Published in final edited form as:

Neuroimage. 2013 August 15; 77: 262–267. doi:10.1016/j.neuroimage.2013.03.072.

Imaging of glutamate in the spinal cord using GluCEST

Feliks Kogan¹, Anup Singh¹, Catherine Debrosse¹, Mohammad Haris¹, Keija Cai¹, Ravi Prakash Nanga¹, Mark Elliott¹, Hari Hariharan¹, and Ravinder Reddy¹

¹Center for Magnetic Resonance and Optical Imaging, Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Glutamate (Glu) is the most abundant excitatory neurotransmitter in the brain and spinal cord. The concentration of Glu is altered in a range of neurologic disorders that affect the spinal cord including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and spinal cord injury. Currently available magnetic resonance spectroscopy (MRS) methods for measuring Glu are limited to low spatial resolution, which makes it difficult to measure differences in gray and white matter glutamate. Recently, it has been shown that Glu exhibits a concentration dependent chemical exchange saturation transfer (CEST) effect between its amine ($-NH_2$) and bulk water protons (GluCEST). Here, we demonstrate the feasibility of imaging glutamate in the spinal cord at 7T using the GluCEST technique. Results from healthy human volunteers (N=7) showed a significantly higher ($p < 0.001$) GluCEST_{asym} from gray matter ($6.6 \pm 0.3\%$) compared to white matter ($4.8 \pm 0.4\%$). Potential overlap of CEST signals from other spinal cord metabolites with the observed GluCEST_{asym} is discussed. This noninvasive approach potentially opens the way to image Glu *in vivo* in the spinal cord and to monitor its alteration in many disease conditions.

Keywords

Chemical Exchange Saturation Transfer; Glutamate; Spinal Cord; MRI

Introduction

Glutamate (Glu) is the most abundant neurotransmitter responsible for excitatory synaptic transmission in the brain and spinal cord. Glutamate is an amino-acid which is stored in vesicles and released in the synaptic space, where it binds to and activates its postsynaptic receptors (Nakanishi, 1992; Petroff, 2002). Under pathological conditions, an excess of glutamate in the synaptic space can trigger a toxic cascade leading to cell death (Lipton and Rosenberg, 1994; Liu et al., 1999). This excitotoxicity due to 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 (Rothstein et al., 1992; Srinivasan et al., 2005; Xu et al., 2004).

© 2013 Elsevier Inc. All rights reserved.

Corresponding Authors Feliks Kogan fkogan@seas.upenn.edu Ravinder Reddy ravi@mail.mmrcc.upenn.edu Center for Magnetic Resonance and Optical Imaging (CMROI) Department of Radiology University of Pennsylvania B1 Stellar-Chance Laboratories 422 Curie Boulevard Philadelphia, PA 19104-6100 Tel: (215) 898-9357 Fax: (215) 573-2113 .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Low concentrations make high resolution imaging of glutamate with conventional spectroscopic based methods impractical. In addition, limitations such as the small size of the cord, the motion artifacts arising from respiration and cardiac and cerebro-spinal fluid (CSF) pulsations make spinal cord imaging challenging. Magnetic resonance spectroscopy (MRS) can detect glutamate's signature groups using short echo times along with spectral editing techniques (Petroff et al., 2000; Srinivasan et al., 2006). In the brain, previous work has shown that MRS can detect higher glutamate content in brain gray matter than white matter. The major shortcoming of MRS is poor spatial resolution. This makes it particularly difficult to measure differences in gray and white matter glutamate. High resolution imaging may enable spatial mapping of Glu in a variety of spinal cord pathologies related to changes in Glu concentration.

Chemical exchange saturation transfer (CEST) is a sensitivity enhancement technique that is able to indirectly detect metabolite content based on their exchange-related properties (Forsen And Hoffman, 1963; Wolff And Balaban, 1990). CEST utilizes the reduction of the bulk water magnetization due to the exchange of saturated magnetization from exchangeable protons of solute metabolites with bulk water. This reduced magnetization manifests as image contrast due to the metabolite. CEST has been used to measure contrast from endogenous mobile proteins and metabolites in biological tissue (Haris et al., 2011; Jones et al., 2006; Zhou and van Zijl, 2006).

