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Cross-sectional and Longitudinal Reproducibility of Rhesus Macaque Brain Metabolites: A Proton MR Spectroscopy Study at 3 T

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Abstract

Non-human primates are often used as preclinical model systems for (mostly diffuse or multi-focal) neurological disorders and their experimental treatment. Due to cost considerations, such studies frequently utilize non-destructive imaging modalities, MRI and proton MR spectroscopy (¹H-MRS). Cost may explain why the *inter*- and *intra*-animal reproducibility of the ¹H-MRS-observed brain metabolites, is not reported. To this end, we performed test-retest three-dimensional brain ¹H-MRS in five healthy rhesus macaques at 3 T. Spectra were acquired from 224 isotropic (0.5 cm)³=125 μL voxels, over 28 cm³ (~35%) of the brain, then individually phased, frequency aligned and summed into a spectrum representative of the entire volume of interest. This dramatically increases the metabolites' signal-to-noise ratios, while maintaining the (narrow) voxel linewidth. The results show that the average *N*-acetylaspartate, creatine, choline and *myo*-inositol concentrations in the macaque brain are: 7.7±0.5, 7.0±0.5, 1.2±0.1 and 4.0±0.6 mM/g wet weight (mean±standard deviation). Their *inter*-animal coefficients of variation (CV) are 4%, 4%, 6% and 15%; and the longitudinal (*intra*-animal) CVs are lower still: 4%, 5%, 5% and 4%, much better than the 22%, 33%, 36% and 45% *intra-voxel* CVs, demonstrating the advantage of the approach and its utility for preclinical studies of diffuse neurological diseases in rhesus macaques.

Keywords

Animal models; Brain; MR Spectroscopy; Longitudinal Metabolites concentration; Rhesus Macaque; reproducibility

INTRODUCTION

Due to similarities in physiology, anatomy and cellular function to its human counterpart, the rhesus macaque brain is used as a “pre-clinical” model system for various diseases, such as ischemic stroke (1,2), Parkinson's disease (3), schizophrenia (4), multiple sclerosis (5), and neuroAIDS (6,7). Due to the cost of such studies, especially if they involve serial observations, non-invasive imaging methods, such as MRI for morphology and proton MR spectroscopy (¹H-MRS) for physiology and metabolism, are often the modalities of choice. Indeed, at the *TE* 40 ms that are often used, *in vivo* ¹H-MRS can monitor the *N*-

acetylaspartate (NAA), creatine (Cr), choline (Cho) and *myo*-inositol (*mI*) signals, the putative markers for neuronal integrity, cellular energy status, membrane turnover rates and glial proliferation, respectively (8–12).

Although several ^1H -MRS studies have reported metabolite concentrations in the healthy macaque brain (6,13–15), all used just single $1 - 3 \text{ cm}^3$ voxels placed over specific isolated brain regions. Since most of the above diseases are diffuse throughout the central nervous system, such single-voxels invariably suffer partial volume contamination *and* miss over 95% of this small, $\sim 85 \text{ cm}^3$, brain (16). Due perhaps to cost and complexity constraints, only one study is known to us to have assessed cross-sectional (*inter*-animal) and longitudinal (*intra*-animal) metabolite *ratios* in (just) several regions of the rhesus macaque brain. Although the reproducibility metric is essential for adequately powered study design and to distinguish real changes from biological and instrumental noise (15), its global average values and their range has not been reported.

The partial volume and coverage issues can be addressed with three dimensional (3D) MRS that can cover extensive (tens percent) portions of the brain at well below 1 cm^3 spatial resolution (17,18). The variable T_1 - and T_2 -weighting incurred at various $TE > 0$ and $TR < 5T_1$ used by different ^1H -MRS sequences, can be accounted for using the metabolites' T_1 and T_2 s that have been reported for this species at various magnetic field (B_0) strengths (19–21). Differences due to specific imager and coil combinations can be addressed by absolute quantification against a reference (22,23), leaving the *inter*- and *intra*-animal “biological” variations as the remaining confounds. Unfortunately, since MRS is intrinsically fraught with noise, the above fluctuations are always exacerbated by instrumental variations that depend on the experimental setup (24).

To reduce the contribution of repositioning and instrumental noise, we summed the phased and frequency-aligned spectra from all the voxels in the volume of interest (VOI) in post processing. (Note, that any specific brain structure(s) or tissue type(s) can also be summed within the 3D ^1H -MRS data set, if necessary depending on the hypotheses tested). Absent a specific hypothesis, whole-VOI summation yields a single average spectrum that is relevant to diffuse brain disorders with the substantial signal-to-noise improvement that is essential for reproducibility (22), at minimal cost in linewidth (spectral resolution) and repositioning errors. Our two primary goals, consequently, are: (i) to establish the macaque brain's average NAA, Cr, Cho and *mI* concentrations and their cross-sectional (*inter*-animal) and longitudinal (*intra*-animal) variations; and (ii) establish and demonstrate the method's sensitivity to distinguish pathological change from biological and instrumental variations. Towards these ends we applied test-retest 3D multivoxel ^1H -MRS to the brain of five healthy rhesus macaques.

MATERIALS AND METHODS

Non-human primates

Five (3 females, 2 males; 4.3 – 5.6 kg weight) healthy 3 years 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 was used to prevent hypothermia. One macaque was subsequently infected by intravenous injection with simian immunodeficiency virus (SIV) and CD8+ lymphocytes were depleted [to facilitate rapid progression to AIDS and SIV

encephalitis (25)] with an antibody targeted against CD8 (cM-T807) at 6, 8 and 12 days post-inoculation and then rescanned 6 weeks later. The protocol was approved by both the Harvard Medical School and Massachusetts General Hospital Institutional Animal Care and Utilization Committees.

