_{ijk} = \frac{V}{S_R} \frac{P_j^{180^\circ}}{P_R^{180^\circ}} S_{ijk} \quad [1]$$

where V is the voxel volume, the S_R is the sphere's voxels' metabolites' signal, $P_j^{180^\circ}$ and $P_R^{180^\circ}$ the RF power for a non-selective 1 ms 180° inversion pulse on the animal and reference.

To account for different relaxation times in vivo (T_1^{vivo} , T_2^{vivo}) and in the reference phantom (T_1^{vitro} , T_2^{vitro}), the Q_{ijk} in Eq. [1] were corrected with a factor for each metabolite, i :

$$Q_{ijk} = \frac{V}{S_R} \frac{P_j^{180^\circ}}{P_R^{180^\circ}} S_{ijk} \frac{T_1^{vivo}}{T_1^{vitro}} \frac{T_2^{vivo}}{T_2^{vitro}} \quad [2]$$

with 316, 177 and 264 ms macaque 3 T T_2^{vivo} values for NAA, Cr and Cho used (40), that represent a 60:40 GM:WM brain fractions in the VOI. For mI , we used the human $T_2^{vivo}=200$ ms value reported by Posse *et al.* (41). We also used the respective macaque 3 T T_1^{vivo} values of 1232, 1238, 1107 and 1170 ms (41,42). No age-related T_1/T_2 differences were anticipated in this cohort of animals of similar ages as those in a previous report

(40,42). The corresponding values measured in the phantom were $T_2^{vitro}=483, 288, 200, 233$ ms and $T_1^{vitro}=605, 336, 235, 280$ ms.

The global tissue concentration of each metabolite in the VOI, C_{ik} , was obtained as:

$$C_{ik} = \frac{1}{N} \sum_{j=1}^N f_{jk} \quad [3]$$

This C_{ik} has the advantage of (number of voxels) $^{1/2} \approx 15$ fold lower variance than the individual voxels' and, hence, expected to yield proportionally better precision, as described by Kreis (43).

Global WM and GM concentrations

Since the CSF does not contribute to the ^1H -MRS signal, the i -th metabolite amount in the j -th voxel in the k -th animal can be modeled as the sum of two compartments' (GM, WM) amounts:

$$f_{jk} = C_{ik}^{GM} V_{jk}^{GM} + C_{ik}^{WM} V_{jk}^{WM} \quad [4]$$

where C_{ik}^{WM} , C_{ik}^{GM} are the (unknown) global WM and GM metabolites' concentrations and the GM and WM f_j -s are given by Eq. [2]. The T_2^{vivo} s used were 325, 178, 274 and 200 ms for NAA, Cr, Cho and *mI* in GM; 311, 181, 255 and 200 ms in WM (40,41). Since no significant GM and WM T_1^{vivo} differences are reported (42), we used the values following Eq. [2]. Although C_{ik}^{WM} and C_{ik}^{GM} cannot both be derived from Eq. [4], since the macaque brain's GM and WM spatial heterogeneity is on a scale smaller than the voxels, each has different, independent V_{jk}^{WM} and V_{jk}^{GM} coefficients. The resulting over-determined system of $j=224$ equations in C_{ik}^{WM} and C_{ik}^{GM} was solved with linear regression. The regression fitting error per voxel was calculated for each metabolite for every animal *pre*- and *post*-infection and averaged. The intra-animal coefficient of variation (CV= standard deviation/mean) of this approach was shown to be under 5% for all metabolites (30).

Statistical Analyses

The metabolite change for each metabolite and the brain volume change in each compartment (i.e., global VOI tissue, WM, and GM) were computed for each animal by taking their *pre*-minus the *post*-infection level so that a positive change reflects a decline over time. The lower and upper limits of a 95% confidence interval for the true mean change from the "*pre*" to "*post*"-infection scans were estimated for each metabolite in each tissue compartment. The 5-animal sample size was insufficient to permit a nonparametric test of whether there was a change in any metabolite for any tissue compartment. As a result, paired sample *t* tests were used to assess MRS as well as brain volume changes from the "*pre*" to "*post*"-infection scans. Significance was tested at the $p<0.05$ level and SAS version 9.0 (SAS Institute, Cary, NC) was used for all calculations.

