In vivo L-COSY MR Distinguishes Glutamate from Glutamine and Shows Neuropathic Pain to Cause a Buildup of Glutamine in the Brain

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Introduction: Jeener introduced 2D magnetic resonance spectroscopy (MRS) in 1971(1) by proposing a simple two-pulse sequence (90x-t1-90x-Acq) which, after 2D Fourier transformation, produced a 2D COSY. This method when applied in vitro to cells an biopsies provided important information on 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 including pain. Unfortunately, due to the strong j-coupling between these two molecules, their peak resonances overlap one another in 1D MR spectra. 2D COSY overcomes this problem because it can measure scalar couplings between molecules and therefore, in theory, can separate the unique glutamate resonance. While the 1D TE-averaging method takes advantage of this physical property, the sequences isolate a single metabolite and cannot assess changes in the other major metabolites.

Objective: Alterations in neurotransmitters have been reported in chronic pain patients with migraine, low back pain spinal cord injury (27). Studies Specific metabolites such as glutamate, aspartate, glycine, and GABA have been found to alter. Our goal is to measure altered brain chemistry in patients with neuropathic pain using 2D COSY with a specific focus on the glutamate and glutamine metabolites.

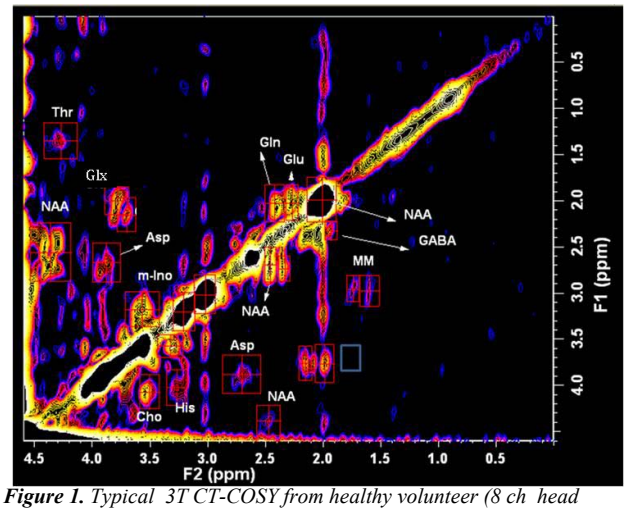

Figure 1. *Typical 3T CT-COSY from healthy volunteer (8 ch head coil)*

Methods: 2D COSY was acquired on a 3T clinical MR scanner (TIM Trio, Siemens, Germany) using an 8 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 96 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 22 minutes.

Standards of creatine (Cr) and Glu and Gln, (physiological concentrations and ph) were measured using the COSY method. **Patients and volunteers:** Five subjects with neuropathic pain and five age-matched controls were examined using the same parameters with a voxel centered at the posterior cingulate gyrus. **Data processing:** Raw COSY data was transferred to Matlab. Commercial 2D spectral processing software (Felix-2007, Accelrys, San Diego, CA, USA) was used for spectral reconstruction 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).

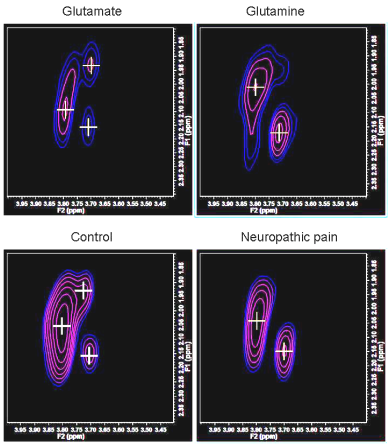

Figure 2. *Expanded region L-COSY F2 (1.6 – 2.4 ppm) and F2 (3.4 – 4.0 ppm) of Glu & Gln standards (top); healthy volunteer bottom left; neuropathic pain patient bottom right.*

Results and Discussion: A typical 2D COSY from a healthy volunteer is shown in Figure 1. A multitude of metabolites, not visible to conventional 1D MRS, such as GABA, amino acids (threonine, histidine, aspartate), and macromolecules as seen. In Figure 2, we expand the F2 (1.6 – 2.4 ppm) and F2 (3.4 – 4.0 ppm) region of the upper quadrant of the COSY spectra. The top row shows 2D COSY from of glutamate (left) and glutamine (right) where glutamate shows three distinct crosspeaks but glutamine only shows two. The crosspeak in glutamate is located lower in F1 (2.10 ppm than glutamine (2.02 ppm) . In the bottom row (Figure 2) the *in vivo* human 2D COSY, are shown one from a healthy control (left) and from a chronic neuropathic pain (trigeminal neuralgia) right. In the healthy control there should be roughly 2:1 glutamate and glutamine ie all the major crosspeaks expected for glutamate and glutamine should be seen which is the case. However in chronic neuropathic pain only two crosspeaks are evidence and these are consistent with glutamine. This finding suggests that the there is glutamergic dysfunction resulting in a buildup of MR visible glutamine.

Conclusion: 2D COSY can measure differences in the MR visible glutamate and glutamine in the human brain associated with chronic neuropathic pain, in a clinically acceptable time of 22 minutes at 3T using an 8 channel head coil.

References:

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