Published in final edited form as:

Magn Reson Med. 2019 May ; 81(5): 2896–2904. doi:10.1002/mrm.27635.

Simian Immunodeficiency Virus transiently increases brain temperature in rhesus monkeys: detection with magnetic resonance spectroscopy thermometry

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Abstract

Purpose: To evaluate brain temperature effects of early Simian Immunodeficiency Virus (SIV) infection in rhesus macaques using proton magnetic resonance spectroscopy (MRS) thermometry and to determine whether temperature correlates with brain choline or myo-inositol levels.

Methods: Brain temperature was retrospectively determined in serial MRS scans that had been acquired at baseline and at 2 and 4 weeks post-SIV infection (wpi) in 16 monkeys by calculating the chemical shift difference between N-acetylaspartate (NAA) and water peaks in sequentially-acquired water-suppressed and un-suppressed point-resolved spectroscopy (PRESS) spectra. Frontal and parietal cortex, basal ganglia, and white matter spectra were analyzed.

Results: At 2 wpi, brain and rectal temperatures increased relative to baseline and normalized at 4 wpi. Brain temperatures correlated with choline levels in several brain areas but not with myo-inositol levels.

Conclusion: These data indicate that SIV transiently increases brain temperature soon after infection and that temperature is correlated with transient changes in choline levels. As choline levels are associated with brain inflammation in SIV-infected monkeys, our findings suggest that the SIV-induced temperature increase reflects brain inflammation. We conclude that MRS thermometry may be informative in Human Immunodeficiency Virus (HIV) models and may be useful for assessing effects of treatments that reduce inflammation. This study also illustrates that existing MRS datasets containing un-suppressed water spectra can be used to measure tissue temperature, an important physiological parameter.

Keywords

MRS thermometry; brain temperature; HIV; SIV; inflammation

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INTRODUCTION

HIV infection of the central nervous system leads to a cascade of processes that result in neuronal injury and apoptosis (1–6), which in later stages can cause neuroAIDS or HIV-Associated Neurocognitive Disorder (HAND) (7). The rhesus macaque Simian Immunodeficiency Virus (SIV) model of HIV is well-established (8) and facilitates studies of disease progression and of experimental treatment efficacy (9–12). Our previous proton MRS studies in SIV-infected macaques detected higher brain choline (Cho) and myo-inositol (mI) levels at 2 weeks post-infection (wpi) (10,13), consistent with other MRS studies that have demonstrated transient or persisting Cho and/or mI elevations with HIV or SIV progression (14–26). Cho and mI are involved in a number of biological processes (27) and Cho and mI elevations have been associated with increased levels of inflammation biomarkers in HIV (28–29) and in cerebrospinal fluid in SIV-infected macaques (13). Collectively, these findings suggest that SIV-induced Cho or mI increases could reflect SIV-induced inflammation (9–10,13).

As infusion of neuroinflammatory substances is associated with brain hyperthermia (30–33), we used Magnetic Resonance Spectroscopy Thermometry to determine whether SIV infection increases brain temperature and whether SIV-induced brain temperature changes are associated with SIV-induced brain Cho or mI changes. Brain temperature measurements can be useful because temperature modulates several neural processes including neurotransmission, blood brain barrier permeability, and cellular morphology (32). MRS thermometry is based on the well-established observation that under physiologic conditions, the tissue water proton chemical shift is proportional to the tissue temperature, while the chemical shifts of metabolites such as N-acetylaspartate (NAA) or creatine are temperature-independent (34–35). In our prior MRS studies of the early effects of SIV infection, water-suppressed MRS spectra and un-suppressed water spectra were sequentially acquired in pairs for the purpose of metabolite referencing to the tissue water signal (10,13). We retrospectively reanalyzed these spectra by measuring the chemical shift difference between the NAA peak in water-suppressed MRS spectra and the water peak in paired water un-suppressed spectra to estimate temperatures over the first 4 weeks of SIV infection. We hypothesized that brain temperature would increase during this period and that there would be an association between brain temperature and brain Cho and/or mI levels.