MRI

All experiments were done in a 3 T MR whole-body imager (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) with a circularly-polarized transmit-receive human knee coil. To guide the ^1H -MRS VOI and for tissue segmentation, sagittal and axial turbo spin echo (TSE) MRI: TE/TR=16/7430 ms, $140\times 140\text{ mm}^2$ field-of-view (FOV), 512×512 matrix, 2.0 mm sagittal and 1.2 mm axial slice thickness, were acquired.

VOI tissue fraction (T_f)

Since the VOI also includes ventricles and sulci (see Fig. 1) correction for their cerebro-spinal fluid (CSF) partial volume is required. To this end the TSE images were segmented with our FireVoxel package (26), as shown in Fig. 2. The process begins with automatic detection of the white matter (WM) signal intensity, I_{WM} , in a periventricular “seed” region. Following selection of all pixels at or above 50% (but below 142.5% 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 142.5% of I_{WM} were classified as CSF. The T_f for each macaque was the tissue volume, V_T , (sum of all non-CSF pixel volumes in the VOI) divided by the 28 cm^3 nominal VOI.

VOI Gray matter (GM) and WM fractions

Following a process similar to the above, all pixels at or above 90% (but below 142.5% to exclude CSF) of I_{WM} , were classified as GM, as shown in Fig. 2c. The GM fraction (GM_f) for each macaque was the product of the number of GM pixels $\times$ the pixel-volume, divided by V_T . Pixels below 90% (but above 50% to exclude air cavities) were classified as WM, as shown in Fig. 2d. The WM fraction (WM_f) was the product of WM pixels $\times$ pixel-volume, divided by V_T .

Multivoxel 3D ^1H -MRS

A 4.0 cm anterior-posterior (AP) $\times$ 3.5 cm left-right (LR) $\times$ 2.0 cm inferior-superior (IS) $=28\text{ cm}^3$ VOI was centered on the corpus callosum of each animal and angled along its genu-splenium line, as shown in Figs. 1a and 1b. The manufacturer’s automatic procedure adjusted the first- and second-order shims to a consistent $26\pm 1\text{ Hz}$ full-width-at-half-maximum (FWHM) VOI water line width. The VOI was then excited using PRESS (TE/TR= 33/1440 ms) with two second-order Hadamard encoded slabs (4 slices) interleaved along the IS direction every TR, as shown in Fig. 1b, for optimal signal-to-noise-ratio (SNR) and spatial coverage (27). Interleaving also allowed us to apply *very* strong, 18 mT/m, Hadamard slice select gradients to reduce the chemical shift displacement between NAA and *mI* to 0.2 mm, 4% of the slice width (28). The slices’ planes were encoded with 16×16 2D-Chemical shift imaging over an $8\times 8\text{ cm}^2$ (LR $\times$ AP) FOV to yield nominal $(0.5\text{ cm})^3=0.125\text{ cm}^3$ voxels. Although the *actual* voxel size (FWHM of the point spread function for uniform 2D phase encoding) was actually $0.55\times 0.55\times 0.5=0.15\text{ cm}^3$ (29,30), for consistency reasons we will refer to its nominal size in the ensuing discussions. This localization grid yielded 8×7 voxels per slice in the VOI, as shown in Fig. 1, in each of the 4 slices. Given the $4.0\times 3.5\times 2.0=28\text{ cm}^3$ (AP $\times$ LR $\times$ IS) dimensions of the VOI, a total of 224 voxels fell within it. The MRS signal was acquired for 256 ms with 512 points at $\pm 1\text{ kHz}$ bandwidth and each $16\times 16\times 4$ scan took 12.5 minutes.

The MRS data was processed offline using in-house software. It was voxel-shifted to align the CSI grid with the NAA VOI, Fourier transformed in the time, AP and LR dimensions and Hadamard reconstructed along the IS direction. The 224 VOI spectra were each frequency-aligned and zero-order phased in reference to the NAA peak, then summed, retaining individual spectra linewidth and improved the SNR by $224^{1/2} \approx 15$, as shown in Fig. 3.

Relative levels of the i^{th} (NAA, Cr, Cho, *mI*) metabolite in the j^{th} subject were estimated from their peak area, S_{ij} , using the SITools-FITT parametric spectral modeling and least-squares optimization software of Soher *et al.* (31) that also provided the linewidth estimate. Analysis of this baseline modeling showed that for 5 Hz spectral linewidth the mean fit errors are 3.4%, 2.3% and 2.8% for NAA, Cr and Cho (24). Seven model functions: NAA, aspartate, glutamate, glutamine, Cr, Cho and *mI*, were used to fit our data. The S_{ij} were then scaled into absolute concentrations, C_{ij} , relative to signals from a 2 L sphere of

$C_i^{vitro} = 12.5, 10.0, 3.0$ and 7.5 mM NAA, Cr, Cho and *mI* in water of physiological ionic strength (to properly load the coil) as (32):

$$C_{ij} = C_i^{vitro} \cdot \frac{S_{ij}}{S_R} \cdot \frac{V_j^{180^\circ}}{V_R^{180^\circ}} \cdot \frac{1}{T_f}, \quad [1]$$

where S_R is the sphere's metabolite signal, $V_j^{180^\circ}$ and $V_R^{180^\circ}$ are the RF voltages needed for a non-selective 1 ms 180° inversion pulse on subject and reference phantom. The C_{ij} s were corrected for relaxation time *in vivo* (T_1^{vivo}, T_2^{vivo}) and *in vitro* (T_1^{vitro}, T_2^{vitro}) with a factor (33):

$$f = \frac{\exp(-TE/T_2^{vitro})}{\exp(-TE/T_2^{vivo})} \cdot \frac{1 - \exp(-TR/T_1^{vitro})}{1 - \exp(-TR/T_1^{vivo})}, \quad [2]$$

using $T_1^{vivo} = 1.3, 1.3$ and 1.1 s, $T_2^{vivo} = 316, 177$ and 264 ms reported for NAA, Cr and Cho at 3 T (20,21,34); and their $T_1^{vitro} = 605, 336$ and 235 ms, $T_2^{vitro} = 483, 288$ and 200 ms measured in the phantom. Since *J*-coupling modulation and low SNR for *mI* prevent obtaining its T_2 , we assumed $f=1$ for it. Finally, the C_{ij} s were divided by T_f to convert into absolute tissue concentration.