More recently, it has been shown that Glu exhibits a concentration dependent CEST effect (GluCEST) between its amine ($-NH_2$) and bulk water protons (Cai et al., 2012). Glu amine protons have an exchange site at ~ 3.0 ppm from water and the CEST effect from glutamate is linearly proportional to the Glu concentration in the physiological pH range. Imaging of glutamate in the brain was demonstrated at 7T, where CEST_{asym} maps showed distinct white matter, gray matter differences.

In this study, we demonstrate the feasibility of imaging glutamate in the human spinal cord using the GluCEST technique. The CEST effect from metabolites present in the spinal cord visible with 1H MRS as well as their potential contribution to the GluCEST effect is addressed. Finally, the advantages and challenges in GluCEST imaging human spinal cords at 7T as well as potential overlap with other metabolites *in vivo* are discussed.

Materials and Methods

CEST Technique

The CEST technique is a result of forward and back exchange of protons between the pool of exchangeable protons on the solute (metabolite) and a much larger pool of bulk water protons (Guivel-Scharen et al., 1998; Wolff And Balaban, 1990). Exchange of saturated magnetization from solute protons with water protons leads to the accumulation of saturated protons in the bulk water pool and results in a proportional decrease in water signal. While the saturation pulse is being applied, this process continues to decrease the observable water signal. Optimal performance of the CEST technique requires that a discrete chemical shift difference () between water and the exchangeable proton on the CEST agent is preserved, and the exchange rate (k_{sw}) is in the slow to intermediate exchange regime (k_{sw}) (Ward et al., 2000).

The factors that confound the CEST technique are the direct saturation (DS) of water and the background magnetization transfer (MT) effects in biological tissues. However, since the direct water saturation effects are symmetric with respect to the water resonance frequency , they can be removed by asymmetry analysis where the water signal from one side of the z-spectrum is subtracted from the other side (van Zijl and Yadav, 2011; Zhou and van Zijl,

2006). MT effects however are not always symmetric and may confound CEST asymmetry measurements. MT asymmetry is dependent on the applied saturation pulse parameters (40). A strong saturation pulse with short duration can be used to remove these asymmetric effects to a large extent. Thus, under certain saturation parameters (Saturation power $>3\mu\text{T}$), asymmetry analysis will also largely mitigate the confounding effects of MT asymmetry. Thus, to isolate the chemical shift of a particular metabolite, the CEST asymmetry ($\text{CEST}_{\text{asym}}$) is computed by subtracting the normalized magnetization signal at the exchangeable solute proton frequency [$M_{\text{sat}}(+)$], from magnetization at the corresponding reference frequency symmetrically at the opposite side of the water resonance [$M_{\text{sat}}(-)$] (Liu et al., 2010):

$$\text{CEST}_{\text{asym}} = \frac{M_{\text{sat}}(-\Delta\omega) - M_{\text{sat}}(+\Delta\omega)}{M_{\text{sat}}(-\Delta\omega)} \quad (1)$$

MT asymmetry, if present would act to reduce the observed $\text{CEST}_{\text{asym}}$ leading to underestimation of labile proton concentration.

The irradiation pulse needs to be strong in order for complete saturation of solute protons to occur, but not too strong to cause significant RF spillover or DS effects. The saturation can be controlled and optimized by using a combination of B_1 field strength and saturation time. As the amplitude and duration of the B_1 irradiation pulse are increased, there will be a maximum $\text{CEST}_{\text{asym}}$ that will occur when saturation transfer is close to its maximum and DS effects are small. These optimal parameters will depend on the exchange rate of protons and the ratio of the populations of the exchanging pools (Sun et al., 2005).

