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Doubly Selective Multiple Quantum Chemical Shift Imaging and T_1 Relaxation Time Measurement of Glutathione (GSH) in the Human Brain *In Vivo*

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Abstract

Mapping of a major antioxidant, glutathione (GSH), was achieved in the human brain *in vivo* using a doubly selective multiple quantum filtering based chemical shift imaging (CSI) of GSH at 3 T. Both *in vivo* and phantom tests in CSI and single voxel measurements were consistent with excellent suppression of overlapping signals from creatine, γ -Amino butyric acid (GABA) and macromolecules. The GSH concentration in the fronto-parietal region was $1.20 \pm 0.16 \mu\text{mol/g}$ (mean $\pm$ SD, $n = 7$). The longitudinal relaxation time (T_1) of GSH in the human brain was $397 \pm 44 \text{ ms}$ (mean $\pm$ SD, $n = 5$), which was substantially shorter than those of other metabolites. This GSH CSI method permits us to address regional differences of GSH in the human brain with conditions where oxidative stress has been implicated, including multiple sclerosis, aging and neurodegenerative diseases.

Keywords

glutathione; human brain *in vivo*; multiple quantum filtered CSI; longitudinal relaxation time (T_1) of glutathione

Introduction

Reduced glutathione (GSH, γ -L-glutamyl-L-cysteinylglycine) is a ubiquitous tri-peptide, composed of L-glutamate, L-cysteine and glycine, and is the most significant and prevalent intracellular nonprotein thiol compound in mammalian cells (1). GSH plays a key role as a major antioxidant in the first line of antioxidant defense against free radicals and the loss of GSH is implicated in normal aging and neurodegenerative diseases. In addition, the concentration of GSH is known to vary depending on brain regions in aging and disease conditions (2,3). Thus the technical development to measure regional distribution of GSH concentration in the human brain *in vivo* is of importance to characterize the selective loss of GSH in brain regions.

The reliable measurement of GSH signals is rather challenging due to the low concentration and other overlapping and dominant signals from e.g., creatine (Cr), γ -Amino butyric acid (GABA) and macromolecules (MM). Only a few techniques have been reported to measure GSH in the human brain *in vivo* using spectral editing via either J-modulation or multiple quantum (MQ) coherence transfer. Most editing techniques target coupled spins of GSH, the cysteine β -CH₂ protons at 2.95 ppm and the cysteine α -CH proton at 4.56 ppm (4–6). When MQ-filtering techniques are combined with a double-band frequency selective pulse during the MQ coherence preparation period, potential contamination from J-coupled molecules such as GABA (3.0 ppm and 1.9 ppm) and MM (3.0 ppm and 1.7 ppm) to GSH (2.87 – 2.96 ppm and 4.56 ppm) can be minimized (6,7), in a similar manner to the GABA measurements as demonstrated previously (8–10).

Among several advantageous properties of MQ-filtering techniques, the versatility of the technique provides a unique advantage over J-difference editing techniques. A single-shot MQ filtering technique can readily be adaptable to two-dimensional MQ spectroscopy (11,12) and MQ chemical shift imaging (CSI) (13,14). In addition, a shortcoming of conventional MQ filtering techniques in lacking reference signals for phase, frequency and quantification was overcome by further development of a conventional MQ-filtering technique into the single-shot two-echo techniques for simultaneous detection of the MQ filtered target signal and other reference signals such as Cr (9,10) or a water navigator (15).

In this study, we sought to develop a CSI technique for GSH measurements in the human brain *in vivo* using a selective multiple quantum filtering technique. Using the proposed doubly selective MQ CSI technique of GSH, we obtained GSH mapping with excellent suppression of the overlapping signals from Cr, GABA and MM. The preliminary account of this work has been published as an abstract (15).