Strategy

To determine *intra*-animal reproducibility, two macaques each underwent three separate ^1H -MRS sessions and one macaque underwent two. To determine *inter*-animal reproducibility, two more macaques each underwent one session (total of 10 scans from 5 animals). To determine the variance that is due to VOI repositioning and increase the metabolites' SNRs, four back-to-back scans were acquired at each session with all experimental parameters kept unchanged for a total of 40 full $16 \times 16 \times 4$ 3D ^1H -MRS data sets. To compare our results with previous single-voxel reports (6), *intra*-voxel coefficients of variation (CV) for each metabolite in each of the 224 voxels in the VOI were also calculated from the four back-to-back scans in every session. Finally, one macaque was infected with SIV, a disease of known widespread diffuse pathology, in order to examine whether the sensitivity gain facilitates identification of metabolic changes that may otherwise be either not statistically significant or obscured by the noise.

Statistical Analyses

Restricted maximum likelihood (REML) estimation of variance components in a random effects model was used to assess the *intra*-session, *inter*-session and *inter*-animal variance components of each metabolite. This method produces statistically optimal variance component estimates (in the sense of maximizing the likelihood of obtaining the data that were actually observed) subject to the constraint that estimates must be non-negative. Each CV was estimated by taking the square root of the relevant variance component estimate and dividing this by the mean value over all measurements, as described by Chard *et al.* and Tedeschi *et al.* (35,36). In addition, the total variation of each metabolite was estimated as the variance among its 40 observations.

The *intra*-voxel variation [the “residual error” term in the model of Chard *et al.* (35)] is the component of the *intra*-animal variance that measures the variation expected between the four replicate measures derived for each of the 224 voxels in the VOI, the *intra*-session variation (a.k.a. *inter*-scan variation) is the component of the *intra*-animal variance that measures the variation that is expected between the metabolite levels derived from different scans within a single imaging session of a single animal. It was estimated by computing the variance among the four observations derived from the four repeat scans within a single imaging session of a single animal and then averaging these variances over all sessions and all animals. The *inter*-session variance is the component of the *intra*-animal variance that measures the variation expected between results derived from different imaging sessions of the same animal. The *inter*-animal component measures the expected variation between the true mean metabolite levels of two different randomly selected animals.

RESULTS

The position and geometry of the VOI for each of the scans are shown in Fig. 1. Typical spectra from the VOI and the metabolic maps generated from them (to demonstrate the quality of the spatial localization), are shown in Figs. 1c and 1d. Similar spectra were obtained from all animals. Spectral modeling produced consistent voxel FWHM linewidth of 7.6 ± 0.8 Hz (mean $\pm$ standard deviation), as shown in Fig. 3a. The metabolites' SNRs from nearly 10,000 voxels (224 voxels/session $\times$ 10 sessions $\times$ 4 scans/session), defined as peak height divided by the root mean square of the noise, for NAA, Cr, Cho and *mI* were 10.2 ± 2.1 , 5.3 ± 1.9 , 4.1 ± 1.5 and 4.5 ± 1.8 .

The 224 phased spectra from the VOI were summed to yield a single spectrum, as shown in Fig. 3b, that is equivalent to a single-voxel PRESS from this entire volume. While it exhibits a dramatic, $\sim 224^{1/2}$, SNR gain due to the summation, it clearly also suffers over $\times 2.5$ fold line broadening resulting from $\mathbf{B}_0$ inhomogeneities over the entire VOI (37). If on the other hand the 224 voxel spectra are pre-aligned, these inhomogeneities are corrected for and their sum then benefits from *both* the improved SNR *and* the narrow typical single-voxel linewidth, (FWHM of 6 and 8 versus 20 Hz), as seen by comparing Figs. 3a and 3c with 3b.

Consequently, the 224 spectra from each of the 4 scans of each of the 10 sessions (2 animals $\times$ 3 sessions + 1 animal $\times$ 2 sessions + 2 animals $\times$ 1 session) were aligned, then summed. These 40 whole-VOI spectra are shown in Fig. 4, superimposed with the model functions used to obtain the S_{ij} s in Eq. [1]. Their SNRs: 173.5 ± 11.1 , 96.1 ± 6.4 , 54.5 ± 6.4 and 45.7 ± 6.6 for the NAA, Cr, Cho and *mI* reflect the (expected) $224^{1/2}$ gain over the single-voxel SNRs *and* their linewidths are similar to the single-voxel counterparts, as shown in Fig. 3. The resultant absolute concentrations, for each metabolite, scan and animal, are compiled in Table 1.

