Imaging of Glutamate in the Spinal Cord using Chemical Exchange Saturation Transfer (CEST) at 7T

Feliks Kogan[1,2], Anup Singh[1], Mohammad Haris[1], Kejia Cai[1], Hari Hariharan[1], and Ravinder Reddy[1]

[1]*Center for Magnetic Resonance and Optical Imaging, University of Pennsylvania, Philadelphia, PA, United States,* [2]*Bioengineering Graduate Group, University of Pennsylvania, Philadelphia, PA, United States*

Introduction: Glutamate (Glu) is the primary neurotransmitter responsible for excitatory synaptic transmission in the brain stem and spinal cord. Glutamate has been implicated in a range of neurologic disorders that affect the spinal cord including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and spinal cord injury[1]. However, current imaging methods are unable to measure these changes with sufficient spatial resolution. Magnetic Resonance Spectroscopy (MRS) has been used to quantify concentrations of glutamate in the spinal cord and has shown that the concentrations are high enough for detection with MR.[2] However, poor spatial resolution and long acquisition times make the use of current MRS challenging for clinical use. In this study, we describe a novel, noninvasive approach for imaging glutamate in the spinal cord by exploiting the chemical exchange of protons between the amino group ($-NH_2$) of Glu and water using a technique known as chemical exchange saturation transfer (CEST)[3]. Previous work has shown that Glu exhibits a CEST effect which is linearly proportional to the Glu concentration[4]. In this work, we demonstrated the CEST effect from Glu *in vivo* in a healthy human spinal cord at 7T.

Methods: All imaging experiments were performed on a 7T whole body scanner (Siemens Medical Systems, Erlangen, Germany) using a 32 Channel transmit and receive head coil. All *in vitro* and *ex vivo* samples were prepared at a physiological pH (7.0) and temperature (37C). The CEST saturation pulse utilized a long irradiation pulse consisting of a series of 100 ms Hanning windowed saturation pulses ($B_{1,rms}$ = 3.6 μT for 1 second) followed by a segmented RF spoiled gradient echo (GRE) readout. Glutamate amine group protons have a chemical shift of 3.0 ppm from the bulk water resonance. $CEST_{asym}$ maps were computed by subtracting the normalized magnetization signal at the Glu proton frequency ($+\Delta\omega$), from the magnetization at the corresponding reference frequency symmetrically at the opposite side of the water resonance ($-\Delta\omega$).[4]

$$CEST_{asym} = \frac{M_{sat}(-\Delta\omega) - M_{sat}(+\Delta\omega)}{M_O} \quad [1]$$

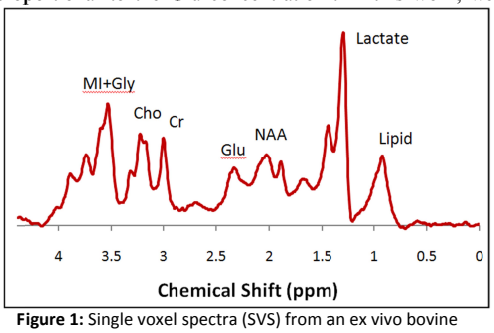

Figure 1: Single voxel spectra (SVS) from an ex vivo bovine spinal cord at 7T

Ex vivo Spectroscopy: A bovine cervical spine specimen was obtained from which the spinal cord was extracted and equilibrated in a bath of PBS solution for scanning. A single voxel proton MR spectrum (SVS) was acquired using a stimulated echo acquisition mode (STEAM) sequence and 100 signal averages.

In vitro Imaging: The CEST effect from all major metabolites present in the spinal cord as observed with MRS were imaged under physiological conditions in a phantom consisting of 10 mM N-acetyl aspartate (NAA), 8 mM choline (Cho), 10 mM myo-inositol (MI), 6 mM creatine (Cr), 6 mM glycine(Gly) and 10 mM Glu. $CEST_{asym}$ Maps were reconstructed according to Eq (1).

In vivo CEST Imaging: CEST imaging was performed on a healthy 26 year old human cervical spinal cord using the CEST imaging sequence described above. Two signal-averaged acquisitions were performed for calculating CEST maps using eq. (1). All CEST images and glutamate CEST contrast maps were corrected for B_0 and B_1 inhomogeneities. The average CEST values were calculated from the spinal cord grey and white matter as well as the cerebrospinal fluid (CSF) as determined by anatomical images.