METHODS

Subjects and SIV infection

This study analyzed MRS spectra previously obtained from sixteen adult (4- to 5-year old) male rhesus monkeys (*Macaca mulatta*). Methods for animal procedures were published in detail in our previous reports (10–12). The studies were approved by the Massachusetts General Hospital (MGH) Subcommittee on Research Animal Care (SRAC) and by the Institutional Animal Care and Use Committee of Harvard University. All studies were performed in accordance with federal laws and regulations, international accreditation standards, and institutional policies.

Animals were housed according to the standards of the American Association for Accreditation of Laboratory Animal Care. Treatment of animals was in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources. Animals were quarantined at the New England Primate Research Center (NEPRC) and transported to the MGH Center for Comparative Medicine (CCM) for the study. They were housed in an area that was inspected and approved by the SRAC and the Director of CCM.

All animals received environmental enrichment and were monitored daily for evidence of disease and changes in attitude, appetite, or behavior suggestive of illness. Specifically, animals were clinically monitored for general health with complete blood counts and physical examinations performed. Weekly observations of body weight, food consumption and stool character were also recorded. Health checks were performed each morning on every animal at the MGH CCM by the animal care staff and if a problem was noted the animal was examined by a veterinarian. Appropriate clinical support was administered under the direction of the attending veterinarian and included analgesics, antibiotics, intravenous fluids, and other supportive care.

After baseline MRS scans, animals were inoculated with SIV-mac251 virus (10 ng SIVp27, i.v.) and their CD8 lymphocytes were depleted with an antibody (cM-T807) administered, 6, 8, and 12 days post-inoculation. MRI scans of the animals were acquired at baseline, 2, and 4 weeks post-inoculation (wpi). During MRI sessions, each animal was tranquilized with 15–20 mg/kg intra-muscular ketamine hydrochloride and intubated to ensure a patent airway. Atropine (0.4 mg/kg injected intravenously) was administered to prevent bradycardia. A continuous infusion of propofol (0.25 mg/kg/min) was maintained throughout the scans via a saphenous vein catheter. Heart rate, oxygen saturation, end-tidal CO₂, and respiratory rate were monitored continuously. A heated water blanket was used to maintain body temperature. Rectal temperatures were recorded once before and once after scan sessions, with a temporal resolution of about 1 minute. After each scan, animals were extubated and recovered and monitored closely to ensure complete recovery.

MRI and MRS

All MRI/MRS scans were performed on a 3 Tesla whole-body imager (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) at the Athinoula A. Martinos Center for Biomedical Imaging at MGH, using a circularly polarized transmit-receive extremity coil. Several MRI scans were acquired to standardize voxel placement (resulting in highly reproducible voxel placement across repeated scans). First, a three-plane localizer scan was acquired to position monkeys in the coil. Subsequently, sagittal, and axial turbo spin echo images were obtained to guide voxel placements. Imaging parameters were, 140 × 140 mm² field of view, 512 × 512 matrix, TE = 16 ms for sagittal and axial images. Slice thicknesses were 2 mm for sagittal images and 1.2 mm for axial images. TR was 4500 ms for sagittal and 7430 ms for axial images. Acquisition times were 3 min for the sagittal and 5 min for the axial turbo spin echo sequence, respectively.

Single-voxel proton (¹H) MRS spectra were acquired using point-resolved spectroscopy (PRESS). PRESS scan parameters were, TR = 2.5 s, TE = 30 ms, 192 averages, spectral

bandwidth = 1.2 kHz, 1024 complex data points, acquisition time = 8 min per spectrum. Spectra were obtained from brain voxels ($1.2 \times 1.2 \times 1.2 \text{ cm} = 1.728 \text{ cm}^3$) placed in the basal ganglia (BG), parietal cortex (PC), frontal cortex (FC), and white matter semiovale (WM). Paired unsuppressed water reference spectra (4 averages) were sequentially acquired as well from the same voxel locations under fully relaxed conditions ($TR = 10 \text{ s}$) allowing the derivation of absolute metabolite concentrations in institutional units. All scans were acquired consistently with regard to timing of each voxel acquisition after anesthesia induction.