The *intra*-voxel, *intra*- and *inter*-animal CVs per metabolite, are compiled in Table 2 and box plots of their concentrations' together with single-voxels' (*inter*-voxel - computed from variations in each of the 224 voxels in the VOI along its 4 back-to-back scans each measurement) for comparison, are shown in Fig. 5. This demonstrates the improved reproducibility of the VOI sums, a consequence of the $\times 15$ improved SNR (22). Specifically, (i) *intra*-voxel CVs are all approximately threefold larger than the mean values reported for 3.4 cm³ voxels in healthy macaques (6). (ii) The *inter*-animal CVs (reflecting the inherent biologic differences among these animals) are: 4.3%, 4.0%, 5.8% and 14.5% for NAA, Cr, Cho and mI, dominating the total variance, as shown in Table 2 and Fig. 5. (iii) The *intra*-animal variance comprise two categories: (iii. a) the *inter*-session CVs: 4.0%, 4.9%, 4.5% and 3.9%; and (iii. b) the *intra*-session CVs: 3.9%, 3.7%, 5.6% and 8.5% for NAA, Cr, Cho and mI, both of comparable magnitude, indicating minimal contribution from serial VOI misregistration. As expected, both *intra*-animal contributions were smaller than the *inter*-animal contribution to the total variation, as shown in Table 2 and respective box plots in Fig. 5.

The sensitivity advantage of this approach is demonstrated by comparing the VOI spectrum of one of these macaques (#1 in Table 1) at baseline and 6 weeks post SIV infection, as shown in Fig. 6. The infection leads to a 30% and 20% decline in NAA and Cho and an 8% increase in mI, all statistically significant based on the *intra*-animal CVs in Table 2. Given the single voxel CVs in Table 2, in contrast, these changes would *not* be significant and possibly not even visible (seen by comparing the 0.125 cm³ voxels spectra in Fig. 1 with the whole VOI sum in Fig. 4).

Examples of GM, WM and CSF VOI masks, segmented from the MRI for estimating T_f, in Eq. [2], are shown in Fig. 2. The resultant GM_f, WM_f and T_f for each animal and session are compiled in Table 1 with box plots of their distribution shown in Fig. 5. Specifically, the *inter*-animal CVs for the GM_f, WM_f and T_f are: 3.6%, 4.6% and 1.1% and their *intra*-animal values, estimates from multiple sessions of the same animal, are smaller: 1.5%, 2.0% and 1.0%. This reflects the minimal partial tissue volume variations in serial (large) VOI placement.

DISCUSSION

As ¹H-MRS becomes more prevalent in preclinical studies in rhesus macaque models, the need for reproducibility metrics becomes pressing in order to, (i) power studies correctly and to weigh (based on cost) numbers of animals versus scans per animal; and (ii) to be able to discern pathogenesis (or treatment) effects from experimental fluctuations. Indeed, CVs of 11%, 8%, 14% and 13% for NAA, Cr, Cho and mI, were reported in the frontal cortex, WM of the centrum semiovale and basal ganglia of rhesus macaques using single 3.4 cm³ voxels and 6.5 minutes acquisition at 1.5 T (6). Spectra from our 0.125 cm³ voxels yield *intra*-voxel CVs that are 3 – 4 fold *larger* (see Table 2 and Fig. 5). What may have kept the CV disparity to just 3–4 fold despite order-of magnitude SNR difference (22), may be that these larger single-voxels also suffered comparably larger partial GM/WM volume and *inter*- and *intra*-animal positioning uncertainty.

It is also noteworthy that increasing the VOI by nearly an order-of-magnitude (from 3.4 cm³ to 28 cm³ at 3 T) helps reduce the *inter*-animal CV by (only) slightly less than a factor of 2. This indicates that at this brain coverage *true* biological variability is the dominating factor that cannot be reduced by further experimental refinements, *e.g.*, SNR or VOI size suggesting that more measurements on healthy animals are unlikely to yield significantly different results. This is of particular importance in model systems, since unlike human diseases, every animal enters these studies healthy. Therefore, given the CVs reported here,

a baseline scan may also enable to detect at the onset, metabolic conditions that may escape the veterinarian's attention but may later confound the outcome of the disease or treatment for which that animal was a model.

Our current approach of synthesizing the VOI spectrum from the sum of all phased and aligned single 0.125 cm^3 elements in the VOI enabled us to address both the reproducibility and precision issues: First it achieves much ($\times 15$ fold) higher metabolite SNRs and consequently $\times 4$ – $\times 11$ fold better CVs (22), by exploiting the B_0 homogeneity (narrow linewidth) across the individual small voxels (37); and correction for regions of B_0 variations (frequency shifts) on a voxel-by-voxel basis. [Although the CV improvement is expected to be linear in SNR, as described by Kreis (38), the observed *intra*-session VOI CVs are all less than $\times 15$ fold better than the *intra*-voxel CVs, likely reflecting a combination of *intra*- and *inter*-voxel variations]. Second, its order-of-magnitude higher spatial coverage: 28 cm^3 ($\sim 35\%$) of the macaque brain, versus 3.4 cm^3 (less than 5%) for single-voxel methods, minimizes partial GM/WM/CSF volume variations, therefore, effectively increasing the *inter*-session VOI placement accuracy, thereby further improving the metabolites' reproducibility (39).

The improvement is demonstrated by the subtle (but diffuse) changes brought about by SIV infection in the macaque brain. Indeed, while a decrease in NAA and NAA/Cr at 6 weeks post SIV infection are consistent with previous reports (40), establishing them with statistical significance using 3.4 cm^3 single voxel ^1H -MRS required four animals. While such changes would have not been significant, indeed possibly even detected given the CVs in Table 2 and Fig. 5, they are both clearly visible and significant with this approach in a single animal.