RESULTS

Example of the VOI position and size, *pre*- and *post*-infection, is shown in Fig. 1. The corresponding GM, WM and CSF VOI masks, segmented from the TSE images for V_{GM} and V_{WM} , are shown in Fig. 2. Spectra from the VOI and metabolic maps generated from them, *pre*- and *post*-infection, are shown in Fig. 3. Note that the lateral ventricles in the MRI in Fig. 1a, a', can be detected in the metabolic map for each of the metabolites, reflecting SNR and localization performance. Shimming yielded voxel linewidth of 5.9 ± 0.9 Hz FWHM (mean $\pm$ standard deviation) in the 2240 voxels (224 voxels/scan $\times$ 2 scans/animal $\times$ 5 animals) and SNRs of NAA: 25 ± 8 , Cr: 16 ± 6 , Cho: 10 ± 3 and *mI*: 10 ± 4 , as shown in Fig. 3. Excellent fit reliability with mean voxel Cramer-Rao lower bounds (CRLBs) below 15% were obtained for NAA, Cr, Cho, and *mI*. To optimize the analyses reliability, VOI voxels were included only if their CRLBs were $<20\%$ for all metabolites. The global regression fitting errors per voxel for GM/WM were: NAA= 0.04 ± 0.02 , Cr= 0.03 ± 0.01 , Cho= 0.002 ± 0.001 , *mI*= 0.04 ± 0.04 mM amongst the five animals and two timepoints.

GM and WM tissue volumes in the VOI were 16.0 ± 0.7 cm³ and 10.1 ± 0.6 cm³ *pre*-infection, and 15.0 ± 1.1 cm³ and 10.6 ± 0.9 cm³ *post*-infection. None of these changes were significant ($p > 0.2$ for both). Since the % WM and GM volumes changes seem to be offsetting, *i.e.*, the total tissue volume in the VOI appears to remain unchanged, these variations probably reflect different tissue sampling from slight VOI misregistration in the follow-up scan of each animal.

The sums of all 224 spectra in the VOI (equivalent to the numerator of Eq. [3]) for each animal *pre*- and *post*-infection, overlaid with their fits, are shown in Fig. 4. They exhibit SNRs of 390 ± 30 , 223 ± 12 , 151 ± 17 and 144 ± 17 for NAA, Cr, Cho and *mI*, a dramatic $\sim 224^{1/2} \approx 15$ fold gain over the source 0.125 cm³ voxels (compare Fig. 4 with Fig. 3). The single-voxels' spectral resolution is also maintained in the sums, as reflected by their 8.2 ± 0.8 Hz FWHM; a result of *pre*-alignment, this further contributes to their SNR increase.

The resultant global (Eq. [3]) as well as GM and WM (Eq. [4]) concentrations for each metabolite in each animal, *pre*- and *post*-SIV-infection, are shown in Fig. 5. They reveal a significant global VOI Cho decline (-13% , $p < 0.03$), with a 95% confidence interval (CI) of $[-0.25, -0.08$ mM] and *mI* increase (11% , $p < 0.03$), CI= $[0.18, 1.38$ mM]. A significant Cho decline (-20% , $p < 0.003$), CI= $[-0.39, -0.16$ mM] was found only in the GM. Slight increases at the "trend" level ($p \sim 0.1$) were also observed in the WM Cr and *mI*, perhaps reflecting the small sample size and smaller VOI WM volume (see above; the WM volume was on average $\frac{2}{3}$ of the GM's). Since NAA is known to always *decline* in all adult neuro-pathologies, we tested at the single-sided level and found a significant global decline in the whole VOI (-9% , $p < 0.04$), CI= $[-1.31, 0.07$ mM] and in the WM (-8% , $p = 0.05$) CI= $[-1.23, 0.13$ mM], but no significant change in GM. No metabolite differences greater than two standard deviations from the mean of all animals were observed between males and females when the group was divided according to gender.

DISCUSSION

Although several SIV-infected macaque models ^1H -MRS studies have been reported to date, these have been limited by low, $>3\text{ cm}^3$, spatial resolution single-voxels that excluded $>95\%$ of the brain and suffered CSF, GM, and WM partial volume (21-23). To overcome such limitations, we combined the data from high spatial resolution (0.125 cm^3) 3D multi-voxel MRSI with tissue segmentation from the MRI. This enabled us to analyze the metabolic and structural data from hundreds of voxels cooperatively, increasing the overall precision, at the cost of sensitivity to possible regional or specific structure variations (30,43).

Due to the sporadic nature and low incidence of HAND and its consequent paucity of postmortem studies, non-human primate models provide an alternative to histopathology for studying its neuropathogenesis. In this context, ^1H -MRS provides a nondestructive tool for detecting brain metabolite abnormalities in intact animals, facilitating longitudinal studies that histopathology does not. Towards this end, this study aims to quantify the global GM and WM changes associated with SIV-infection in order to test the hypothesis that global neuro-axonal injury and glial cell activation develop in an accelerated nonhuman primate model of HAND.