All spectra were processed offline using LCModel (36) to estimate quantities and chemical shifts of the metabolites N-acetylaspartate and N-acetylaspartylglutamate (collectively referred to as NAA), creatine-containing compounds (referred to as Cr), choline-containing compounds (referred to as Cho), and myo-inositol (mI).

Brain Temperature Estimation

Because LC model does not provide chemical shifts for water or metabolites, they were determined independently with custom code using the following procedures. Water-suppressed metabolite spectra and paired un-suppressed water reference spectra were read into Matlab (R2017b; The MathWorks, Inc., Natick, Massachusetts) using customized code that adapted a Siemens RDA-format reader function (37) from the VESPA simulation software (38) project. Chemical shifts of the NAA and water peaks were identified for each spectrum pair. Temperatures were estimated from the resonant frequencies of the H₂O and NAA peaks, in ppm, according to the following modeling equation (35):

$$T_{\text{brain}}(^{\circ}\text{C}) = 36.0 - 103.80 \times (H_2O(\text{ppm}) - NAA(\text{ppm}) - 2.6759), \quad [1]$$

where the chemical shift difference ($H_2O(\text{ppm}) - NAA(\text{ppm})$) was computed for each pair of spectral data. Because it took about 10 minutes to acquire each spectrum pair, the temporal resolution for brain temperature measurements is about 10 minutes per region. We used paired un-suppressed water peak spectra to determine water chemical shifts rather than using the water chemical shift in water-suppressed spectra to avoid a potential chemical shift source of inaccuracy induced by the water-suppression pulse train.

Statistical Methods

All statistical analyses were performed using Stata (Stata SE 14.2, StataCorp, College Station, Texas). The temporal patterns of temperature, Cho, and mI were examined using repeated-measures ANOVA. Post-hoc comparisons were performed at 2 and 4 wpi versus baseline (BL). The resultant P-values also were corrected for multiple comparisons (4 brain regions assessed) using the Bonferroni approach and the less conservative Sidak-Holm correction (39).

Association patterns between temperature and both Cho and mI levels were assessed with Random-intercept Generalized Least Squares (GLS) regression to account for multiple measurements per subject, using the xtreg command in Stata (40). The xtreg regression

command provides the within-subjects Pearson correlation for clustered data, which also was independently estimated using the Bland-Altman procedure (41), provided as a custom Stata program by Gregory Stoddard (42). Both the custom implementation and the Random-intercept GLS regression using the xtreg command resulted in the same values for the cluster-corrected Pearson correlation coefficient.

RESULTS

Effects of SIV infection on rectal and brain temperatures and on Cho and mI levels

Spectrum line widths and signal to noise ratios averaged (SD) 6.1 ± 1.2 Hz and 18.1 ± 2.5 , respectively. NAA levels declined on the order of 5–10% during the first 4 wpi under the conditions studied (10) but this did not impair the ability to measure NAA chemical shifts.

Following SIV inoculation, brain and rectal (pre-scan) temperatures transiently increased versus baseline at 2 wpi and normalized by 4 wpi (Figure 1A, Table 1). During the week 0, 2, and 4 scans, despite the use of warming blankets, rectal temperatures declined during scans by comparable magnitudes of 0.89 ± 1.00 , 0.94 ± 0.20 , and 0.82 ± 0.68 °C, respectively (Supporting Information Table S1). Cho levels transiently increased (Figure 1B, Table 1) while 2 wpi mI increases tended to be sustained at 4 wpi (Figure 1C, Table 1). Tests of temperature increases in the FC at 2 wpi relative to baseline remained significant after Bonferroni correction for multiple comparisons (Table 1). Using the less conservative Sidak-Holm correction, temperature increases at the 2 wpi time point remained significant relative to baseline in FC and PC.

