Indication of oxidative stress in Multiple Sclerosis using proton MR spectroscopic imaging at 7T

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Introduction

Oxidative stress has been implicated in inflammatory demyelinative diseases such as Multiple Sclerosis (MS). The glutathione S-transferase (GST) supergene family encodes isoenzymes involved in Glutathione [GSH] metabolism that appear to be critical in protection against oxidative stress and affects long term prognosis in MS [1]. Genetic markers of GST were differentially expressed in chronic and acute plaque tissue relative to MS white matter [2]. Hence, *in-vivo* detection of glutathione might be a marker for disease phenotype and oxidative status in MS. GSH is present in low concentrations in the brain and overlaps strongly with creatine in the ^{1}H MR spectrum. GSH detection therefore benefits from improved SNR and increased spectral separation at 7T. Here we develop an acquisition scheme for the unambiguous detection of GSH over a two-dimensional (2D) region and use it to investigate GSH concentrations in normal controls and MS patients in the white and gray matter.

Methods: Design and implementation: A high pass filter (stopband width = 300 Hz) was designed to suppress GSH at 4.56 and refocus its coupled partner at 2.95 ppm within a spectral editing scheme. To maintain high selectivity the transition width was set to 100Hz to allow a +50Hz tolerance to any B0 induced frequency shift before the passband resonance of Glx at 3.7 ppm is affected. The resulting 20ms RF pulse was incorporated into the BASING [3] acquisition scheme. The robustness of the editing scheme to generate an unobstructed GSH resonance was validated in a phantom containing creatine, GSH and NAA at an echo time of 1/J (=136 ms).
Data Acquisition: Data were acquired on a 7T GE Signa scanner (GE Healthcare, Waukesha) using an 8-channel phased array (Nova Medical) coil. Two-dimensional spectroscopic ^{1}H proton spectroscopic imaging data were acquired at a resolution of 1.2x1.2x2.0 cm using the BASING acquisition scheme with VAPOR water suppression at a TR = 2s. With elliptical k-space sampling and a NEX = 11 the scan time was ~ 17 mins. A high resolution image (512x512, 18cm FOV, NEX = 3, TE/TR = 11/250 ms, 2mm/6mm slice/gap) was acquired to prescribe the single spectral slice above the corpus callosum and used for segmentation. A custom shimming routine [4] was iterated twice to compensate for B0 inhomogeneities. Two healthy controls and two MS patients (1M/1F) were scanned using this protocol.
Spectral Quantification: LCmodel was used to fit the edited spectrum using a basis set that consisted of GSH and the co-edited aspartyl resonance of NAA (asp-NAA). Relative signal intensities of fitted GSH and asp-NAA calibrated from a phantom containing equi-molar (=12mM) concentrations of GSH and NAA was used to estimate *in-vivo* GSH concentrations from the signal intensity of fitted GSH and asp-NAA. B1 maps were generated using the ratio of two flip angle (60^o/120^o) images [5] at a TR of 10s over the spectral slice. The deviations from nominal flip angle obtained from the B1 maps for each voxel were used to adjust the gain of the spectra prior to LCmodel quantification to account for non-uniformities in excitation.
Estimation of GSH concentration in white and gray matter: The high resolution images were segmented into gray matter (GM), white matter (WM), and cerebro-spinal fluid (CSF) and re-grid to the spectroscopic resolution to yield the percent WM and GM within each voxel. By modeling the metabolite concentrations as a linear function of WM content, "pure" GM and WM metabolite concentrations were extrapolated from the end points of the linear fit [6] for each subject. Spectroscopic voxels were included in the linear fit only if the GSH estimate was within 20% Cramer-Rao bounds.