The $<10\%$ *inter*-animal CVs in Table 2 indicate good cross sectional similarity between healthy animals, *i.e.*, metabolite concentrations differences larger than 10% (20% for mI) are unlikely or, alternatively, if occurring they may be indicative of an abnormal animal. The longitudinal *inter*-animal reproducibility is even better, exhibiting CV's below 10% across the board. Both these results are encouraging given past reports of NAA changes larger than 25% within lesioned areas after ischemic stroke (2), in frontal cortical GM after acute simian immunodeficiency virus (SIV)-infection (41,42), within acute and chronic lesions caused by experimental allergic encephalitis (5) and in nigrostriatal dopamine neurons in a macaque model of Parkinson's disease (3); as well as Cho changes $>20\%$ in simian acute EAE lesions (5). Specifically, given the CVs in Table 2, one would require fewer than 10 animals to detect metabolic changes of this magnitude (15).

Admittedly, the study also encountered several limitations due to the methodology used. First, the sensitivity to metabolic changes reported here, derived from the high SNR shown in Fig. 4, come at the cost of localization. Any focal or regional differences in the VOI are averaged out in the sum. Although the original localized information is still available (see Fig. 1), the proposed strategy reflects a choice of maximum sensitivity to monitor the global effects of diffuse pathogenesis or treatment responses, especially for the weaker mI signal. Second, although our results correct for T_1 and T_2 relaxation using literature values, possible effects of pathology on either remains unknown. Use of short TE 40 ms and of $TR \approx T_1$, however, reduces the sensitivity of metabolic quantification to T_1 and T_2 variations smaller than 10% of nominal to below the voxel SNR, as shown by Liu *et al.* and Zaaraoui *et al.* (21,34).

CONCLUSION

Both the cross-sectional *and* longitudinal *average* metabolite levels in the healthy rhesus macaque brain can be assumed to vary less than $\pm 10\%$ from the nominal values reported here. Since unlike investigations of human neurological disorders, all animals entering these studies are *healthy* at baseline, these CVs can be used to compute power tables geared to detect (and identify as “real”) predetermined metabolic changes that are due to either disease progression or treatment response. Given the CVs reported here, these tables will then be subject to more mundane cost constraints to determine the number of animals versus that of the number of scans on each needed in order to detect the desired changes.

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References

1. Hirouchi Y, Suzuki E, Mitsuoka C, Jin H, Kitajima S, Kohjimoto Y, Enomoto M, Kugino K. Neuroimaging and histopathological evaluation of delayed neurological damage produced by artificial occlusion of the middle cerebral artery in Cynomolgus monkeys: establishment of a monkey model for delayed cerebral ischemia. *Exp Toxicol Pathol*. 2007; 59(1):9–16. [PubMed: 17596924]
2. Roitberg B, Khan N, Tuccar E, Kompolti K, Chu Y, Alperin N, Kordower JH, Emborg ME. Chronic ischemic stroke model in cynomolgus monkeys: behavioral, neuroimaging and anatomical study. *Neurol Res*. 2003; 25(1):68–78. [PubMed: 12564129]
3. Brownell AL, Jenkins BG, Elmaleh DR, Deacon TW, Spealman RD, Isacson O. Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease. *Nat Med*. 1998; 4(11):1308–1312. [PubMed: 9809556]
4. Bertolino A, Knable MB, Saunders RC, Callicott JH, Kolachana B, Mattay VS, Bachevalier J, Frank JA, Egan M, Weinberger DR. The relationship between dorsolateral prefrontal N-acetylaspartate measures and striatal dopamine activity in schizophrenia. *Biol Psychiatry*. 1999; 45(6):660–667. [PubMed: 10187995]
5. Richards TL, Alvord EC Jr, Peterson J, Cosgrove S, Petersen R, Petersen K, Heide AC, Cluff J, Rose LM. Experimental allergic encephalomyelitis in non-human primates: MRI and MRS may predict the type of brain damage. *NMR Biomed*. 1995; 8(2):49–58. [PubMed: 7547186]
6. Ratai EM, Pilkenton SJ, Greco JB, Lentz MR, Bombardier JP, Turk KW, He J, Joo CG, Lee V, Westmoreland S, Halpern E, Lackner AA, Gonzalez RG. In vivo proton magnetic resonance spectroscopy reveals region specific metabolic responses to SIV infection in the macaque brain. *BMC Neurosci*. 2009; 10:63. [PubMed: 19545432]
7. Lentz MR, Westmoreland SV, Lee V, Ratai EM, Halpern EF, Gonzalez RG. Metabolic markers of neuronal injury correlate with SIV CNS disease severity and inoculum in the macaque model of neuroAIDS. *Magn Reson Med*. 2008; 59(3):475–484. [PubMed: 18306400]
8. Ross, B. Magnetic Resonance Spectroscopy Diagnosis of Neurological Diseases. 1999.
9. Benarroch EE. N-acetylaspartate and N-acetylaspartylglutamate: neurobiology and clinical significance. *Neurology*. 2008; 70(16):1353–1357. [PubMed: 18413589]
10. Michel V, Yuan Z, Ramsubir S, Bakovic M. Choline transport for phospholipid synthesis. *Exp Biol Med* (Maywood). 2006; 231(5):490–504. [PubMed: 16636297]
11. Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. *Brain Res Bull*. 2008; 76(4):329–343. [PubMed: 18502307]

12. Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci.* 1993; 13(3):981–989. [PubMed: 8441018]
13. Juchem C, Logothetis N, Pfeuffer J. High-resolution (1)H chemical shift imaging in the monkey visual cortex. *Magn Reson Med.* 2005; 54(6):1541–1546. [PubMed: 16206143]
14. Pfeuffer J, Juchem C, Merkle H, Nauerth A, Logothetis N. High-field localized 1H NMR spectroscopy in the anesthetized and in the awake monkey. *Magn Reson Imaging.* 2004; 22(10):1361–1372. [PubMed: 15707786]
15. Greco JB, Sakaie KE, Aminipour S, Lee PL, Chang LL, He J, Westmoreland S, Lackner AA, Gonzalez RG. Magnetic resonance spectroscopy: an in vivo tool for monitoring cerebral injury in SIV-infected macaques. *J Med Primatol.* 2002; 31(4–5):228–236. [PubMed: 12390545]
16. Cheverud J, Falk D, Vannier M, Konigsberg L, Helmkamp R, Hildebolt C. Heritability of brain size and surface features in rhesus macaques (*Macaca mulatta*). *J Hered.* 1990; 81(1):51–57. [PubMed: 2332614]
17. Juchem C, Merkle H, Schick F, Logothetis N, Pfeuffer J. Region and volume dependencies in spectral line width assessed by 1H 2D MR chemical shift imaging in the monkey brain at 7 T. *Magn Reson Imaging.* 2004; 22(10):1373–1383. [PubMed: 15707787]
18. Gonen O, Liu S, Goelman G, Ratai EM, Pilkenton S, Lentz MR, Gonzalez RG. Proton MR spectroscopic imaging of rhesus macaque brain in vivo at 7T. *Magn Reson Med.* 2008; 59(4):692–699. [PubMed: 18302225]
19. Liu S, Gonen O, Fleysher L, Fleysher R, Soher BJ, Pilkenton S, Lentz MR, Ratai EM, Gonzalez RG. Regional metabolite T2 in the healthy rhesus macaque brain at 7T. *Magn Reson Med.* 2008; 59(5):1165–1169. [PubMed: 18429024]
20. Liu S, Gonen O, Fleysher R, Fleysher L, Babb JS, Soher BJ, Joo CG, Ratai EM, Gonzalez RG. Metabolite proton T(2) mapping in the healthy rhesus macaque brain at 3 T. *Magn Reson Med.* 2009; 62(5):1292–1299. [PubMed: 19780178]
21. Liu S, Fleysher R, Fleysher L, Joo CG, Ratai EM, Gonzalez RG, Gonen O. Brain metabolites B1-corrected proton T1 mapping in the rhesus macaque at 3 T. *Magn Reson Med.* 2010; 63(4):865–871. [PubMed: 20373387]
22. Kreis R, Slotboom J, Hofmann L, Boesch C. Integrated data acquisition and processing to determine metabolite contents, relaxation times, and macromolecule baseline in single examinations of individual subjects. *Magn Reson Med.* 2005; 54(4):761–768. [PubMed: 16161114]
23. Jansen JF, Backes WH, Nicolay K, Kooi ME. 1H MR spectroscopy of the brain: absolute quantification of metabolites. *Radiology.* 2006; 240(2):318–332. [PubMed: 16864664]
24. Soher BJ, Young K, Maudsley AA. Representation of strong baseline contributions in 1H MR spectra. *Magn Reson Med.* 2001; 45(6):966–972. [PubMed: 11378873]
25. Williams K, Westmoreland S, Greco J, Ratai E, Lentz M, Kim WK, Fuller RA, Kim JP, Autissier P, Sehgal PK, Schinazi RF, Bischofberger N, Piatak M, Lifson JD, Masliah E, Gonzalez RG. Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. *J Clin Invest.* 2005; 115(9):2534–2545. [PubMed: 16110325]
26. Mikheev A, Nevsky G, Govindan S, Grossman R, Rusinek H. Fully automatic segmentation of the brain from T1-weighted MRI using Bridge Burner algorithm. *J Magn Reson Imaging.* 2008; 27(6):1235–1241. [PubMed: 18504741]
27. Goelman G, Liu S, Hess D, Gonen O. Optimizing the efficiency of high-field multivoxel spectroscopic imaging by multiplexing in space and time. *Magn Reson Med.* 2006; 56(1):34–40. [PubMed: 16767711]
28. Goelman G, Liu S, Fleysher R, Fleysher L, Grossman RI, Gonen O. Chemical-shift artifact reduction in Hadamard-encoded MR spectroscopic imaging at high (3T and 7T) magnetic fields. *Magn Reson Med.* 2007; 58(1):167–173. [PubMed: 17659608]
29. Mareci T, Brooker H. Essential considerations for spectral localization using indirect gradient encoding of spatial information. *J Magn Reson.* 1991; 92:229–246.
30. Brooker HR, Mareci TH, Mao JT. Selective Fourier transform localization. *Magn Reson Med.* 1987; 5(5):417–433. [PubMed: 3431402]