Materials and Methods

Pulse sequence

The double-band frequency selective MQ pulse sequence (Fig. 1A) for a navigated GSH CSI is based on a two-echo acquisition scheme with the MQ filtering GSH CSI part followed by the single quantum (SQ) water navigator part. The parts of water suppression and outer volume suppression are not shown in the figure. The MQ filtering GSH CSI part consists of (a) preparation of MQ coherence, (b) coherence conversion from the MQ to the SQ status, (c) the double quantum (DQ) gradient filtering and (d) the phase encoding gradients for CSI. During the MQ coherence preparation period, a double-band frequency selective 180° pulse (double-band optimized Gaussian pulse, 20 ms) is inserted between two slice-selective 90° pulses for the frequency selective MQ transition. The two selection bands of the double-band frequency selective pulse was set at 2.95 ppm to select the cysteine β -CH₂ protons and at 4.56 ppm to select the cysteine α -CH proton of GSH (Fig. 1B). The bandwidth of each selection band was 64 Hz (full width at half maximum, FWHM) and the coupled partner of GABA resonance at 1.93 ppm was completely outside of the selection band. The selective MQ coherence preparation of the J-coupled protons of GSH for an MQ state minimizes the contribution of signals from other J-coupled molecules such as GABA and MM to the GSH in each single scan. For the coherence conversion from the MQ to the SQ status, a semi-selective 90° pulse ($45^\circ_x - 45^\circ_x$ hard pulses, 100 μ s) is used to generate a 90° rotation on the cysteine α -CH proton (4.56 ppm) and 0° rotation on the cysteine β -CH₂ protons of GSH (2.95 ppm). To select DQ coherences, two DQ filtering gradients are positioned before the semi-selective 90° pulse and after the slice selective refocusing 180° pulse at a 1:2 ratio (9,16).

The three-dimensional volume selection was performed using two slice-selective 90° pulses (five-lobe sinc pulse, 4 ms) in the x- and y-axes during the MQ coherence preparation, and a slice-selective 180° pulse (five-lobe sinc pulse, 4 ms) in z-axis positioned before MQ acquisition. The localization in the x- and y-axes, in principle, is not essential for CSI; however, it limits the selected volume of interest in the brain providing the advantage of suppressing signals from extra-cerebral regions such as subcutaneous lipids. The slice-selective 180° pulse was placed prior to the MQ acquisition for chemical shift rephasing of the cysteine β -CH₂ protons of GSH and for selecting the CSI slice in the z-axis. Phase encoding gradients were placed in the x- and y-axes following the MQ gradient and a crusher, and prior to the MQ data acquisition. The SQ water navigator CSI was acquired as an optional module following the MQ acquisition, with the same phase encoding steps. The flip angle of the water navigator pulse (five-lobe sinc pulse, 4 ms) was set to approximately 5° to minimize perturbation of T_1 relaxation and potential signal loss of GSH.

To optimize the editing efficiency of GSH measurements, phantoms containing various combinations of GSH, Cr, NAA, GABA, acetate and lactate were used with each metabolite at 10 mM concentration. The echo time of the MQ CSI part was empirically determined to optimize the GSH signal yield. The duration of the double-band frequency selective pulse was optimized to ensure robust suppression of overlapping resonances and to make the GSH signal yield less sensitive to frequency variations in the CSI slice.

T_1 measurements

The T_1 of GSH was measured by an inversion recovery method using the MQ filtering GSH sequence in the single voxel mode (voxel size = $4 \times 4 \times 4$ cm³) with an inversion pulse. The voxel was located in the fronto-parietal regions of the brain. The T_1 measurement was performed on five subjects. The pulse was a non-slice selective adiabatic inversion pulse with a 1.65 kHz bandwidth (broadband hyperbolic secant (HS) pulse, 6 ms) (17) used to invert the z-magnetization in the entire sensitive volume of the coil. The relaxation delay after each excitation was set to 1.7 – 2.2 s, which was at least four times of an estimated T_1 value of GSH. A total of seven data sets with inversion times (TI) of 60, 115, 221, 424, 814, 1563 and 3000 ms were acquired. The T_1 of GSH in a solution phantom was measured using the same pulse sequence with the relaxation delay set to 5 – 10 s. The T_1 value of GSH was calculated with a three-parameter exponential curve fit using a nonlinear curve fitting routine in Origin software (OriginLab Corporation, Northampton, MA).