CEST Imaging on 7.0 T Whole Body MR Scanner

All imaging experiments were performed on a 7T whole body scanner (Siemens Medical Systems, Erlangen, Germany). A 32 channel ^1H Head coil (Nova Medical, Wilmington, MA) was used for phantom imaging. CEST imaging experiments utilized a max B_1 of 250 Hz ($B_{1\text{rms}}$ of 153 Hz ($3.6\mu\text{T}$)) and a 700 ms long saturation pulse train consisting of a series of 100 ms Hanning windowed saturation pulses followed by a segmented RF spoiled gradient echo (GRE) readout with centric phase encoding order. Localized shimming was performed to keep B_0 inhomogeneity less than ± 0.3 ppm. Water saturation shift reference (WASSR) images and B_1 maps were collected, as described previously (Kim et al., 2009; Singh et al., 2012). Glu amine protons have a chemical shift of 3.0 ppm down field from water and thus CEST images were collected in a frequency range of 2.5 ppm to 3.5 ppm with a 0.25 ppm step size to allow for adequate B_0 inhomogeneity correction.

Phantom Studies

The CEST effect from all major metabolites present in the central nervous system as observed with MRS (Jansen et al., 2006) were imaged under physiological conditions (37°C & $\text{pH}=7.0$) in a phantom consisting of samples 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 prepared to observe any potential contributions to the GluCEST effect. The imaging parameters were as follows: slice thickness = 10 mm, flip angle = 15° , $\text{TR} = 7.3$ ms, $\text{TE} = 3.6$ ms, field of view = $100 \times 100 \text{ mm}^2$, matrix size = 192×192 , with one saturation pulse train every 10 seconds. A Styrofoam holder was utilized to maintain the phantom temperature at $37 \pm 1^\circ$ for 45 minutes. $\text{CEST}_{\text{asym}}$ Maps were reconstructed according to Eq (1).

Human Studies

All studies were conducted under an approved Institutional Review Board protocol of the University of Pennsylvania. Informed consent from each volunteer was obtained after explaining the study protocol.

An 8 channel ^1H Head coil (Rapid Biomedical GmbH, Rimpf, Germany) was utilized for spinal cord imaging. The subjects were positioned such that the base of their brain stem was at iso-center. Localized shimming was performed in a voxel of the upper portion of the cervical spinal cord by manually adjusting first and second order shims to minimize the water linewidth in order to keep B_0 inhomogeneity less than ± 0.3 ppm. CEST imaging of the cervical spine was performed between the C1 and C2 vertebra at 7T on 7 healthy volunteers (3 female, 4 male, ages 24–62 years). In addition to GluCEST_{asym} maps, a full z-spectra was acquired on one subject from -5 ppm to 5 ppm with a step size of 0.25 ppm. Spinal cord CEST imaging was performed with the same CEST saturation pulse described for the phantoms and the following imaging parameters: slice thickness = 10 mm, flip angle = 15° , TR = 6.1 ms, TE = 2.9 ms, field of view = 160×160 mm², matrix size = 192×192 . The total scan time to acquire the GluCEST_{asym} map was ~ 8 minutes.

Data Processing

All image processing and data analysis was performed using in-house written MATLAB (Mathworks Inc., Natick, MA, version 7.5, R 2007b) scripts. Base images used to create CEST_{asym} maps, B_0 maps and B_1 Maps were registered employing non-rigid transformations using NIH ImageJ software (developed by Wayne Rasbands, National Institutes of Health, Bethesda, MD) prior to data processing. B_1 maps were created from two images obtained using preparation square pulses with flip angles of 30° and 60° . A B_1 calibration curve for the spinal cord tissue was developed from spinal cord CEST data at varying saturation amplitudes and used in conjunction with B_1 maps to correct for B_1 inhomogeneities (Singh et al., 2012). Similarly, B_0 maps were used to generate corrected CEST images (± 3.0 ppm.) using the WASSR method (Kim et al., 2009). CEST contrast was computed using equation (1) described above. Z-spectra were computed by plotting the B_0 corrected water magnetization as a function of saturation frequency offset. Similarly, asymmetry curves were calculated by applying equation [1] to each frequency acquired in the z-spectra. A region of interest was manually drawn by a single user (FK) around perceived areas of higher intensity to segment the cord and gray matter on anatomical GRE based images and applied to CEST_{asym} maps in order to determine differences in the CEST_{asym} in the gray matter (GM), white matter (WM) and cerebro-spinal fluid (CSF). A Paired student t-test between mean GM and WM GluCEST_{asym} values across the 7 subjects was performed in Matlab to measure statistical significance between GM/WM GluCEST_{asym}. Coefficient of variation of GluCEST from each volunteer was calculated as the standard deviation over mean.