WM neuronal dysfunction

Indeed, we find significant NAA decline in WM that appears earlier or is more profound than in GM. Although evidence of focal neuronal damage in frontal cortex was seen with single-voxel ^1H -MRS (22), it is possible that global GM dysfunction does not occur until later stages of infection, as suggested previously (27). Nonetheless, a finding of lower WM NAA is consistent with *ex vivo* studies showing NAA/Cr decline in centrum semiovale and frontal WM in acute- and chronically-infected animal and human samples (44). Several *in vivo* animal studies also found 5-10% WM NAA/Cr decreases 4 weeks *post*-infection (23,45,46), consistent with us, and some even showed WM NAA decline 27 days *post*-infection was proportional to synaptic and dendritic integrity deterioration and neuronal loss in quantitative immunohistochemistry (23,46).

Diffuse GM and WM glial activation

Increased global *mI* levels support our hypothesis of diffuse glial activation (28,29,47). Previous HIV studies have also suggested a role for gliosis, reflected by elevated *mI* in both WM and GM of patients with mild HIV-dementia and clinically asymptomatic patients (12,48); these fit well with immunohistochemical studies showing astrocytic and microglial hypertrophy in both the WM and GM of HIV patients with, or without, dementia (49). In SIV-infected macaque models, elevated *mI* has been reported in several GM and WM regions using single voxel ^1H -MRS (22). Evidence seems to suggest a correspondence with increased levels of glial fibrillary acidic protein and ionized calcium binding adaptor molecule-1, immunohistochemistry markers of astrogliosis and microglial activation, at 4 (or 8) weeks *post*-infection (46).

GM host immune response

The observed 20% global GM Cho decline (see Fig. 5) is consistent with a host immunological response to developing SIV encephalitis, reported previously in the basal ganglia (50). That is, immunopathological evidence has shown that macaques with basal ganglia Cho/Cr near uninfected level or below at 4 weeks *post* infection (after an initial increase at 2 weeks *post* infection) revealed no later signs of encephalitis, whereas those whose levels remained elevated did (50). Moreover, studies of both the traditional (21) as well as accelerated (46) SIV macaque models of HAND have also demonstrated large reductions in Cho and Cho/Cr (after initial elevation) 2 to 4 weeks *post*-infection, consistent with our findings of decreased Cho at 4 weeks.

Although it has not been established what role this return to *pre*-infection levels plays in the context of disease progression, it is reasonable to assume that an immune response is involved given the similar Cho/Cr changes reported after neuroprotective treatment in SIV-infected macaques (22,45), and following antiretroviral treatment in HIV-infected patients (51). Furthermore, both treatments have been shown to support host immune defense through: (i) downregulation of microglial activation in the former (52); and (ii) reduction of infected monocyte trafficking into the central nervous system in the latter (23).

Finally, increased energy demand may also occur due to glial activation, as reflected by slight increases in WM Cr. Evidence for this can be found in a previous study (46) that showed significant Cr elevation at 8-weeks *post*- compared with *pre*-infection and a correspondence of this change with greater histopathological evidence of gliosis.

Caveats

This study is also subject to several limitations. First, the decision to maximize the sensitivity to diffuse, global changes, especially for the weaker *mI* signal, comes at the cost of localization. Although the original MRSI data is available, regional analyses may suffer 10× lower sensitivity to metabolic changes due to 22-45% intra-voxel CVs (53). Consequently, a ~25% decline in parietal cortex neuron number 4 weeks after SIV-infection (46) would go undetected. Second, due to the air-tissue interfaces (*i.e.*, paranasal sinuses) in the frontal region that cause severe B_0 field inhomogeneity (54)—leading to signal dropout—our VOI placement was limited to exclude most of the frontal brain - an area known to be affected in HAND. However, since our method assesses *global* GM and WM, exclusion or inclusion of specific region(s) should not affect the findings in diffuse diseases significantly. For example, given their relatively modest, 2.2 cm³, 14% (55) fraction of the VOI's 16 cm³ GM volume and the fact that changes there are of the order of % (see Fig. 3), areas vulnerable to high viral burden, *e.g.*, basal ganglia, cannot in of themselves explain the global GM changes observed. Third, although we account for T_1 and T_2 in *healthy* macaques, possible pathology effects on either remain unknown. Determining these effects was too costly and time-consuming, especially since pathology-related changes may vary over the 4-6 week infection period. However, use of short, $TE=33$ ms and $TR \approx T_1$ has been shown to reduce the metabolic quantification variations from T_1 and T_2 variations of up to 10% to below the ~5% voxel SNR (42,56). Fourth, because viral and host immune function can vary over a 2-week period (23), use of two *post*-infection scan times could have

obscured some effects. Due to cost constraints and lack of preliminary differences between the two groups, we combined their data to improve statistical power. Logistical constraints notwithstanding, future studies would benefit from more animals scanned at multiple times, including closer to infection, when alterations in cerebral metabolism due to initial viral activity may be more evident.

