Detection of glutamate, glutamine, and glutathione by RF suppression and TE optimization at 7T

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Target audience: Scientists and clinicians who are interested in the *in vivo* measurement of glutamate (Glu), glutamine (Gln), and glutathione (GSH) using magnetic resonance spectroscopy (MRS).

Purpose: Glu and Gln play important roles in brain chemical and neurobiological processes. Accurate measurement of Glu and Gln using *in vivo* MRS is hampered by spectral overlap. A TE-optimized PRESS (point-resolved spectroscopy) pulse sequence has been proposed to resolve and measure Glu and Gln at 7T (1). In this study, we optimized the TE values of the pulse sequence, validated the numerical simulations through phantom experiments, and performed MRS scans on eight healthy volunteers for *in vivo* quantification of glutamate, glutamine and other metabolites such as GSH.

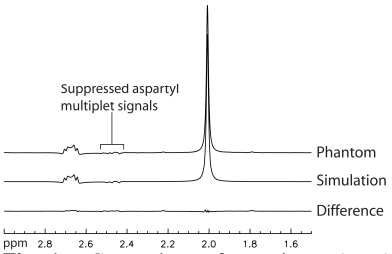

Fig. 1. Comparison of experimental and numerically simulated NAA spectra using the J-suppression pulse.

Methods:

Pulse sequence optimization: The pulse sequence consists of PRESS localization with a J-suppression RF pulse inserted at the midpoint between the two refocusing pulses. The J-suppression pulse is a frequency selective sinc-Gauss RF pulse (bandwidth = 158 Hz) placed at the resonance frequency of the aspartyl CH proton of N-acetyl-aspartate (NAA) at 4.38 ppm, which alters the J-evolution of the NAA aspartyl CH_2 multiplet at 2.49 ppm. Density matrix simulations using experimental RF pulse shapes with 3D localization were performed to find an optimized set of values for TE_1, TE_2, and the J-suppression pulse flip angle to minimize the NAA multiplet at 2.49 ppm and thus reduce interference in the detection of the C4 protons of Gln (2.45 ppm) and GSH (2.54 ppm). The excitation and refocusing pulses were all amplitude-modulated and their bandwidths were 3.1 kHz and 2.0 kHz respectively. In Ref. (1), TE_1 = 70 ms, TE_2 = 40 ms, and J-suppression pulse flip angle = 90° were used. Using an improved optimization process in this study, we found that TE_1 = 69 ms, TE_2 = 37 ms, and J-suppression pulse flip angle = 90° resulted in reduced NAA multiplet signals at 2.49 ppm while retaining near-maximum peak amplitudes for Glu, Gln, and GSH C4 protons.

Phantom experiments: A 50 mM NAA phantom built in-house was scanned at 37ºC on a Siemens 7T scanner equipped with a 32-channel receiver head coil using the proposed pulse sequence with TR = 2.5 s, TE_1 = 69 ms, TE_2 = 37 ms, J-suppression pulse flip angle = 90°, spectral width = 4000 Hz, number of data points = 2048, and number of transients = 16. Water suppression was accomplished using eight RF pulses of ~350 Hz bandwidth. The reconstructed spectrum of the phantom was fitted with a density matrix simulated NAA signal.

In vivo experiments: Eight healthy volunteers, who gave informed consent in accordance with procedures approved by the local institutional review board, were scanned using similar sequence parameters as those of the phantom experiments except that the number of transients was increased to 128. For each subject, MRS data were collected from two 2×2×2 cm^3 voxels, one in the medial prefrontal cortex and the other in the right frontal cortex. After spectra were reconstructed, a linear combination fitting program developed in-house and a simulated basis set containing NAA, N-acetyl-aspartyl-glutamate (NAAG), γ-aminobutyric acid (GABA), Glu, Gln, GSH, aspartate (Asp), creatine + phosphocreatine (tCr), glycerophosphocholine + phosphocholine (tCho), and myo-inositol (mI) were used to fit the spectral data between 1.8-3.3 ppm. Cramer-Rao lower bounds (CRLB) for the quantifications were also computed.

Table 1. Mean metabolite ratios in the frontal lobe WM and GM regions of eight healthy volunteers.