Results

After optimization, for *in vivo* imaging with the 8 channel head coil, the optimal saturation parameters for GluCEST that satisfied scanner constraints as well as Food and Drug Administration's (FDA) Specific Absorption Rate (SAR) guidelines were a peak B_1 of 250 Hz (root mean squared $B_{1\text{rms}} = 153$ Hz [$3.6 \mu\text{T}$]) for a total duration of 700 ms. Figure 1 shows the CEST effect from the major metabolites detected by ^1H MRS at their physiological concentrations (10 mM NAA, 8 mM Cho, 10 mM MI, 6 mM Cr, 6 mM Gly, 10 mM Glu). With the saturation parameters described, glutamate is responsible for the majority of the CEST effect. A 4.3% ($\pm 0.4\%$) CEST_{asym} was observed from 10 mM Glu

with small contributions from Cr ($\sim 0.4 \pm 0.1\%$), and negligible contributions from all other metabolites.

Glutamate CEST maps of spinal cord from one volunteer are shown in figure 2. Local shimming of the main magnetic field in the spinal cord region created a fairly uniform B_0 field ($< \pm 0.2$ ppm) as seen in the B_0 field map in figure 2B. Similarly, the B_1 map shows small variation across the spinal cord region ($< 10\%$ of the reference B_1) (fig. 2c). The B_0 and B_1 corrected GluCEST_{asym} map of the spinal cord overlaid on the anatomical image is shown in figure 2d. Gray matter and white matter regions were manually segmented from anatomic images to determine the GluCEST_{asym} from each region. The segmented GluCEST_{asym} for GM and WM overlaid on the anatomic image are shown in figures 2e and 2f, respectively. Minimal CEST_{asym} was observed from the CSF ($< 0.5\%$).

Z-spectra and CEST asymmetry curves from GM and WM regions of the spinal cord were broad and showed a maximum asymmetry at ~ 1.5 ppm (Fig 3). The broadness in asymmetry is due in part to the faster exchange rate of Glu at a pH of 7.0, which shifts the line peak towards the water resonance due to a chemical shift averaging effect (Cai et al., 2012). Additionally, there is a potential contribution from the CEST effect from Cr which appears at 1.8 ppm (Singh et al., 2011; Sun and Sorensen, 2008).

Table 1 shows the mean ($\pm$ Standard Deviation (SD)) GluCEST_{asym} values and coefficients of variation (CV) from GM and WM regions for 7 health human subjects. The mean mask size of gray matter and white matter was 28.7 ± 6.6 pixels (19.9 ± 4.6 mm²) and 97.7 ± 7.0 pixels (67.8 ± 4.9 mm²), respectively. Differences in GM and WM GluCEST_{asym} values were statistically significant with $p < 0.001$.

Discussion

The results of this work provide the first evidence that the GluCEST method can be used to noninvasively image Glu in the spinal cord with good spatial resolution.

In vitro CEST contrast from a phantom with different spinal cord metabolites at their physiological concentration and pH of 7.0 demonstrate that, with these saturation parameters, Glu is responsible for the majority of the CEST contrast. Of the other metabolites present in the spinal cord, Cho does not have any exchangeable protons while NAA has an amide proton but does not exhibit any CEST effects at neutral pH. MI has exchangeable hydroxyl ($-\text{OH}$) protons and only exhibits a CEST effect at chemical shifts less than 1 ppm. Cr and Gly both have amine protons that exhibit a CEST effect. However, at physiological pH, the Cr CEST effect occurs around 1.8 ppm and its contribution at 3 ppm was also not appreciable ($< 0.5\%$). Gly amine group protons on the other hand have a much faster exchange rate than Glu and a very broad CEST peak at 2.8 ppm, which require a stronger saturation pulse and thus have minimal contributions under these parameters.