31. Soher BJ, Young K, Govindaraju V, Maudsley AA. Automated spectral analysis III: application to in vivo proton MR spectroscopy and spectroscopic imaging. *Magn Reson Med*. 1998; 40(6):822–831. [PubMed: 9840826]
32. Inglese M, Li BS, Rusinek H, Babb JS, Grossman RI, Gonen O. Diffusely elevated cerebral choline and creatine in relapsing-remitting multiple sclerosis. *Magn Reson Med*. 2003; 50(1):190–195. [PubMed: 12815694]
33. Kirov, Patil V, Babb JS, Rusinek H, Herbert J, Gonen O. MR spectroscopy indicates diffuse multiple sclerosis activity during remission. *J Neurol Neurosurg Psychiatry*. 2009; 80(12):1330–1336. [PubMed: 19546105]
34. Zaaraoui W, Fleysheer L, Fleysheer R, Liu S, Soher BJ, Gonen O. Human brain-structure resolved T(2) relaxation times of proton metabolites at 3 Tesla. *Magn Reson Med*. 2007; 57(6):983–989. [PubMed: 17534907]
35. Chard DT, McLean MA, Parker GJ, MacManus DG, Miller DH. Reproducibility of in vivo metabolite quantification with proton magnetic resonance spectroscopic imaging. *J Magn Reson Imaging*. 2002; 15(2):219–225. [PubMed: 11836781]
36. Tedeschi G, Bertolino A, Campbell G, Barnett AS, Duyn JH, Jacob PK, Moonen CT, Alger JR, Di Chiro G. Reproducibility of proton MR spectroscopic imaging findings. *AJNR Am J Neuroradiol*. 1996; 17(10):1871–1879. [PubMed: 8933871]
37. Li BS, Regal J, Gonen O. SNR versus resolution in 3D 1H MRS of the human brain at high magnetic fields. *Magn Reson Med*. 2001; 46(6):1049–1053. [PubMed: 11746567]
38. Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. *NMR Biomed*. 2004; 17(6):361–381. [PubMed: 15468083]
39. Li BS, Babb JS, Soher BJ, Maudsley AA, Gonen O. Reproducibility of 3D proton spectroscopy in the human brain. *Magn Reson Med*. 2002; 47(3):439–446. [PubMed: 11870829]
40. Ratai EM, Bombardier JP, Joo CG, Annamalai L, Burdo TH, Campbell J, Fell R, Hakimelahi R, He J, Autissier P, Lentz MR, Halpern EF, Masliah E, Williams KC, Westmoreland SV, Gonzalez RG. Proton magnetic resonance spectroscopy reveals neuroprotection by oral minocycline in a nonhuman primate model of accelerated NeuroAIDS. *PLoS One*. 2010; 5(5):e10523. [PubMed: 20479889]
41. Gonzalez RG, Cheng LL, Westmoreland SV, Sakaie KE, Becerra LR, Lee PL, Masliah E, Lackner AA. Early brain injury in the SIV-macaque model of AIDS. *AIDS*. 2000; 14(18):2841–2849. [PubMed: 11153665]
42. Tracey I, Lane J, Chang I, Navia B, Lackner A, Gonzalez RG. 1H magnetic resonance spectroscopy reveals neuronal injury in a simian immunodeficiency virus macaque model. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997; 15(1):21–27. [PubMed: 9215650]

Fig. 1.

Left: Axial (**a**) and sagittal (**b**) T_2 -weighted TSE MRI of a macaque head shows the location and geometry of the $4.0 \times 3.5 \times 2.0 \text{ cm}^3$ VOI (solid white frame) as well as the location of the two second-order Hadamard slabs (4 slices) in the IS direction (**1b**).

Top right (**c**): Real part of the $7 \times 8 \text{ (LR} \times \text{AP) cm}^2$ ^1H spectra matrix from the VOI in **a**, on common chemical shift and intensity scales. Note the spectral resolution and SNR (10.2 ± 2.1 , 5.3 ± 1.9 , 4.1 ± 1.5 and 4.5 ± 1.8 for NAA, Cr, Cho and *mI*) obtained in these 0.125 cm^3 voxels in 12.5 minutes of 3D ^1H -MRS acquisition.

Bottom right (**d**): Metabolic maps for NAA, Cr, Cho, *mI* obtained from the spectra in **c**. Note the reproducibility of the gross anatomical features, *e.g.*, lateral ventricles in the MRI in **a**, for each of the metabolites, reflecting the SNR *and* localization performance.

Fig. 2.

Top, left: **a:** Axial T_2 -weighted TSE image shows the anatomical coverage of the 4.0×3.5 cm^2 VOI. **b – d:** same as **a**, overlaid with the CSF, GM and WM masks generated by the FireVoxel package and used to obtain tissue, GM and WM fractions in the VOI for Eq. [2]. Note the accurate CSF and each different tissue type differentiation.

Fig. 3. Top, **a**: Single 0.125 cm³ voxel spectrum from a single scan in one of the animals. Center, **b**: Sum of the 224 spectra in the VOI (equivalent to its single-voxel acquisition). Note the improved SNR but increased linewidths reflecting **B₀** variations in the VOI. Bottom, **c**: Same as **b** but the spectra were pre-aligned before summation. Note the high SNR as in **b** but linewidth similar to **a** reflecting the advantage of pre alignment to minimize susceptibility effects in the VOI and retain single-voxel spectral resolution.

Fig. 4.

Real part of the 224 aligned and summed spectra from the entire 28 cm³ VOI from each of the 5 animals (**1**, **2**...**5**), serial sessions (up to three per animal: **a**, **b**, **c**) and the 4 back-6to-back scans in every session (*i*, *ii*, ... *iv*) (thin black line), corresponding to the labels in Table 1. Each spectrum is overlaid with its fitted model function (thick gray line). All spectra are on common intensity and chemical shift scales. Note, (*i*) the higher (174 ± 11 , 96 ± 6 , 55 ± 6 and 45 ± 7 for the NAA, Cr, Cho and *mI*) SNRs for the sums compared with Fig. 1; (*ii*) the quality of the spectral fit; and (*iii*) the similarity of the spectra within scans, sessions and macaques, reflecting *inter*- and *intra*-animal reproducibility.

Fig. 5.

Top, right: Box plots displaying the 25th, median, 75th (box) and $\pm 95\%$ -tiles (whiskers) of the NAA, Cr, Cho and mI concentrations distributions for all voxels of a scans (*inter-voxel*), sessions across all macaques (*inter-animal*), sessions of an animal (*intra-animal*) and all scans for a session (*intra-scan*). Note the $\times 4$ to $\times 11$ fold, improvement in both *inter*- and *intra*-animal reproducibility of the sums versus with the single voxels. Insert: Box plots of the GM, WM and tissue fractions (Gm_f, Wm_f, and T_f) distributions in the VOIs of all animals. Note the narrow distribution of tissue types, indicating the minimal GM/WM/CSF partial volume repositioning error of the proposed approach.