	Medial prefrontal cortex		Right frontal cortex	
	Metabolite ratios (/[Cr])	CRLB (%)	Metabolite ratios (/[Cr])	CRLB (%)
Glu	1.17±0.07	0.8±0.1	1.06±0.09	1.2±0.1
Gln	0.25±0.03	4.8±0.7	0.20±0.04	8.8±1.7
GSH	0.21±0.02	4.8±0.5	0.27±0.03	5.4±0.9
NAA	1.52±0.14	0.5±0.1	1.90±0.18	0.5±0.1
NAAG	0.12±0.04	3.0±1.6	0.35±0.09	1.4±0.5
tCr	1	0.5±0.1	1	0.7±0.1
tCho	0.30±0.03	0.6±0.1	0.35±0.03	0.7±0.1

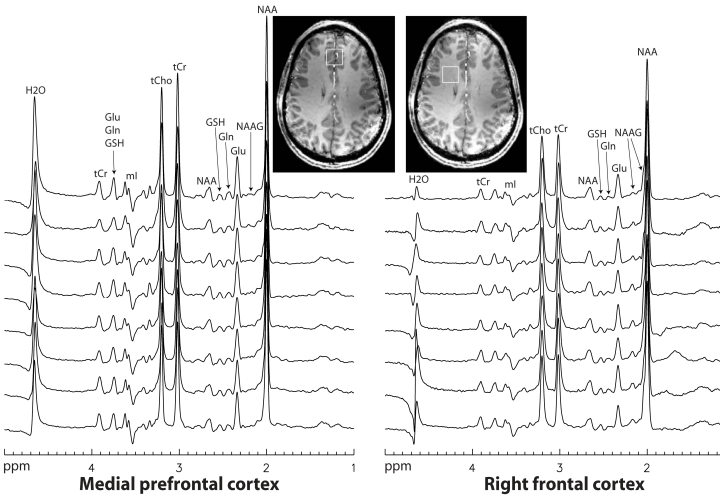

Fig. 2 Stack plots of spectra from the medial prefrontal cortex (GM voxel) and right frontal cortex (WM voxel) of eight healthy volunteers.

Results: The reconstructed spectrum of the NAA phantom is plotted in Fig. 1, along with the numerically simulated spectrum of NAA and the difference between the two. The experimental spectrum agrees very well with the simulated spectrum and confirms that our proposed pulse sequence results in very low NAA multiplet signals at 2.49 ppm. The reconstructed spectra of the eight volunteers are displayed in Fig. 2 with the height of each spectrum normalized by the total area of the NAA and NAAG peaks. In both medial prefrontal cortex and right frontal cortex, the Glu, Gln, and GSH peaks have well defined shapes and are adequately separated. On average, the Glu peak at 2.35 ppm and the Gln peak at 2.45 ppm are noticeably larger in the medial prefrontal cortex than in the right frontal cortex. Quantification results from the eight volunteers for Glu, Gln, GSH, NAA, NAAG, tCr, and tCho as ratios to tCr are given in Table 1. The Glu and Gln to tNAA ratios were also computed. The Glu/tNAA ratio was 0.72±0.05 in the medial prefrontal cortex and 0.47±0.04 in the right frontal cortex. Corresponding values for Gln/tNAA were 0.15±0.03 and 0.09±0.02. Both of the Glu/tNAA and Gln/tNAA ratios were significantly higher (two tailed t-test, $p < 0.001$) in the medial prefrontal cortex than in the right frontal cortex. Since tNAA is slightly higher in GM than WM (2), it can be concluded that the absolute concentrations of Glu and Gln are significantly higher in frontal lobe GM than in frontal lobe WM, which agrees with previous findings from neurochemical analysis of brain tissues (3).

Discussion and Conclusion: Phantom experiments and *in vivo* studies of eight healthy volunteers demonstrated that the TE-optimized PRESS sequence modified with a J-suppression pulse minimized the NAA aspartyl multiplet signals at 2.49 ppm while retaining excellent spectral resolution and peak amplitude for Glu, Gln, and GSH. In the medial prefrontal cortex, the Glu, Gln, and GSH to tCr ratios were found to be 1.17±0.07, 0.25±0.03, and 0.21±0.02, respectively. Corresponding values in the right frontal cortex were found to be 1.06±0.09, 0.20±0.04, and 0.27±0.03. To the best of our knowledge, this is the first report of significantly higher concentration of Gln in frontal lobe GM than in frontal lobe WM based on spectrally resolved Gln signal.

References:

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