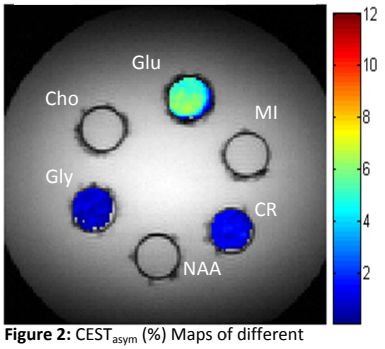

Figure 2: $CEST_{asym}$ (%) Maps of different metabolites present in the spinal cord (Corrected for B_0 & B_1). [10 mM NAA, 8 mM Cho, 10 mM MI, 6 mM Cr, 6 mM Gly and 10 mM Glu]

Results and Discussion: The high resolution single voxel spectra taken on the bovine spinal cord demonstrates that there are several metabolites with concentrations sufficient enough to be detected by current CEST methods (figure 1). Figure 2 shows that with the experimental parameters used to optimize the Glu CEST contrast, glutamate is still responsible for most of the CEST effect. A 6% CEST effect was observed from 10 mM glutamate with small contributions from glycine (~0.4 %) and Cr (~0.8%), and negligible contributions from all other metabolites. Of these metabolites, Cho does not have any exchangeable protons while NAA has an amide proton and at neutral pH does not exhibit any CEST effects. MI has exchangeable –OH protons and only exhibits a CEST effect at ≤1ppm. Cr and Gly both have amine protons that exhibit a CEST effect. However, at physiological pH, the Cr CEST effect occurs around 1.8ppm while Gly protons have much faster exchange rates than Glu and thus have minimal contributions when the optimal parameters for the Glu CEST effect are used at 3ppm. Finally, the CEST effect from amide protons, which has been demonstrated in the spinal cord in previous literature, has a much a slower exchange rate requiring a low saturation power and long saturation duration and will not be seen with the parameters used for Glu CEST[5]. Thus, these results demonstrate that with these particular parameters, the majority of the CEST contrast is due to glutamate. The glutamate CEST map obtained from a healthy human cervical spinal cord demonstrates a distinct gray (GM) and white (WM) matter distribution pattern (figure 3). As shown in table 1, the average $CEST_{asym}$ in the GM was 6.0% compared to 4.1 % in the WM. As supported by the phantom data, it is expected that the majority of the CEST contrast is from glutamate in the spinal cord with minor contributions from Cr and Gly. Thus these differences are due mainly to differences in the glutamate concentrations in GM and WM which have been previously shown in the brain[2]. A minimal CEST signal (0.8±0.7%) was observed in the cerebrospinal fluid (CSF) indicating that B_0 inhomogeneities, which are a major concern for CEST imaging at 7T, were adequately corrected for. The findings of this preliminary study suggests that the *in vivo* high resolution mapping of Glu is feasible using the CEST technique, which provides a new method to detect changes in Glu concentration in spinal cord disorders.

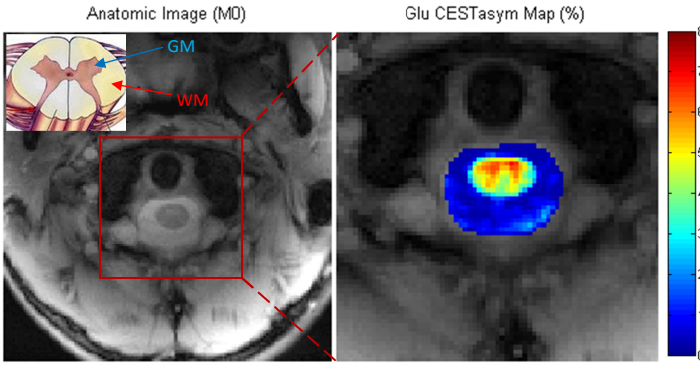

Figure 3: Anatomical image (left) and overlaid $CEST_{asym}$ map (right) of a healthy human cervical spinal cord showing significant distinction between WM, GM and CSF. (Spinal Cord anatomy is shown in upper left)

Table 1: Mean and (SD) Glu $CEST_{asym}$ (%) from the cervical spinal cord

	GM	WM	CSF
$CEST_{asym}$ (%)	6.0 (0.5) %	4.1 (0.9) %	0.8 (0.7) %

Conclusion: In this work, we demonstrated that it is feasible to detect the CEST effect from glutamate at 7T with high spatial resolution. We showed that with the optimal parameters for Glu CEST contrast, the majority of the CEST effect is due to Glu with minor contributions from Cr and Gly. CEST contrast from glutamate can be used to study relative distribution and any changes in Glu in the spinal cord *in vivo*.

References: [1] Leu et al. *Neuroscience*. 93(1999): 1383-1389, [2] Hurd *et al.Magn Reson Med.* 51(2004): 435-440, [3] Wolff et al. *J. Magn. Reson*. 86(1990): 164–169, [4] Cai et al. *ISMRM Proceedings* 2781, 2011, [5] Dula et al. *ISMRM Proceedings* 407, 2011