Associations between study measures

Cho, but not mI, levels were associated with brain temperature during the first 4 weeks post infection. Correlation plots for each voxel in each animal illustrate that the majority of subjects exhibited a positive association between temperature and Cho but not between temperature and mI (Supporting Information Figures S1–S8). A regression over all subjects, taking into account the clustered nature of the data (three repeated measurements per animal and two metabolite comparisons), was performed using Random-intercept Generalized Least Squares (GLS) regression. The mean slopes (regression coefficients) all were positive and significant for Cho in the FC, PC, and WM voxels (Table 2). The BG regression coefficient did not attain significance on the GLS regression but was significant on the Pearson Correlation Coefficient, which also accounted for within-subject clustering (Table 2). None of the associations between brain temperature and mI were significant (Table 2). Cho associations with temperature in FC, PC, and WM remained significant after Bonferroni and Sidak-Holm corrections (Table 2). Rectal temperatures were associated with regional brain temperatures (Table 2).

DISCUSSION

We used MRS thermometry to retrospectively identify a transient brain temperature increase in rhesus monkeys, two weeks after SIV infection, which normalized by four weeks after infection. We also found associations between brain temperature and Cho levels in several brain areas over the time frame studied. We conclude that in early SIV infection, brain

temperature changes tend to correlate with brain Cho changes. MRI images of the voxels from which metabolite and thermometry data were obtained all appeared normal at all time points, suggesting that tissue compositions were within the normal physiological range. Thus, the MRS thermometry changes we observed in this study likely are due to brain temperature changes. We did not measure spectrometer frequency between scans and so our temperature estimations are subject to errors associated with magnetic field drift (43). However, because each water-suppressed and unsuppressed spectrum pair was acquired sequentially, the total acquisition time per spectrum pair consistently took about 10 minutes, and because frequency drift tends to be linear with time (43), frequency drift effects likely were small and were distributed comparably across all timepoints and brain regions.

Given that brain Cho increases are associated with or induced by HIV or SIV infection (10–26,29–30), that we previously reported that SIV infection is associated with a cerebrospinal fluid pro-inflammatory monocyte chemoattractant protein 1 (MCP-1) response that is correlated with brain Cho levels (13), and that brain temperature increases are induced by infusions of inflammatory mediators (30–31,33), the brain temperature increases we detected could reflect SIV-induced neuroinflammation. However, brain temperature also is increased by sensory and affective stimuli, by psychoactive drugs, and by oxidative stress (32–33,44). Rectal temperature also transiently increased in this study and rectal temperatures were correlated with brain temperatures. Accordingly, we cannot rule out the possibility that processes other than or in addition to neuroinflammation induced or contributed to the temperature increases we detected in SIV-infected monkeys.

Using the same MRS thermometry methodology, we previously detected a frontal cortex temperature increase in a transgenic mouse model of HIV, iTat mice (44), which conditionally express HIV-transactivator of transcription (Tat) protein in brain (45). Tat protein expression is key to HIV transcription and proliferation and is produced by and plays an analogous role in SIV infection (46). However, in iTat mice, we did not detect either a Cho or mI increase (44). Instead, we found higher frontal cortex levels of glutathione, the most abundant small molecule antioxidant (47), and an association between temperature and glutathione levels, suggesting that oxidative stress may have contributed to the Tat-induced brain temperature increase (44). The apparent discrepancy between these HIV models could result from species differences or disease model differences: iTat mice selectively and conditionally model the effects of Tat expression while SIV-infected monkeys more closely model HIV itself, including by expressing multiple SIV proteins that induce complex effects.