Our results reveal global tissue injury and recovery while also distinguishing GM- from WM-related dysfunction during the later stages of SIV infection in an accelerated macaque model of HAND. Although host immunological response appears a likely explanation to some of the changes observed during viral infection, its disparate effects in GM and WM suggest distinct pathological pathways that need additional study. These, for example, might examine how neuroprotective intervention in conjunction with anti-inflammatory agents affect the GM and WM and whether alone, or taken together, might be better strategies for treatment of the disease.

Acknowledgments

We thank Drs. Andrew A. Maudsley and Brian J. Soher for their SITools-FITT software and Dr. Joanne Morris and Ms. Shannon Luboyeski for animal veterinary care.

Supported by NIH Grants EB01015, NS050520, NS050041, NS051129, NS059331, NS040237 and NS059331. The Athinoula A. Martinos Center for Biomedical Imaging is supported by National Center for Research Resources grant number P41RR14075. Assaf Tal acknowledges support from the Human Frontiers Science Project's Cross-Disciplinary Fellowship.

Abbreviations Used

HIV	human immunodeficiency virus
HAND	HIV-associated neurocognitive disorders
SIV	simian immunodeficiency virus
GM	gray matter
WM	white matter
CSF	cerebrospinal fluid
MRSI	magnetic resonance spectroscopic imaging
VOI	volume-of-interest
FWHM	full-width at half-maximum
NAA	<i>N</i> -acetylaspartate
Cho	choline
Cr	creatine
<i>mI</i>	<i>myo</i> -inositol
CD8	cluster of differentiation 8

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Fig. 1.

Left (*pre*-infection): Axial (**a**) and sagittal (**b**) T_2 -weighted turbo spin echo (TSE) MRI of a female macaque head showing the location and size of the $4.0 \times 3.5 \times 2.0 \text{ cm}^3$ volume-of-interest (VOI) and localization grid (solid white frame) and two second-order Hadamard slabs, }1 and }2, in the IS direction (**b**).

Right (**a'**, **b'**): Same as (**a**, **b**) six weeks *post*-SIV infection in that animal. Note similar VOI placement and brain coverage.

Fig. 2.

Left: Axial T_2 -weighted TSE image shows the anatomical coverage of the $4.0 \times 3.5 \text{ cm}^2$ VOI, overlaid with the: **a**: CSF, **b**: GM and **c**: WM masks generated by the FireVoxel package. Note the tissue type differentiation performance.

Right: **a'-c'**: Same as **a-c**, *post*-infection of the same female macaque.

Fig. 3.

Top: Real part of the 7×8 (LR×AP) ¹H spectra matrices from the VOI in Fig. 1a and a', on common chemical shift and intensity scales *pre-* (**a**) and six weeks *post-* (**a'**) SIV infection. Note the SNR in those $(0.5)^3=0.125\text{ cm}^3$ voxels: NAA: 25 ± 8 , Cr: 16 ± 6 , Cho: 10 ± 3 and *mI*: 10 ± 4 and spectral resolution, leading to excellent fit reliability reflected by voxel CRLBs below 15% for these four metabolites.

Bottom (**b**, **b'**): NAA, Cr, Cho, and *mI* metabolic maps from the spectra in **a**, **a'**. Note the gross anatomical features reflecting spatial localization.

Fig. 4.

Real part of the aligned and summed ^1H spectra from all VOI voxels (thin black lines) representing Eq. [3], for each of the animals *pre-* (left) and 4 (animals 4 and 5) or 6 (animals 1-3) weeks *post-SIV-infection* (right), superimposed with their fitted model functions (thick gray lines), on common intensity and chemical shift scales. Animals 1 and 2 are female; 3 – 5 male. Note the excellent SNRs (reflected by CRLBs of less than 2% for the four metabolites) and spectral resolution compared with individual voxels .

Fig. 5.

NAA, Cr, Cho and *ml* concentrations' line-plots for all animals, *pre-* and 4 (animals 4 - 5) or 6 (animals 1-3) weeks *post-SIV-infection*, for the whole VOI and its GM and WM. Note significant >10% declines in VOI Cho and NAA, elevated *ml*, as well as GM Cho and WM NAA declines.