The GluCEST_{asym} observed from Glu in phantom experiments was lower than what we observed in *in vivo* experiments. This is due to our definition of CEST_{asym} which uses the $M_{\text{sat}}(-)$ for normalization as it has a higher dynamic range. As the T_2 of Glu phantoms is long (~ 800 ms), there is very little direct water saturation and as a result $M_{\text{sat}}(-)$ is very close to M_0 , the magnetization without selective saturation. Additionally, the exchange rate of Glu amine protons is expected to be somewhat slower in the spinal cord compared to that in phantoms, which provides higher CEST_{asym} for the same saturation parameters.

In previous work, the CEST effect from amide protons has been used to map protein concentrations *in vivo* in the spinal cord (Dula et al., 2011). Amide protons have a chemical shift of 3.5 ppm downfield of water, which is in close proximity of Glu amine protons (0.5

ppm downfield). However, the exchange rate of amide protons is $\sim 30 \text{ s}^{-1}$ is much slower than that of Glu amine protons (Zhou et al., 2004). Amide proton transfer (APT) utilizes low power ($<1\mu\text{T}$) and long duration ($> 2 \text{ s}$) saturation pulses to optimize CEST contrast for slow exchanging spins (Zhao et al., 2011). However, with high power and shorter duration saturation pulse train utilized in this study, the CEST effect from amide protons is expected to be minimal.

Another possible confounder to GluCEST contrast are asymmetric MT effects. MT contrast is due to magnetization exchange between water molecules bound to larger macromolecules in solid or semisolid phases and free water (Wolff and Balaban, 1989). These MT effects are not always symmetric due to the small chemical shift difference between bound and free water molecules and as a result may confound CEST asymmetry measurements. However, this MT asymmetry has been shown to be dependent on the applied saturation pulse and is minimized by using a strong saturation pulse ($> 3\mu\text{T}$) with short duration as was the case in this study (Hua et al., 2007; Zhao et al., 2011). Thus MT asymmetry effects are expected to be minimal. Finally, any MT asymmetry effects would compete with Glu CEST effects and would result in an underestimation of actual $\text{GluCEST}_{\text{asym}}$.

Glutamate concentration is expected to be greater in the GM compared to the WM due to the higher neuronal density in GM which should result in higher GluCEST in GM compared to WM (Inglese et al., 2008). The average $\text{GluCEST}_{\text{asym}}$ across 7 healthy subjects from gray matter was $6.6 \pm 0.3\%$ compared to $4.8 \pm 0.4\%$ in white matter. This yields a GluCEST ratio from GM and WM ROIs of ~ 1.4 . In the brain, this ratio was reported to be 1.6 and was in agreement with the Glu concentration ratio from ^1H MRS Data (Cai et al., 2012). This difference between the GluCEST ratio in the spinal cord determined in this work and the GluCEST ratio in the brain maybe due to differences in glutamate distribution between the brain and the spinal cord. The relatively small size of the cord and even smaller area of the gray matter tracks makes imaging challenging and results in partial volume effects. Additionally, a high slice thickness was used in this study (10 mm) in order increase the signal to noise ratio (SNR) which may also lead to partial volume effects as the GM tracks may vary in the z-direction. These partial volume effects result in a blurring of gray matter regions and makes quantifying gray and white matter differences more challenging. However, $\text{GluCEST}_{\text{asym}}$ maps shows noticeably increased intensity around the center of the cord where gray matter tracks reside. Also, a statistically significant ($p < 0.001$) difference in $\text{GluCEST}_{\text{asym}}$ was observed between GM and WM regions manually segmented from anatomic images.