Yet, taken together, the data from our studies suggest that MRS thermometry may be a useful noninvasive method for assessing effects of HIV, SIV, effects of related proteins, and possibly effects of novel HIV treatments. It is procedurally straightforward and temporally economical to add brief un-suppressed water scans to existing MRS protocols and to measure the water peak chemical shift. If the tissue being studied is not substantially compromised in terms of its microstructure or its ionic, protein, or macromolecule contents, as such abnormalities could affect thermometry measurements independent of temperature, this thermometry method and other methods (e.g., 48, 49) can be used to assess tissue temperature, an important physiological parameter (32), as well as to assess tissue

temperature relationships with MRS metabolites or other measures. In addition, already-completed MRS studies that acquired un-suppressed water spectra for metabolite referencing to tissue water could be retrospectively analyzed to assess tissue temperature, as we did, as long as the assumptions noted above regarding normal tissue composition hold. Retrospective data analyses are common and encouraged in the field of functional MRI research, e.g., the Human Connectome Project, but are less common in MRS research. Although rectal temperatures predicted and thus could serve as surrogate markers for brain temperatures in SIV-infected monkeys under the conditions we studied, brain temperatures do not always correlate with peripheral temperature measures (32,33). Thus, brain temperature can be a useful independent measure and could be informative in other brain disorders including in major depression, which is associated with inflammation (50–52), as well as in addiction disorders (32,53–54), psychotic disorders (55–57), seizure disorders (58), and stroke (59–60).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from National Institutes of Health grants R01DA039044 (MJK), R21NS059331 (EMR), R01NS050041 (RGG), from grants R01NS040237, R01NS37654, and RR00168 (New England Primate Research Ctr. Base Grant), and from the McLean Hospital Brain Imaging Center. The anti-CD8 depleting antibody (human recombinant, cM-T807) used in these studies was provided by the NIH Nonhuman Primate Reagent Resource (R24RR016001, N01AI040101). Portions of these data were presented in abstract form at the 2018 International Society for Magnetic Resonance in Medicine (ISMRM) meeting in Paris, France.

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FIGURE 1.

Panel A: Average pre-scan rectal and brain temperature profiles (degrees Celsius) during the first 4 weeks post SIV infection in the frontal (FC) and parietal cortex (PC), in white matter (WM), and in basal ganglia (BG). Panel B: Average Cho levels (institutional units from LCModel analysis) in the same brain regions. Panel C: Average mI levels (institutional units from LCModel analysis) in the same brain regions. Values are plotted as means $\pm$ SEMs for brain temperature and MRS Cho and mI levels (N=16) and rectal temperature (N=11). Reported P-values (uncorrected; Table 1) are from comparisons at 2 and 4 wpi

versus baseline. Asterisks indicate significances between timepoints versus baseline. For clustered points, a single symbol represents the lowest P value: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. †: applies only to WM and BG points.

Table 1.

Effects of Early SIV Infection on Brain Temperature, Choline (Cho), and Myo-inositol (mI) Levels 2 and 4 Weeks Post Infection (wpi) Versus Baseline

Measure	Comparison	voxel	Uncorrected P-value	Bonferroni-corrected P-value	Sidak-Holm-corrected P-value
Brain Temperature	BL – 2wpi	FC	<0.001	0.012	0.008
		PC	0.003	0.077	0.038
		WM	0.006	0.147	0.065
		BG	0.026	0.618	0.188
Brain Temperature	BL – 4wpi	FC	0.514	>0.990	0.885
		PC	0.156	>0.990	0.639
		WM	0.861	>0.990	0.969
		BG	0.825	>0.990	0.969
Cho	BL – 2wpi	FC	<0.001	<0.001	<0.001
		PC	<0.001	0.001	0.001
		WM	<0.001	0.007	0.005
		BG	<0.001	0.011	0.008
Cho	BL – 4wpi	FC	0.308	>0.990	0.842
		PC	0.367	>0.990	0.842
		WM	<0.001	0.018	0.011
		BG	0.019	0.461	0.165
mI	BL – 2wpi	FC	<0.001	<0.001	<0.001
		PC	0.002	0.038	0.022
		WM	0.018	0.429	0.165
		BG	<0.001	0.001	0.001
mI	BL – 4wpi	FC	<0.001	<0.001	<0.001
		PC	0.002	0.055	0.029
		WM	<0.001	<0.001	<0.001
		BG	0.042	>0.990	0.26

