Different Types of COSY Applied To Study Glutamate and Glutamine in a Clinical Scanner

Saadallah Ramadan[1], Hui Jun Liao Liao[2], Alexander Lin[2], and Carolyn Mountford[2,3]

[1]Centre for MR in Health, School of Health Sciences, University of Newcastle, Callaghan, New South Wales, Australia, [2]Center for Clinical Spectroscopy, Brigham and Women's Hospital, Boston, MA, United States, [3]Centre for MR in Healt, University of Newcastle, Callaghan, NSW, Australia

Introduction:
Two-dimensional (2D) magnetic resonance spectroscopy (MRS) was introduced by Aue et al (1) in 1976 by a simple two-pulse sequence (90x-t1-90x-Acq) which, after 2D Fourier transformation, produced a 2D COrrelation SpectroscopY (COSY) spectrum. This method when applied in vitro to cells and biopsies provided important information on the chemistry behind the development of human diseases (2). The COSY method has now been extended to examine the human brain *in vivo* and utilizes the L-COSY(3). Glutamate (Glu) is the primary excitatory neurotransmitter in the human cerebral cortex and its coupling with glutamine (Gln) is imperative for normal brain function. The ability to measure steady state glutamate and glutamine could provide insight to the molecular mechanisms of neurological diseases. Unfortunately, due to the strong j-coupling within these two molecules, and their structural similarity, their resonances overlap in 1D MR spectra as well as 2D COSY spectra. However, due to the additional dispersion in 2D COSY, there is a higher probability of discrimination between glutamate and glutamine, or at least determine their relative ratio. In order to better understand the ration of glutamate and glutamine in the human brain in vivo we first chose to evaluate the capacity of three different types of COSY acquisitions to measure the relative ratio of glutamate to glutamine in phantoms. The techniques used were the adiabatic localized COSY (AL-COSY) (3), constant-time COSY (CT-COSY) (4), and localized-COSY (L-COSY) (5).

Methods:
2D COSY: was acquired on a 3T clinical MR scanner (TIM Trio, Siemens, Germany, VB17A) using a 12 channel head coil. A 3x3x3 cm^3 voxel was localized on a 3D MRI with: RF carrier frequency at 2.0 ppm, TR 1.7 s, weak water suppression using WET, spectral width=2000 Hz, increments size of 0.8 ms in 64 t1 increments giving an indirect spectral width of 1250 Hz, 8 averages per increment, and 512 data points were acquired in 256 ms. Scan time was 13 minutes. Three types of COSY sequences were used; AL-COSY(3), CT-COSY (4) and L-COSY(5). Five different phantoms (250 ml each at physiological pH) were used: P1 (Glu=12mM, Gln=6 mM), P2 (Glu=18mM, Gln=6mM), P3 (Glu=12mM, Gln=12mM), P4(Glu=6mM, Gln=6mM), P5 (Glu=12mM, Gln=3mM), P6 (Glu=24mM, Gln=6mM),. Notice that P1, P2 and P4 have constant [Gln]=6mM, while P1, P3, P5 have constant [Glu]=12mM. All phantoms have a constant creatin concentration of 10 mM. In addition, one dimensional spectra were acquired from all phantoms.
Data processing: Commercial 2D spectral processing software (Felix-2007, Accelrys, San Diego, CA, USA) was used for spectral processing and analysis. Zero-padding to double the original data size, followed by apodization with skewed sine-squared window functions, in both dimensions was applied prior to magnitude two-dimensional Fourier transform (2DFT). The 2D spectra were chemical shift referenced and scaled to the prominent singlet diagonal peak of Cr (F2 = F1 = 3.02 ppm). Glx (Glu+Gln) cross peaks at (F2=3.7, F1=2.1)ppm and (F2=2.1, F1=3.72)ppm were integrated as shown in Figure 1. Peak ratios from each phantom were calculated and compared. Also, peaks integral from P2 and P3 ([Glx]=24mM) were computed and compared. All peak integrals were normalized to constant creatine.

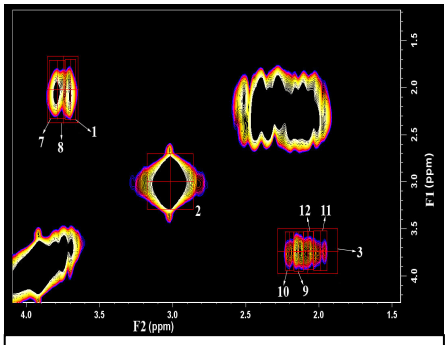

Figure 1. Typical Glu/Gln phantom showing picked cross peaks.

Results and Discussion:
Average integrals of diagonal peak of creatine (3.02,3.02) ppm were identical, within experimental errors, in all phantoms from the AL-COSY and L-COSY protocols, but was 11% higher in CT-COSY.