There are several potential limitations in this study. The coil used in this study was a head coil and not a coil designed specifically for imaging of the neck. Although, the imaging slice was able to be positioned close to the iso-center of the coil, coil loading was not optimal. This resulted in higher reference voltages which limited the power and duration of the saturation pulse train that could be used within scanner limits and FDA SAR restrictions. This setup also led to differences in the effective transmit B_1 field leading to lower saturation power ($\sim 75\%$ - $100\% B_{1\text{rms}}$) and a lower observed $\text{GluCEST}_{\text{asym}}$. These differences in effective transmit B_1 field were detected in the B_1 field map and addressed using the described B_1 correction method. In the brain, it was shown that this method of B_1 correction was able to accurately correct $\text{GluCEST}_{\text{asym}}$ for a B_1 inhomogeneity of $\sim 50\%$ in the brain and thus it is expected that this method of B_1 correction was able to adequately correct for differences in effective transmit field in the spinal cord. Nevertheless, a coil designed specifically for the neck could lower the reference voltage and allow a for a more favorable saturation pulse to optimize GluCEST contrast. Additionally, a dedicated neck coil would increase SNR allowing for higher resolution and decreased slice thickness to minimize partial volume effects and optimize GM/WM contrast.

Another concern is movement of the cord due to respiration or pulsatile flow of CSF. This was addressed by registration of base CEST, WASSR and B₁ Map images. However, registration utilized rigid body transformations and did not account for any out of plane movement. Respiratory and cardiac gating in addition to a holder that contours to the neck may remedy these concerns as well as any dynamic field inhomogeneity changes and provide better resolution to GM/WM differences in the cord.

In summary, the findings of this preliminary study suggest that it is feasible to detect the CEST effect from glutamate in the spinal cord at 7T with high spatial resolution without exceeding SAR. In vitro phantoms demonstrated that the majority of the observed GluCEST effect is due to Glu with minor contributions from Cr. Additionally, statistically significant differences in GluCEST contrast were observed between gray and white matter regions of the cord. Future work with an optimized dedicated spine coil is expected to enable further improvement in resolution of GluCEST maps that may be able to better resolve and characterize differences in gray and white matter Glu distribution.

Acknowledgments

We gratefully acknowledge Dr. Tom Connick for his help implementing and optimizing the MR hardware used in this study. We thank D. Reddy for his help with human studies. This work was supported by a NIBIB supported P41 resources center grant EB015893, R21-DA032256 and T32EB009384, and a pilot grant from TBIC of ITMAT of the University of Pennsylvania.

References

- Cai K, Haris M, Singh A, Kogan F, Greenberg J, Hariharan H, Detre J, Reddy R. Magnetic resonance imaging of glutamate. *Nature Medicine*. 2012; 18:302–306.
- Dula, A.; Dortch, R.; Landman, B.; Gore, J.; Smith, S. Application of Chemical Exchange Saturation Transfer (CEST) Imaging to Examine Amide Proton Transfer (APT) in the Spinal Cord at 3T. *Proceedings of the 19th ISMRM; Montreal, Canada*. 2011. p. 407
- Forsen S, Hoffman R. Study of moderately rapid chemical exchange reactions by means of nuclear magnetic double resonance. *Journal of Chemical Physics*. 1963; 39:2892–2901.
- Guivel-Scharen V, Sinnwell T, Wolff S, Balaban R. Detection of proton chemical exchange between metabolites and water in biological tissues. *Journal of Magnetic Resonance*. 1998; 133:36–45. [PubMed: 9654466]
- Haris M, Cai K, Singh A, Hariharan H, Reddy R. In vivo mapping of brain myo-inositol. *Neuroimage*. 2011; 54:2079–2085. [PubMed: 20951217]
- Hua J, Jones C, Blakeley J, Smith S, van Zijl P, Zhou J. Quantitative description of the asymmetry in magnetization transfer effects around the water resonance in the human brain. *Magnetic Resonance in Medicine*. 2007; 58:786–793. [PubMed: 17899597]
- Inglese M, Rusinek H, George IC, Babb JS, Grossman RI, Gonen O. Global average gray and white matter N-acetylaspartate concentration in the human brain. *NeuroImage*. 2008; 41:270–276. [PubMed: 18400521]
- Jansen JFA, Backes WH, Nicolay K, Kooi ME. ¹H MR Spectroscopy of the Brain: Absolute Quantification of Metabolites. *Radiology*. 2006; 240:318–332. [PubMed: 16864664]
- Jones C, Schlosser M, van Zijl P, Pomper M, Golay X, Zhou J. Amide proton transfer imaging of human brain tumors at 3T. *Magnetic Resonance in Medicine*. 2006; 56:585–592. [PubMed: 16892186]
- Kim M, Gillen J, Landman B, Zhou J, van Zijl P. Water Saturation Shift Referencing (WASSR) for Chemical Exchange Saturation Transfer (CEST) Experiments. *Magnetic Resonance in Medicine*. 2009; 61:1441–1450. [PubMed: 19358232]
- Lipton SA, Rosenberg PA. Excitatory amino-acids as a final common pathway for neurologic disorders. *New England Journal of Medicine*. 1994; 330:613–622. [PubMed: 7905600]