Fig. 6.

Left: Real part of the VOI (aligned and summed) spectrum from a healthy macaque (black line) superimposed with its spectral fit function (thick gray line). The arrows next to each peak indicate their respective *intra*-animal CVs ($\pm 4\%$, $\pm 5\%$, $\pm 5\%$, $\pm 4\%$ for NAA, Cr, Cho and mI). Right: The spectrum from same animal after 6 weeks of simian immunodeficiency virus (SIV) infection and CD8⁺ lymphocyte depletion. Both spectra are on common intensity and frequency scales. Note the marked NAA and Cho decline post SIV infection but that the Cr, although visually lower is still within the CV whereas the mI is elevated. These changes would have most likely not been significant and possibly not even detectable at the much lower sensitivity of single voxels (compare corresponding box plots in Fig. 5).

Volumetric (MRI) and metabolic (MRS) data for each of the 5 animals (labeled **1**, **2**, ... **5**), in every serial session (up to 3 per animal – labeled **a**, **b**...**c**) and 4 back-to-back scans each session (labeled: *i*, *ii*, ... *iv*) in this study.

Table 1

Animal (session)	Scan	MRI				^a MRS			
		VOI WM fraction	VOI GM fraction	VOI Tissue fraction	NAA	Cr	Cho	mI	
1 (a)	<i>i</i>	0.43	0.57	0.96	7.8	7.4	1.1	3.6	
	<i>ii</i>				8.1	7.7	1.2	4.2	
	<i>iii</i>				7.5	7.2	1.1	4.2	
	<i>iv</i>				7.6	7.5	1.2	4.0	
(b)	<i>i</i>	0.45	0.55	0.97	7.9	7.6	1.3	4.2	
	<i>ii</i>				7.2	7.2	1.2	3.1	
	<i>iii</i>				7.1	7.1	1.1	4.0	
	<i>iv</i>				7.3	7.0	1.3	3.7	
(c)	<i>i</i>	0.43	0.57	0.95	7.6	7.1	1.1	4.1	
	<i>ii</i>				7.7	7.2	1.1	4.0	
	<i>iii</i>				7.5	7.1	1.2	4.0	
	<i>iv</i>				7.3	7.1	1.1	4.0	
2 (a)	<i>i</i>	0.45	0.55	0.97	8.8	7.5	1.2	4.4	
	<i>ii</i>				8.0	7.3	1.2	4.1	
	<i>iii</i>				8.7	7.8	1.1	4.2	
	<i>iv</i>				8.1	7.5	1.2	4.1	
(b)	<i>i</i>	0.45	0.55	0.97	8.6	7.8	1.1	4.5	
	<i>ii</i>				8.7	7.5	1.1	4.1	
	<i>iii</i>				8.7	7.6	1.2	4.5	
	<i>iv</i>				8.9	7.6	1.2	4.7	
(c)	<i>i</i>	0.40	0.60	0.95	7.4	6.6	1.3	4.3	
	<i>ii</i>				7.5	6.1	1.4	5.4	
	<i>iii</i>				8.4	7.3	1.3	5.0	
	<i>iv</i>				7.5	6.4	1.2	4.0	
3 (a)	<i>i</i>	0.48	0.52	0.97	7.3	6.0	1.1	3.2	

Animal (session)	Scan	MRI				^a MRS			
		VOI WM fraction	VOI GM fraction	VOI Tissue fraction	NAA	Cr	Cho	mI	
(b)	ii				8.0	6.9	1.1	2.7	
	iii				7.0	6.3	1.2	2.6	
	iv				7.1	6.0	1.1	2.7	
	i	0.44	0.56	0.97	7.4	6.6	1.2	2.5	
	ii				7.6	6.5	1.1	3.5	
	iii				7.6	6.6	1.0	3.5	
	iv				7.3	6.4	1.2	3.3	
4 (a)	i	0.41	0.59	0.96	7.4	7.0	1.4	4.5	
	ii				6.9	6.6	1.3	4.4	
	iii				7.3	6.8	1.4	4.4	
	iv				7.5	7.0	1.4	4.4	
5 (a)	i	0.43	0.57	0.95	8.1	7.4	1.3	4.3	
	ii				7.7	7.2	1.2	4.0	
	iii				7.6	7.0	1.2	4.2	
	iv				7.8	7.1	1.2	4.3	
Mean±SD		0.44±0.02	0.56±0.02	0.96±0.01	7.7±0.5	7.0±0.5	1.2±0.1	4.0±0.6	

^a Absolute average concentration in the VOI in millimoles/g wet weight.

Coefficients of variation (CVs in %) associated with the *intra*-voxel [“the residual error” term in the model of Chard *et al.* (35)], *inter*-animal and *intra*-animal (*inter*- and *intra*-session) reproducibility of all metabolite concentrations in the VOI.

Table 2

	<i>Intra</i> -voxel (“Error”)	<i>Inter</i> -animal	<i>Intra</i> -animal		Total variance
Metabolite			<i>Intra</i> -session ^a		<i>Inter</i> -session
NAA	22.0	4.3	3.9	4.0	6.9
Cr	32.8	4.0	3.7	4.9	7.0
Cho	35.9	5.8	5.6	4.5	8.3
<i>mI</i>	44.7	14.5	8.5	3.5	15.9

^aBack-to-back scans.