Uncorrected (“P-value”) and corrected (Bonferroni, Sidak-Holm) P-values for comparisons at 2 and 4 wpi versus baseline (BL). P-values in bold face are significant. Legend: FC: Frontal Cortex; PC: Parietal Cortex; WM: White Matter; BG: Basal Ganglia.

Table 2.

Correlations between MRS metabolites and MRS thermometry and rectal temperatures

Correlations between Cho or mI and regional brain temperatures									
	Voxel	Pearson R				Linear coefficient, β , of GLS regression			
		R	$P^{(a)}$			$\beta^{(b)}$	$P^{(c)}$		
			Uncorrected	Bonferroni	Sidak-Holm		Uncorrected	Bonferroni	Sidak-Holm
Cho	FC	0.67	<0.001	<0.001	<0.001	3.7 ± 1.1	<0.001	0.008	0.007
	PC	0.63	<0.001	<0.001	<0.001	6.1 ± 1.2	<0.001	<0.001	<0.001
	WM	0.48	0.004	0.079	0.050	4.2 ± 1.0	<0.001	<0.001	<0.001
	BG	0.36	0.036	0.729	0.233	3.3 ± 2.1	0.114	>0.990	0.571
mI	FC	0.34	0.061	0.484	0.185	0.34 ± 0.33	0.302	>0.990	0.929
	PC	0.34	0.050	0.399	0.185	0.50 ± 0.37	0.170	>0.990	0.674
	WM	0.22	0.221	>0.990	0.338	0.54 ± 0.44	0.215	>0.990	0.674
	BG	0.23	0.187	>0.990	0.338	0.54 ± 0.42	0.198	>0.990	0.674
Correlations between Cho or mI levels and pre-scan rectal temperatures (N=11)									
Cho	FC	0.73	<0.001	0.001	0.001	3.1 ± 0.6	<0.001	<0.001	<0.001
	PC	0.56	0.005	0.092	0.054	3.2 ± 0.7	<0.001	<0.001	<0.001
	WM	0.55	0.006	0.117	0.063	3.1 ± 0.8	<0.001	0.003	0.002
	BG	0.55	0.006	0.121	0.063	3.0 ± 0.8	<0.001	0.003	0.001
mI	FC	0.45	0.033	0.651	0.233	0.29 ± 0.27	0.282	>0.990	0.929
	PC	0.22	0.311	>0.990	0.462	-0.05 ± 0.25	0.849	>0.990	0.929
	WM	0.16	0.478	>0.990	0.462	0.15 ± 0.29	0.600	>0.990	0.929
	BG	0.49	0.018	0.370	0.155	0.49 ± 0.26	0.061	>0.990	0.435
Correlations between pre-scan rectal and regional brain temperatures (N=11)									
Rectal Temperature	FC	0.73	<0.001	0.001	0.001	0.90 ± 0.17	<0.001	<0.001	<0.001
	PC	0.76	<0.001	<0.001	<0.001	1.00 ± 0.21	<0.001	<0.001	<0.001
	WM	0.62	0.002	0.030	0.021	0.82 ± 0.14	<0.001	<0.001	<0.001
	BG	0.41	0.053	>0.990	0.264	0.74 ± 0.18	<0.001	0.001	<0.001

^(a) Pearson correlation coefficient P-value accounting for within-subject clustering;^(b) Coefficient value ± Robust Standard Error;^(c) GLS regression P-value of the linear coefficient accounting for within-subject clustering; Legend: FC: Frontal Cortex; PC: Parietal Cortex; WM: White Matter; BG: Basal Ganglia. Multiple comparisons corrections were computed for a multiplicity of 20.