- Liu D, Xu GY, Pan E, McAdoo DJ. Neurotoxicity of glutamate at the concentration released upon spinal cord injury. *Neuroscience*. 1999; 93:1382–1389.
- Liu G, Gilad AA, Bulte JWM, van Zijl PCM, McMahon MT. High-throughput screening of chemical exchange saturation transfer MR contrast agents. *Contrast Media & Molecular Imaging*. 2010; 5:162–170. [PubMed: 20586030]
- Nakanishi S. Molecular diversity of glutamate receptors and implications for brain-function. *Science*. 1992; 258:597–603. [PubMed: 1329206]
- Petroff OA, Mattson RH, Rothman DL. Proton MRS: GABA and glutamate. *Advances in Neurology*. 2000; 83:261–271. [PubMed: 10999208]
- Petroff OAC. GABA and glutamate in the human brain. *Neuroscientist*. 2002; 8:562–573. [PubMed: 12467378]
- Rothstein JD, Martin LJ, Kuncel RW. Decreased glutamate transport by the brain and spinal-cord in amyotrophic-lateral-sclerosis. *New England Journal of Medicine*. 1992; 326:1464–1468. [PubMed: 1349424]
- Singh A, Cai K, Haris M, Hariharan H, Reddy R. On B1 inhomogeneity correction of in vivo human brain glutamate chemical exchange saturation transfer contrast at 7T. *Magnetic Resonance in Medicine*. 2012 DOI: 10.1002/mrm.24290.
- Singh, A.; Haris, M.; Cai, K.; Hariharan, H.; Reddy, R. Chemical exchange transfer imaging of creatine. *Proceedings of the 19th ISMRM; Montreal, Canada*. 2011. p. 2771
- Srinivasan R, Cunningham C, Chen A, Vigneron D, Hurd R, Nelson S, Pelletier D. TE-averaged two-dimensional proton spectroscopic imaging of glutamate at 3 T. *Neuroimage*. 2006; 30:1171–1178. [PubMed: 16431138]
- Srinivasan R, Sailasuta N, Hurd R, Nelson S, Pelletier D. Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. *Brain*. 2005; 128:1016–1025. [PubMed: 15758036]
- Sun P, Sorensen A. Imaging pH using the chemical exchange saturation transfer (CEST) MRI: Correction of concomitant RF irradiation effects to quantify CEST MRI for chemical exchange rate and pH. *Magnetic Resonance in Medicine*. 2008; 60:390–397. [PubMed: 18666128]
- Sun P, van Zijl P, Zhou J. Optimization of the irradiation power in chemical exchange dependent saturation transfer experiments. *Journal of Magnetic Resonance*. 2005; 175:193–200. [PubMed: 15893487]
- van Zijl PCM, Yadav NN. Chemical Exchange Saturation Transfer (CEST): What is in a Name and What Isn't? *Magnetic Resonance in Medicine*. 2011; 65:927–948. [PubMed: 21337419]
- Ward K, Aletras A, Balaban R. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). *Journal of Magnetic Resonance*. 2000; 143:79–87. [PubMed: 10698648]
- Wolff S, Balaban R. NMR imaging of labile proton-exchange. *Journal of Magnetic Resonance*. 1990; 86:164–169.
- Wolff SD, Balaban RS. Magnetization transfer contrast (mtc) and tissue water proton relaxation in vivo. *Magnetic Resonance in Medicine*. 1989; 10:135–144. [PubMed: 2547135]
- Xu GY, Hughes MG, Ye ZM, Hulsebosch CE, McAdoo DJ. Concentrations of glutamate released following spinal cord injury kill oligodendrocytes in the spinal cord. *Experimental Neurology*. 2004; 187:329–336. [PubMed: 15144859]
- Zhao X, Wen Z, Huang F, Lu S, Wang X, Hu S, Zu D, Zhou J. Saturation power dependence of amide proton transfer image contrasts in human brain tumors and strokes at 3 T. *Magnetic Resonance in Medicine*. 2011; 66:1033–1041. [PubMed: 21394783]
- Zhou J, van Zijl P. Chemical exchange saturation transfer imaging and spectroscopy. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2006; 48:109–136.
- Zhou J, Wilson D, Sun P, Klaus J, van Zijl P. Quantitative description of proton exchange processes between water and endogenous and exogenous agents for WEX, CEST, and APT experiments. *Magnetic Resonance in Medicine*. 2004; 51:945–952. [PubMed: 15122676]

