

## In vivo L-COSY MRS of Healthy Brain and Glioblastoma

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**Introduction:** The 2D COSY method was first applied to cells in 1984, animal tissue in 1988, and human tissue in 1993, human brain in vivo in 1994 and human immune deficiency in vivo in 2008. The first 'localized' in vivo human brain COSY in 1994 took 102 minutes with a region of interest (ROI) of 240 cm<sup>3</sup> at 2T (1). Independently in 2001, Thomas et al refined this method using a ROI of 27 cm<sup>3</sup> in 35 minutes at 1.5T using a 3-inch surface coil(2), whereas Ziegler et al(3) implemented the method in vivo on a 3T using a quadrature transceiver coil, and a ROI of 27 cm<sup>3</sup>. For review see Ramadan(4).

**Objective:** To develop in vivo two dimensional (2D) localized correlated spectroscopy (L-COSY) to study the biochemistry of the human brain and the pathology of diseases, such as glioblastoma (GBM), in a clinically applicable time at 3T using a 32 channel head coil; to determine the appropriate post processing parameters to allow diagnostic and prognostic molecules to be inspected; and ascertain how much information, recorded in vivo under these experimental conditions, is comparable with the COSY from malignant cultured cells.

**Methods:** L-COSY were acquired on a TIM Trio scanner (Siemens AG, Erlangen, Germany, VB17A), equipped with a receive-only commercially available 32 channel head coil. The L-COSY sequence employed used three shaped and spatially selective RF pulses. The excitation pulses were sinc RF pulses and the 180 RF pulses were optimized Mao pulses(5). The first two RF pulses generate a spin-echo, and then the third RF acts as a spatially selective and coherence transfer pulse. The sinc-shaped 90° and Mao-shaped 180° RF pulses durations were 2.6 and 4.6 ms, respectively. The TE (initial) was 30ms, TR:1.25 sec, 8 averages per increment, spectral width in F2 was 2000 Hz, t1 increment size of 0.8 ms, indirect spectral width used was 1250 Hz and the number of increments was 64. The "WET" water suppression method (6) was applied before the acquisition sequence generating an acquisition time of 11 min. L-COSY was applied to healthy controls (N= 6) and GBM (N=6). Spectra were processed, analyzed and peak volume ratios tabulated. The statistical significance values of all assigned cross and diagonal peaks were calculated by using a t-test. The data used was shown to be normally distributed by passing the Shapiro-Wilk normality test(7). The pathology and grade of each GBM was determined following biopsy or surgery. The study had IRB approval and was HIPAA compliant. The ROI was chosen following the contrast enhanced T1 weighted imaging. Localized shimming was performed by automatic adjustment of first- and second-order shim gradients using the automatic 3D Bo-field mapping technique (Siemens AG, Erlangen, Germany). Manual adjustment of the above mentioned shim gradients was undertaken to achieve a real peak width of water at half-maximum to be 10 Hz or less(8). Following frequency adjustment, water-selective suppression was optimized using the WET-technique.

**Data Processing:** Raw L-COSY data was transferred to Matlab (9), for signal combination from multiple elements followed by row concatenation into a 2D matrix and reformatting. Felix (10) was used for spectral processing and analysis. The processing parameters were deduced as described in Delikatny et al (11). The data was post processed to maximize visibility of those molecules with intermediate and long T2 relaxation values (11) with F2 domain (skewed sine-squared window, 2048 points, magnitude), F1 domain (sine-squared window, linear prediction to 90 points, zero-filling to 512 points, magnitude). The creatine methyl resonance at 3.02 ppm was used as an internal chemical shift reference in F1 and F2 (12).

**Results and Discussion:** the L-COSY method was collected in 11 minutes and a typical 2D L-COSY from a patient with a GBM is shown in Figure 1. When the cross peak volume ratios to creatine from healthy brain and GBM were compared, a statistical significance test (P-value < 0.05) showed lipid(+), alanine(+), N-Acetylaspartate(-),  $\gamma$ -aminobutyric acid(-), glutamine and glutamate(+), glutathione(+), aspartate(-), lysine(+), threonine(+),

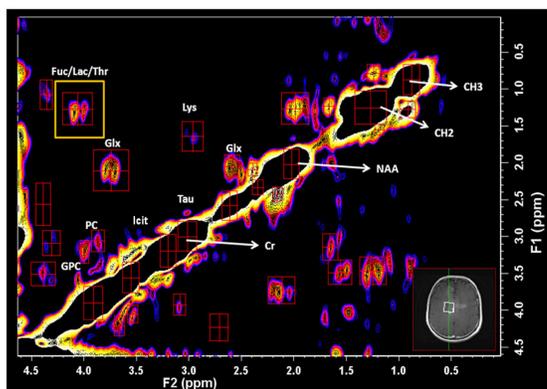


Figure 1: 3T L-COSY, 68yo GBM patient (voxel size shown in inset 25x25x20 mm3), increment size 0.8ms, increments 64, 8 averages per inc, TR 1.25 sec, total experimental time 11 min, acquired vector: 1024 points, acquisition time: 512 ms, spectral width in F2: 2000 Hz, spectral width in F1: 1250 Hz. Tumor filled voxel hence the contribution from normal brain tissue is minimal.

total choline(+), glycerophosphorylcholine(+), inositol(-), imidazole(+), uridine diphosphate glucose(+), iso-citrate(+), lactate(+), fucose(+), to be statistically different between the healthy and GBM cohorts, where (+) and (-) indicate increase or decrease of a particular metabolite, respectively, in GBM in comparison to healthy brain tissue. Of interest is a difference in the L-COSY spectra, between GBM and healthy controls in the methyl-methine coupling region -CH(OH)-CH<sub>3</sub> (F2: 1.00-1.60 ppm; F1: 3.90-4.50 ppm) (yellow square in Figure 1). In this region in healthy brain only the amino acid threonine is visible. In the glioma multiple resonances are seen and are comparable with the low and high

tumorigenic cell lines (13). These cross peaks are tentatively assigned to lactate, threonine, and a range of cell surface fucosylated species reported previously to be representative of loss of cellular differentiation (14) and hence a prognostic marker.

**Conclusion:** The L-COSY, acquired in vivo from GBM patients and healthy controls, at 3T using a 32 channel head coil within an acceptable clinical time and acquisition protocol and post processing parameters were acquired in 11 minutes. The excellent signal to noise of the in vivo GBM spectra allowed a comparison to be made with the biologically well documented cell lines grown in vitro which was unexpected.

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