NMR methods

The proposed MQ sequences were implemented on a 3 T MR scanner (80 cm bore horizontal magnet, Magnex Scientific, Abingdon, UK) interfaced to an SMIS console (SMIS, Surrey, UK). The system was equipped with an actively shielded gradient coil (38 cm inner diameter, Magnex Scientific, Abingdon, UK) capable of switching to 40 mT/m with a rise time of 400 μ s. An in-house built circularly polarized ¹H radiofrequency (RF) helmet coil (diameter ~21 cm) was used, which conforms to the human head and provides a high filling factor and coil sensitivity (18). Automated localized (19) and slice (20) shimming techniques were used to adjust DC currents in all first- and second-order shim coils. After shimming, a short-echo PRESS (point resolved spectroscopy) sequence (echo time, TE = 26 ms; repetition time, TR = 2 s) (9) was used to measure water linewidth from a 27 cm³ voxel located at the center of the CSI slice, resulting in 6.6 ± 0.5 Hz (FWHM). The frequency of Cr was also measured using the PRESS sequence to set the RF transmitter frequency of the MQ CSI. The acquisition parameters of the MQ CSI were: field of view (FOV) = 20×20 cm, slice thickness = 3 cm, 8×8 phase encoding steps, and number of transients (NT) = 8, 12, or 16, spectral width = 2500 Hz, and number of samples = 256. In this study, MQ filtered GSH spectra were acquired using either the two-echo acquisition

scheme for simultaneous measurements of MQ CSI of GSH and SQ CSI of water navigator or the one-echo acquisition scheme for MQ CSI of GSH only.

In vivo study

A total of 13 healthy subjects were studied (28 ± 9 years old, mean $\pm$ SD) according to the consent approved by the Institutional Review Board of the Nathan Kline Institute. All subjects were positioned supine with the head inside the helmet coil (18). Transverse T_1 -weighted images of the brain were acquired using an inversion prepared three-dimensional gradient echo sequence, magnetization-prepared rapid acquisition gradient echo (MPRAGE), with parameters of FOV = 20×20 cm, matrix = $256 \times 160 \times 120$, slab thickness = 15 cm, TR = 2 s, RAGE TR = 11.8 ms, TE = 5.4 ms and effective TI = 1.1 s (21). The T_1 -weighted images were used to calculate the brain tissue volume fraction in each CSI voxel. The axial slice of CSI was positioned superior to the lateral ventricle covering the fronto-parietal regions of the human brain. Following GSH CSI, mapping of the radiofrequency magnetic field (B_1) was performed using a dual excitation gradient echo sequence (22) to correct the effect of B_1 inhomogeneity on GSH signals.

Quantification

GSH concentration was calculated using the external reference method that compares the integral of the MQ GSH signal from the human brain with that from a spherical phantom containing 10 mM GSH and 10 mM Cr (diameter = 17 cm, pH = 7.2). The loading of the phantom was matched to the human head by adding a phosphate buffer solution. The external reference scan was performed by placing the spherical phantom in the helmet RF coil in a similar manner as in in vivo scans using an MRI visible marker attached on the RF coil. GSH CSI data were processed with home-written software in IDL 6.3 (RSI, Boulder, CO). Data processing included a spatial Fourier transformation with one time zero padding, resulting in the final matrix size of 16×16 , and a time domain Fourier transform with line broadening of 2 Hz and zero padding of the data to 8192 points. The line shape of the GSH signals at ~ 2.9 ppm was approximated with two Lorentzian peaks separated by 4 Hz. Co-edited signals from NAA appearing at around 2.6–2.8 ppm were approximated by three Lorentzian peaks. Spectral fitting was performed using a Levenberg-Marquardt least-squares minimization algorithm implemented in the MPFIT curve fitting package (Craig Markwardt).

Corrections for the varying brain tissue volume in each CSI voxel, i.e., atrophy corrections, were performed by measuring the brain tissue volume fraction in each voxel. The detailed procedures of atrophy correction have been described previously (13). In brief, FAST (FMRIB's automated segmentation tool) software in the FSL package (Oxford University, Oxford, UK) was used to analyze the MPRAGE images for segmenting brain tissues into three categories: gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The volume fractions of each tissue type in each CSI voxel were calculated from tissue composition in the corresponding voxels in the segmented MPRAGE images. The brain tissue volume fraction was calculated by adding the GM and WM volume fractions that are calculated by the ratio of the GM and WM volume to the total volume in each voxel. High resolution anatomical segmented images were down-sampled to match the resolution of CSI data based on the point spread function (PSF) correction algorithm as described in the previous studies (13,23). The brain tissue volume fraction was used to calculate the GSH concentration in brain tissues by assuming the GSH concentration in CSF is negligible.