Cross peaks 8 and 3 were the most intense in the 2D spectrum, as shown in Figure 1. Cross peak 3 (pk3) and cross peak 8 (pk8) represent a mix of glutamate and glutamine crosspeaks and responded linearly to changes in [Glx], [Glu] and [Gln]. Integrals of all chosen cross peaks increased as the [Glx] increased indicating that Glx cross peaks can be used as a reliable indicator for Glx concentration. This can be seen in Figure 2.

The plot of various cross peaks for P2 (Glu=18mM, Gln=6mM, Glu/Gln =3, Glx=24mM) and P3 (Glu=12mM, Gln=12mM, Glu/Gln =1, Glx=24mM) acquired in AL-COSY, CT-COSY and L-COSY revealed that relative peak volumes did not change as a result of different ratios of Glu/Gln. Data from L-COSY is shown in Figure 3, and data from CT-COSY and AL-COSY behaved similarly. This observation confirms that while COSY can be used to monitor Glx, it cannot be used to gain an insight into separate glutamate and glutamine pools under these experimental conditions. Additionally, ratios of various peaks to each other in different phantoms were not able to predict the Glu/Gln ratio. This confirms the above finding in P2 and P3. Thus, frequency domain fitting seems incapable of discriminating Glu/Gln concentrations.

Frias-Martinez et al (6) reported superiority of ProFit (7) to LCModel (8) for reporting various cerebral metabolites including Glu and Gln with higher confidence limits. ProFit, however, is complicated and requires a large number of basis sets and prior knowledge for optimal fitting, and is based on both frequency and time domain fitting. We wanted, in this work, to focus on traditional frequency domain fitting processing techniques.

Using a statistical peak-detection and object segmentation algorithm developed by them, Cocuzzo et al (9) reported high P-values when attempting to correlate cross peak volumes with concentration of glutamate and glutamine, but low P-values for total glutamate/glutamine concentration when studying phantoms with different glutamate and glutamine concentrations. The explanation for these and our results requires further investigation.

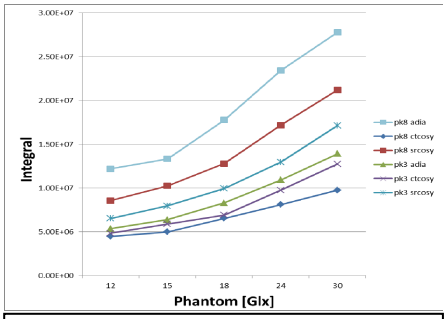

Figure 2. Pk3 and Pk8 cross peaks plotted versus different Glx concentrations.

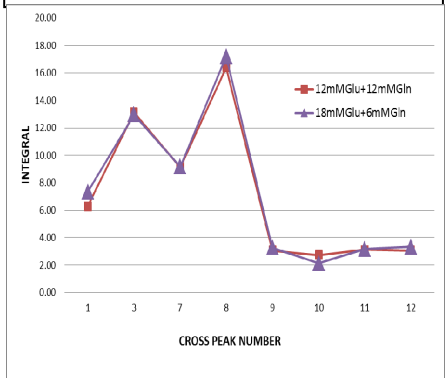

Figure 3. Plot of all cross peaks obtained in L-COSY spectra from two phantoms with same [Glx] but different Glu/Gln ratios.

Conclusion: 2D COSY can measure differences in the MR visible total glutamate and glutamine in phantoms but was unable to provide a reasonable estimate of glutamate and glutamine separately as individual component at 3T using a 12 channel head coil.

References: **1**. Aue WP, Bartholdi E, Ernst RR.. J Chem Phys 1976;64:2229-2246. **2**. Ramadan S, Mountford C. Two-Dimensional Magnetic Resonance Spectroscopy in Biopsy and In Vivo. In: Webb GA, ed. Annu Rep NMR Spectrosc. Volume 65. Burlington: Academic Press, 2009; 161-199. **3**. Ramadan S, Mountford C. J Magn Reson Imaging 2011;33:1447-1455. **4**. Ramadan S, Mountford C. Proc Intl Soc Mag Reson Med; 2009; Honolulu, USA. p 2357. **5**. Thomas MA, Yue K, Binesh N, et al. 2001;46(1):58-67. **6**. Frias-Martinez E, Rajakumar N, Liu X, et al. Proc Intl Soc Mag Reson Med; 2008; Toronto, Canada. p 691. **7**. Schulte RF, Boesiger P. NMR Biomed 2006;19(2):255-263. **8**. Provencher SW. NMR Biomed 2001;14:260-264. **9**.Cocuzzo D, Keshava N, Lin AP, Ramadan S, Mountford C. 33rd Annual International IEEE EMBS Conference; 2011; Boston, MA.