HIGHLIGHTS

- GluCEST detection is feasible in the human spinal cord at ultra high fields (7T)
- GluCEST_{asym} measured from Gray Matter was significantly higher than in White Matter
- The observed GluCEST_{asym} in the spinal cord is primarily from glutamate

Figure 1.

Phantom consisting of nmr tubes with solutions of different metabolites at their physiological concentrations [10 mM NAA, 8 mM Cho, 10 mM MI, 6 mM Cr, 6 mM Gly, 10 mM Glu] under physiological conditions [37°, pH=7.0]. (a) B_0 and (b) B_1 maps used for inhomogeneity correction. (c) $CEST_{asym}$ map around 3.0 ppm with a saturation pulse train with $B_{1rms} = 153$ Hz (3.6 μ T) and a 700 ms duration.

Figure 2. GluCEST imaging of a healthy human cervical spinal cord. **(a)** Anatomical proton image of the axial slice. Enlarged (red box) **(b)** B_0 and **(c)** B_1 maps for the same slice used for inhomogeneity correction. **(d)** The B_0 and B_1 corrected GluCEST_{asymp} map of the spinal cord and cerebrospinal fluid (CSF) overlaid on the anatomical image. Finally, the segmented **(e)** gray matter (GM) and **(f)** white matter (WM) GluCEST_{asymp} maps overlaid on the anatomical image. Colorbar represents percentage GluCEST_{asymp}.

Figure 3.

The *in vivo* (a) z-spectra and corresponding (b) asymmetry curves for gray matter (GM) and white matter (WM) from a healthy human cervical spinal cord. A dotted line in the asymmetry curve represents $GluCEST_{asym}$ at 3.0 ppm. Error bars represent the standard deviation in the normalized signal intensity (M_{sat}/M_0) and $CEST_{asym}$ respectively.

Table 1

The mean ($\pm$ SD) GluCEST_{asym} as well as the coefficient of variation (CV) in gray matter (GM) and white matter (WM) in seven normal volunteers.

Subject	Age	GluCEST _{asym} (mean (SD)) (%)		CV (SD/Mean)	
		GM	WM	GM	WM
1	32	6.7 (0.7)	4.9(1.8)	0.10	0.37
2	26	6.5 (2.1)	4.2 (2.4)	0.32	0.57
3	33	6.5 (0.8)	4.8(1.5)	0.12	0.31
4	25	6.4(1.1)	5.2 (2.2)	0.17	0.42
5	62	6.0(1.6)	4.4 (2.2)	0.27	0.50
6	29	7.0(1.3)	5.4(1.9)	0.19	0.35
7	24	6.8(1.0)	4.9(1.9)	0.15	0.45