To calibrate signal variations caused by the B_1 inhomogeneities and residual static magnetic field (B_0) variations in the CSI slice, GSH signals were measured from a phantom containing 10 mM GSH and 10 mM Cr at various B_1 values and transmitter offset frequency

values. Standard curves for B_1 (Fig. 2A) and B_0 corrections factors (Fig. 2B) were determined by a four parameter Lorentzian curve fit and a three parameter Gaussian curve fit of the GSH yield values measured over a wide range of B_1 and frequency offset values deviated from the optimal values, respectively. Both B_1 and B_0 correction curves provided an excellent approximation for the measurement of GSH yield values ($R^2 = 0.97$). For the B_1 correction, the acquired B_1 maps were used to calculate correction factors in each pixel using the standard curve for B_1 correction. The B_1 correction factor map was transformed to match the resolution of the CSI data based on the similar approach used for the atrophy correction. The B_0 correction was performed using the frequency offset values of GSH signals in each CSI voxel determined from the GSH curve fitting. The B_0 correction factor was calculated using the frequency offset value and the standard curve in each voxel.

The effect of relaxation time differences between *in vivo* and phantom GSH signals was incorporated in calculating GSH concentrations. The correction factor for T_1 difference was calculated using the measured T_1 values of GSH in phantoms and the human brain. The correction factor for transverse relaxation time (T_2) difference was determined based on the assumption that the T_2 relaxation rate ($1/T_2$) difference of GSH is similar to that of Cr (~3 ppm) in phantoms and in the human brain. This assumption was made because measurement of T_2 of metabolites with J-coupling such as GSH is challenging due to J-modulation during T_2 relaxation and currently the T_2 values of GSH are not available at 3T. If the T_2 relaxation rate difference of GSH significantly differs from that of Cr in phantoms and in the human brain, the T_2 correction would lead to systematic over- or underestimation of the GSH levels. The T_2 values of Cr signals used in the calculation were 154 ms for *in vivo* (24) and 909 ms for phantoms. GSH concentrations were obtained from the central $5 \times 5 \text{ cm}^2$ region of the CSI data, where the magnetic field (B_0 and B_1) are the most homogeneous.

Results

The GSH signals were successfully measured in both phantoms and human brains with excellent selectivity using the selective MQ filtering technique with a double-band frequency selective refocusing pulse. The selectivity of the GSH measurement using the proposed sequence was first validated in phantoms containing various concentrations of GSH, Cr, NAA, GABA, acetate and lactate. When the double-band frequency selective pulse was replaced by a non-frequency selective pulse, signals from other resonances of GSH and other chemicals such as GABA and lactate were detected (Fig. 3A). However, contamination from singlets such as signals from Cr and acetate was not observed due to the effective MQ filtering as expected based on the SQ nature of the signals. Please note that the signals from GABA and NAA were much smaller than those from GSH due to the frequency selectivity provided by the semi-selective 90° pulse and the echo time optimized for GSH measurement. When the double-band frequency selective 180° pulse was used, an unequivocal detection of the GSH resonance at 2.95 ppm was achieved with an excellent suppression of all other metabolites (Fig. 3B). For the MQ GSH sequence part, the theoretical optimal echo time of the GSH signals of the cysteine $\beta\text{-CH}_2$ protons would be $1/2J$, which is in the range of 66 ms for the β_b proton to 93 ms for the β_a proton. However, we empirically determined the echo time of 120 ms by changing echo time to achieve the optimal GSH signal yield considering the inhibition and/or interference of long RF pulses on the J-evolution process.

In vivo ^1H PRESS magnetic resonance spectroscopy (MRS) shows the dominating Cr resonance at 3 ppm with the same echo time of 120 ms used for the GSH CSI (Fig. 4A, $\text{TR} = 3 \text{ s}$), which masks the detection of the cysteine $\beta\text{-CH}_2$ protons of GSH that resonate at ~3 ppm. The GSH spectrum was acquired from a single voxel (27 mL, $\text{NT} = 512$) in the fronto-parietal area using the selective MQ filtering GSH technique (Fig. 4B). A clear GSH signal

in the human brain *in vivo* was observed at 2.95 ppm with a separation of 4.0 ± 0.9 Hz (mean $\pm$ SD, $n = 7$) with a well-defined baseline and without contamination from other interfering resonances. When the single voxel MQ filtering GSH technique was developed into the GSH CSI, the GSH spectrum from a CSI voxel (nominal voxel size: 4.7 mL) showed a similar quality of resolution and the signal to noise ratio (SNR) with that of a single voxel with a consistent spectral pattern (Fig. 4C). The signals near GSH resonance at 2.6–2.8 ppm are assigned to co-edited NAA resonances.