Results: As shown in **Figure 1(A)** from a control, GSH is well detected in the edited spectrum in each voxel from the two-dimensional ROI shown in **Figure 1B**. The GSH resonance at 2.95 ppm is distinct from creatine resonance at 3.0 ppm (**Figure 1C:** dashed line) and is well separated from the co-edited aspartyl resonance. SNR of the *in-vivo* GSH resonance is shown in (A) for each voxel, was found to be similar to the SNR of Glutamate at 3T in our previous studies [7]. The linewidth of NAA in the reference spectrum is ~ 14Hz indicating that good B0 homogeneity was obtained. **Figure 2** shows the 2D GSH metabolite map from a control (top) and an MS patient (bottom). An overall reduction in GSH concentration is seen in the WM and GM in MS relative to controls but the statistical significance of this reduction will be determined with more subjects. **Figure 3** shows the linear fit to estimate "pure" WM/GM concentrations using all data from controls (black) and MS patients (red). Using the end points of this fit, the GSH levels in MS normal appearing white matter (NAWM)/normal appearing gray matter (NAGM) = 2.9/2.4 mM was reduced relative to control WM/GM = 3.4/2.7 mM. Signal variations due to effects of non-uniform excitation on coupled spins is a possible source of variability in the fit.

Conclusions: These data demonstrate the unambiguous detection of GSH at 7T with sufficient SNR to detect gray and white GSH differences. The level of detection from GSH which is present at low concentrations in the human brain demonstrates sensitivity and potential of 7T MRSI. While these preliminary data indicate that oxidative stress is present in MS brains and could contribute to neurodegeneration, more data will be acquired to confirm this trend.

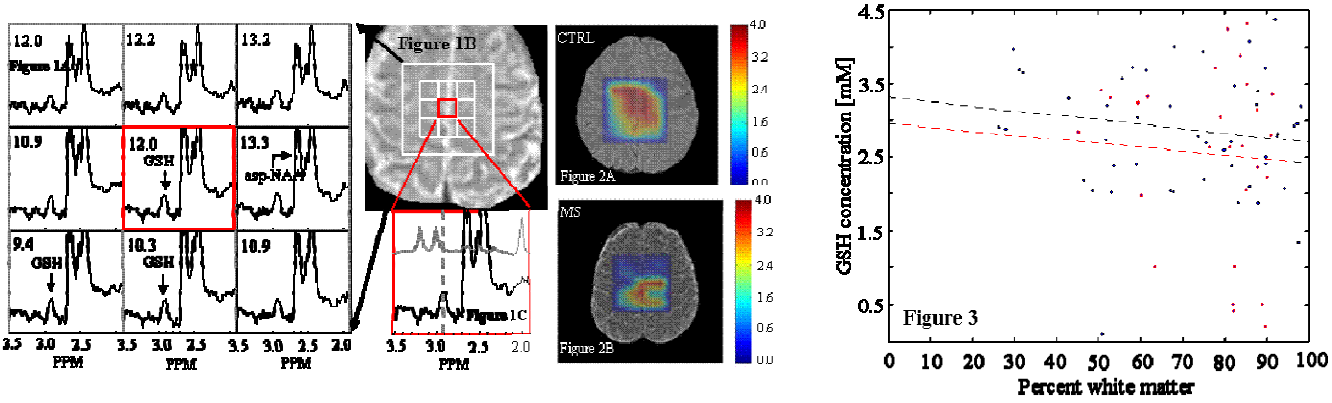

Figure 1: Spectral grid (A) from a control demonstrating the unobstructed detection of GSH resonance at 7T from the two-dimensional ROI (B). The dashed line in (C) indicates that GSH in the edited spectrum is separate from its overlapping creatine resonance and well separated from co-edited asp-NAA. SNR of GSH is reported for each voxel. **Figure 2**: GSH map from a control (top) and an MS patient (bottom). Overall the GSH concentration appears to be lower in MS relative to controls but the statistical significance will be determined with more subjects. **Figure 3**: Linear fit of the GSH concentration to the % WM in each voxel for controls (Black) and MS (red). The "pure" WM/GM GSH concentration, extrapolated from the end points of the fit, is lower in MS relative to controls.

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