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

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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 multiple sclerosis (1), and neuroAIDS (2). Because of the prohibitive cost of performing destructive studies, especially if they involve serial observations, non-invasive imaging methods, such as MRI for morphology and proton MR spectroscopy (^{1}H-MRS) for metabolism, are often the modalities of choice. Indeed, *in vivo* ^{1}H-MRS can monitor the *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho) and *myo*-inositol (*m*I) signals, the putative markers for neuronal integrity, cellular energy status, membrane turnover rates and glial proliferation, respectively (3). Although the reproducibility metric is essential for adequately powered study design and to distinguish real changes from biological and instrumental noise (4), its global average values for this species and their range have not been reported. Our two primary goals, consequently, are: *(i)* to establish the macaque brain's average NAA, Cr, Cho and *m*I concentrations and their cross-sectional (*inter*-animal) and longitudinal (*intra*-animal) variations in a large, 28 cm^3 (~35%) volume of interest (VOI) in the macaque brain; and *(ii)* demonstrate the method's sensitivity to distinguish pathological change from biological and instrumental variations. Towards these ends we applied test-retest 3D multivoxel ^{1}H-MRS to the brain of five healthy rhesus macaques at 3 T.

Methods: All experiments were done in a 3-T MR imager (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) with a circularly-polarized transmit-receive human knee coil. To guide placement of the ^{1}H-MRS VOI, sagittal and axial turbo spin echo MRIs (TE/TR=16/7430 ms, 140×140 mm^2 field-of-view (FOV), 512×512 matrix, 2.0 mm sagittal and 1.2 mm axial slice thickness) were acquired. A 4.0 cm anterior-posterior (AP) × 3.5 cm left-right (LR) × 2.0 cm inferior-superior (IS) =28 cm^3 ^{1}H-MRSI VOI was then centered on the corpus callosum. The VOI was excited using PRESS (TE/TR=33/1440 ms) with two 2nd-order Hadamard encoded slabs (4 slices) interleaved within every TR. These slices' planes were encoded with 16×16 2D-CSI over an 8×8 cm^2 (LR×AP) FOV to yield 224 voxels, (0.5 cm)^3 each, in the VOI. 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 SNR by $224^{½} \approx 15$. Relative levels of the i^{th} (NAA, Cr, Cho, *m*I) metabolite in the j^{th} animal were estimated from their peak areas, S_{ij}, using parametric spectral modeling and least-squares optimization software by Soher *et al.* (5). The S_{ij} were then scaled into absolute concentrations relative to signals from a 2 L sphere of 12.5, 10.0, 3.0 and 7.5 mM NAA, Cr, Cho and *m*I in water, by phantom replacement as described previously (6).

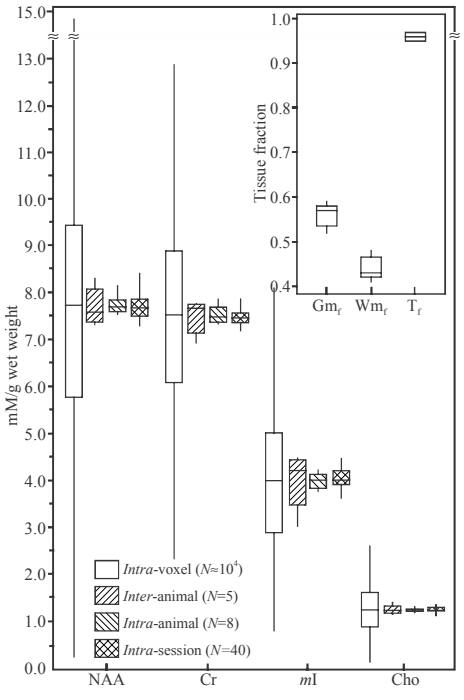

Fig. 1. Box plots displaying the 25th, median, 75th (box) and ±95%-tiles (whiskers) of the NAA, Cr, Cho and *m*I concentrations distributions for all voxels of all scans (*intra*-voxel), sessions across all macaques (*inter*-animal), sessions of an animal (*intra*-animal) and all scans for a session (*intra*-session). Note the ×4 to ×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 fraction (GM$_f$, WM$_f$, and T$_f$) distributions in the VOIs of all animals. Note the narrow distribution of tissue types, indicating minimal GM/WM/CSF partial volume repositioning error in the proposed approach.

Five healthy adult (3 females, 2 males; all 3 years old; 4.3 – 5.6 kg weight) rhesus macaques were scanned one to three times. Each was tranquilized as described previously (7). One macaque was subsequently infected by intravenous injection with simian immunodeficiency virus (SIV) and then rescanned 6 weeks later. Animals were under constant veterinary supervision.

To determine *intra*-animal reproducibility, two macaques each underwent three separate ^{1}H-MRS sessions and one macaque underwent two. To determine *inter*-animal reproducibility, two more macaques each underwent one session (total of 10 sessions from 5 animals). To determine the variance due to VOI repositioning, four back-to-back scans were acquired at each session with all experimental parameters kept unchanged for a total of 40 full 16×16×4 3D ^{1}H-MRS data sets.

Results: Mean NAA, Cr, Cho and *m*I concentrations in the macaque brain were: 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) were 4%, 4%, 6% and 15%; and the *intra*-animal CVs were lower still: 4%, 5%, 5% and 4%, much better than the 22%, 33%, 36% and 45% *intra*-voxel CVs.

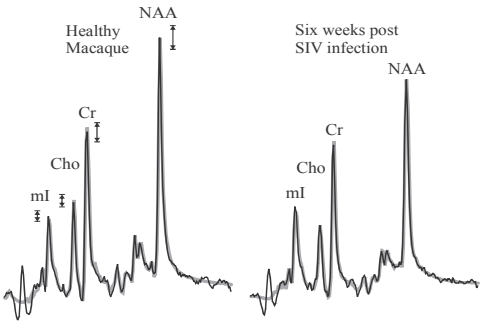

Fig. 2. Left: Real part of the VOI (aligned and summed) spectrum from a healthy macaque (black line) superimposed with its spectral fit function (thick gray line). The arrows next to each peak indicate their respective *intra*-animal CVs (±4%, ±5%, ±5%, ±4% for NAA, Cr, Cho and *m*I). Right: The spectrum 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 *m*I is elevated.

Discussion: As shown in Fig. 1, summing all phased and aligned elements in the VOI achieves ×4 - ×11 fold better CVs than a single-voxel method by exploiting $\mathbf{B_0}$ homogeneity (narrow linewidth) across individual 0.125 cm^3 voxels**.** The improvement is demonstrated by the subtle (but diffuse) changes brought about by SIV infection in a macaque brain 6 weeks post infection (Fig. 2); changes are both clearly visible and significant with this approach in a single animal. The ‹15% *inter*-animal CVs indicate good cross sectional similarity between healthy animals. The longitudinal *intra*-animal reproducibility is even better, exhibiting CV's below 10% across the board. 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.

References: (1) Richards TL *et al. NMR Biomed* 1995 (2) Ratai EM *et al.BMC Neurosci* 2009 (3) Urenjak J *et al. J Neurosci* 1993 (4) Greco J *et al. J Med Primatol* 2002 (5) Soher BJ *et al. Magn Reson Med* 1998 (6) Inglese M *et al. Magn Reson Med* 2003 (7) Liu S *et al. Magn Reson Med* 2009