The potential contribution of the C4 methylene protons of the GABA signal was assessed by applying the double-band frequency selective 180° pulse at 3 ppm and 1.9 ppm with the echo time (TE) of 120 ms for a GABA phantom solution and the human brain. The residual signals of GABA at TE = 120 ms were negligible in both the human brain *in vivo* and the phantom (data not shown).

The inversion recovery data for calculating the T_1 value of GSH in the human brain are shown in Figure 5. The T_1 value of *in vivo* GSH was calculated using the three parameter exponential fit and the resulting T_1 value of 397 ± 44 ms (mean $\pm$ SD, $n = 5$) was substantially shorter than those of other metabolites at 3 T, $\sim 1 - 1.4$ s (24). Taking advantage of the short T_1 value of GSH, the TR of GSH measurements was set to 1.5 s to reduce the total scan time. The T_1 value of GSH in a solution phantom was 339 ± 10 ms and was used for calculating the correction factor for T_1 relaxation.

Figure 6 shows an *in vivo* GSH CSI of the human brain with a nominal voxel size of 4.7 ml after one time zero-filling. The phase of each CSI voxel was corrected based on the phase of navigator water signals from the corresponding voxel. The GSH signals were clearly detected throughout the GSH CSI slice. The quantified GSH concentration after brain atrophy, B_1 and B_0 corrections in central 4×4 voxels covering the fronto-parietal area was 1.2 ± 0.16 mM (mean $\pm$ SD, $n = 7$).

Discussion

Excellent selectivity of the doubly selective MQ filtering technique for GSH was demonstrated in both single voxel and CSI measurements. The *in vivo* GSH doublet at 2.96 ppm was consistent with that of *in vitro* GSH indicating unequivocal detection of GSH without any interfering resonances. Furthermore, the similarity of the spectral pattern of GSH *in vivo* and phantoms suggests effective suppression of other overlapping signals such as Cr, GABA and MM using the double-band frequency selective pulse.

The T_1 value of GSH was significantly shorter than that of other neurochemicals including NAA, Cr, choline, *myo*-inositol and glutamate. The difference is thought to be due to the presence of neighboring sulfur in the cysteine moiety of GSH. This short T_1 value of GSH provides an opportunity to increase the SNR of GSH measurement by shortening TR by 25% compared to the generally used TR of ~ 2 s in clinical ^1H MRS of the human brain. In the current study, the TR value was set to 1.5 s to accommodate the time required water suppression and outer volume suppression. However, TR could be further shortened to about three times of the T_1 value of GSH, 1.2 s, to maximize the SNR of GSH by shortening the duration of water suppression and outer volume suppression.

The levels of metabolites in health as well as their alterations in disease have been shown to be tissue type specific, e.g., GM and WM. When the tissue types are not considered in assessing the levels of metabolites, clinical interpretation could be compromised due to significant differences in metabolite levels between GM and WM. For example, when NAA levels were used to assess the severity of neuronal loss, varying tissue type composition in the voxel could introduce undesirable variability in NAA levels, thus possibly leading to

clinical misinterpretation (25). Measurement of tissue type specific GSH levels can be achieved, in principle, using high resolution single voxel MRS provided that the voxel size is small enough to select only a single tissue type. However, the convoluted shape of human GM and WM requires the single voxel size too small to provide the adequate SNR of GSH for reliable quantification, which is particularly true for GM. An alternative approach for measuring tissue type specific GSH levels is using GSH CSI in conjunction with brain tissue segmentation and a regression analysis. This approach has been successfully utilized to measure tissue-type specific concentrations of metabolites with dominating MR signals including NAA, Cr and choline (26). Recently, a similar approach has been applied to estimate GM and WM concentrations of a metabolite with low concentration such as GABA (13). The GABA measurement was performed using an MQ filtering GABA CSI technique, which is similar to the proposed MQ filtering GSH CSI technique, and utilized tissue segmentation and non-linear regression. For this purpose, a two-echo MQ filtering CSI technique with concomitant measurements of Cr could be advantageous in providing a concentration reference (13). However, the purpose of this study was to implement a simple water navigator CSI in order to estimate the frequency and phase variations of the proposed GSH CSI technique.

CSI techniques provide advantages over single voxel ^1H MRS by simultaneous acquisition of MRS data from multiple voxels. One of the major advantages of MQ CSI of metabolites with J-coupled spins and low concentrations such as GSH and GABA (13) is the efficient scan time use for multiple volumes of interest. When the SNR of metabolite signals is sufficiently high (e.g., NAA, Cr and Cho), single voxel measurement requires a short averaging scan time, while CSI measurement requires much longer scan time due to spatial encoding steps. Thus, additional scan time is needed for CSI compared with single voxel MRS. In contrast, MQ CSI of metabolites with low concentrations (e.g., GSH and GABA) requires almost identical scan time with single voxel MRS, while providing metabolite concentrations in multiple regions.

Quantification of metabolite concentrations in the multiple CSI voxels is challenging due to variations of B_0 , B_1 , and RF coil sensitivity across the CSI volume. Although significant technical advances in B_0 shimming capability and RF coil designs have been made, the residual variations of B_0 and B_1 can alter GSH signals in the CSI voxels. The signal variations due to the B_0 inhomogeneity occurs mainly due to the use of the double-band frequency selective pulse that is essential for removing unwanted J-coupled resonances from other metabolites. To overcome the effect of GSH signal variations, we used an approach that uses phantom calibration experiments with varying transmitter frequency shifts to generate a standard calibration curve. The effect of B_1 inhomogeneities on GSH signals is caused by the flip angle variations for excitation and refocusing pulses. The use of local transmit/receive coils as with the helmet coil provides better sensitivity compared with the larger volume coils at a cost of the reduced B_1 homogeneity. The effect of the B_1 inhomogeneity on GSH signals can also be corrected using a similar approach for the B_0 inhomogeneity correction. The combination of B_0 and B_1 correction approaches provided consistent GSH concentrations across CSI voxels as validated in phantoms.

The acquisition time required for the reliable measurement of GSH CSI was ~13 – 19 min at a clinical field strength of 3 T. In our experience, this scan time requirement was well tolerated by young and older adults in health and disease (27). When the GSH measurement needs to be performed in more challenging populations of patients that require a further decrease in scan time, the use of advanced RF technology could be an option. Multi-channel receiver coils in combination with homogeneous volume coil transmission can provide a significant SNR gain for GSH measurement compared to that using conventional transmit/receive RF coils. The SNR gain, i.e., signal enhancement, can offer the shorter scan time by

reducing the number of signal averages. Advanced hardware technology is readily available in clinical scanners and is expected to reduce the total scan time for GSH measurement to 5 – 10 min.

Conclusions

In this study, we established a doubly selective MQ filtering CSI technique for measurement of GSH with an SQ water navigator in the human brain *in vivo*. Improved selectivity was illustrated by the distinctive spectral pattern of the GSH signal at 2.96 ppm with a clean baseline. We have demonstrated that the MQ filtering GSH CSI in the human brain is feasible in ~15 min with a reasonable SNR for reliable quantification and consistent reproducibility of the signal at 3 T. The capability of GSH detection in the human brain *in vivo* should allow us to monitor the progression of diseases related to the concept of oxidative stress and the effects of pharmaceutical interventions directed at antioxidant treatments.

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Abbreviations

B₀	static magnetic field
B₁	radiofrequency magnetic field
Cr	creatine
CSF	cerebrospinal fluid
CSI	chemical shift imaging
FAST	FMRIB's automated segmentation tool
FOV	field of view
FWHM	full width at half maximum
GABA	γ-aminobutyric acid
GM	gray matter
GSH	glutathione
HS	hyperbolic secant
MM	macro molecules
MPRAGE	magnetization-prepared rapid acquisition gradient echo
MQ	multiple quantum
MRS	magnetic resonance spectroscopy
NAA	N-acetylaspartate
NT	number of transients
PRESS	point resolved spectroscopy
PSF	point spread function
SD	standard deviation

SQ	single quantum
T₁	longitudinal relaxation time
T₂	transverse relaxation time
TE	echo time
TR	repetition time
VOI	volume of interest
WM	white matter

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Figure 1. Pulse sequence diagram for the double-band frequency selective MQ CSI of GSH

(A) The MQ CSI of GSH with a two-echo acquisition scheme consists of the MQ filtered GSH (FID 1) and the SQ navigated water (FID 2) CSI parts. The slice-selection and refocusing gradients were used to select the CSI slice in the z-axis and the in-planar VOI in the x- and y-axes. Double quantum coherence selection gradients (gray color) were applied in a 1:2 ratio and spoiler gradients (black color) to dephase transverse magnetization.

(B) The refocusing profile of the double-band frequency selective pulse (Gaussian 5% truncation, 20 ms, BW = 65 Hz) was calculated using a Bloch equation simulation program. The double-band frequency selective pulse was generated by superimposing two Gaussian pulses with a frequency separation of 205 Hz. The Gaussian pulse with 5% truncation was

used to create broader pass bands to improve the immunity of GSH measurement to residual B_0 inhomogeneity. The positions of other resonances of GABA (3.02 ppm and 1.89 ppm) and Cr (3.03 ppm) are marked with arrows.

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Figure 2. Calibration curves for B_1 and B_0 inhomogeneity corrections

(A) GSH signal yields were measured at various B_1 values and normalized with the value at the optimal B_1 . The calibration curve was obtained by a non-linear fit of the data to a Lorentzian curve and the resulting fit parameters were used to determine a B_1 correction factor for each CSI voxel.

(B) GSH signal yields were measured at various B_0 values and normalized with the value at the optimal B_0 . The calibration curve was obtained by a non-linear fit of the data to a Gaussian curve and the resulting fit parameters were used to determine a B_0 correction factor for each CSI voxel.

Figure 3. Selectivity of MQ filtering GSH CSI with doubly selective refocusing pulse

(A) The efficiency of singlet signal suppression using MQ filtering was tested using a non-frequency selective refocusing pulse on a phantom containing Cr, GSH, NAA and lactate with 10 mM concentration for each. J-coupled resonances from lactate, GABA and NAA were detected while no singlet signals such as Cr and NAA could pass MQ filtering.

(B) The GSH spectrum was acquired using a doubly selective refocusing pulse on the same phantom used in (A). No other signal except GSH resonances at ~3 ppm are detectible, demonstrating the selectivity of the doubly selective MQ filtering technique for GSH.

Figure 4. Measurement of GSH in the human brain *in vivo*

(A) Localized conventional ¹H MRS was performed using a PRESS sequence with TE = 120 ms. GSH resonances were not detectable due to the dominant signals of Cr at 3 ppm (voxel size = 3 × 3 × 3 cm³, TR = 2 s, TE = 120 ms).

(B) *In vivo* GSH spectrum acquired with the MQ filtering GSH CSI sequence in the single voxel mode (voxel size = 3 × 3 × 3 cm³, TE = 120 ms, TR = 1.5 s, NT = 512)

(C) *In vivo* GSH spectrum acquired with the MQ filtering GSH sequence in the CSI mode (FOV = 20 × 20 cm, matrix size = 8 × 8, slice thickness = 3 cm, TE = 120 ms, TR = 1.5 s, NT = 10).

Figure 5. T_1 measurement of GSH in the human brain

The T_1 of GSH was measured from the human brain *in vivo* using the inversion recovery method. The solid line is the best three-parameter exponential fit to the corresponding data. The filled circles indicate the normalized intensity of *in vivo* cerebral GSH (2.96 ppm) signals. The inset shows GSH spectra at inversion times of 60, 115, 221, 424, 814, 1563 and 3000 ms (voxel size = $4 \times 4 \times 4$ cm³, TE = 120 ms)

Figure 6. MQ filtering GSH CSI of the human brain *in vivo*

(A) Partial view of GSH mapping in the human brain acquired using the MQ filtering GSH CSI technique (FOV = 20×20 cm, matrix size = 8×8 , slice thickness = 3 cm, TE = 120 ms, TR = 1.5 s, NT = 10, nominal voxel size = $1.25 \text{ cm} \times 1.25 \text{ cm} \times 3 \text{ cm}$ after $1 \times$ zero padding).

(B) Corresponding partial view of water navigator CSI acquired simultaneously using the MQ filtering GSH CSI.