

# Measurement of Reduced Glutathione (GSH) in Human Brain Using LCModel Analysis of Difference-Edited Spectra

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**The concentration of reduced glutathione (GSH), an antioxidant, may be altered in various brain diseases. MEGA-PRESS was used to edit for the  $^1\text{H}$  NMR signal from GSH in the occipital lobe of 12 normal humans. In all studies, GSH was clearly detected with a spectral pattern consistent with spectra acquired from a phantom containing GSH. Retention of singlet resonances in the subspectra, a key advantage of this difference-editing technique, provided an unambiguous reference for the offset and phase of the edited signal. Linear combination model (LCModel) analysis provided an unbiased means for quantifying signal contribution from edited metabolites. GSH concentration was estimated from the *in vivo* spectra as  $1.3 \pm 0.2 \mu\text{mol/g}$  (mean  $\pm$  SD,  $n = 12$ ). Magn Reson Med 50:19–23, 2003. © 2003 Wiley-Liss, Inc.**

**Key words:** GSH; human; MEGA; LCModel

Glutathione ( $\gamma$ -glutamyl cysteinyl glycine) is a major intracellular antioxidant (1). Present at high levels ( $\sim 1$ – $5$  mM) in animal cells, glutathione cycles between a reduced thiol form (GSH) and an oxidized form (GSSG). The ratio of GSH to GSSG in most cells is greater than 500:1 (2), so GSH is, under most circumstances, the most concentrated form of glutathione. GSH plays a fundamental role in the detoxification of reactive oxygen species, which is critical to the normal function of the human brain (3). Compromised astroglial GSH metabolism may contribute to oxidative stress, which has been implicated in many neurological disorders, as well as in schizophrenia (3,4).

Despite the high reported concentration of GSH in the CNS (5), its measurement by  $^1\text{H}$  NMR spectroscopy has been elusive, partly because of spectral overlap with other signals from more concentrated compounds. Recently, using LCModel analysis of short echo time spectra, a concentration of GSH on the order of  $1 \mu\text{mol/g}$  was reported in rat brain (6), which was reduced in quinolinic acid-lesioned rat striatum (7) in agreement with biochemical reports (8). In the human brain, the detection of GSH using spectral editing based on double quantum coherence filtering has been reported (9,10). The strongly coupled cysteine  $\beta$ - $\text{CH}_2$  protons of GSH resonating at 2.95 ppm, which are J-coupled to cysteine  $\alpha$ -CH (4.56 ppm), were found to be suitable for edited detection. Brain metabolites resonating in the vicinity of 2.95 ppm include: (phospho)creatine (3.03 ppm), aspartate (2.82 ppm), GABA (3.01 ppm), and macromolecules (2.98 ppm) (6).

While the single shot nature of multiple-quantum editing is advantageous, editing based on J-difference spectroscopy offers the advantage of unambiguous determination of the frequency and phase in the edited spectra from the simultaneously detected singlet resonances in the non-edited subspectra. In addition, the singlet resonances in the subspectra can be exploited for frequency and phase correction of each acquisition, which results in improved spectral quality (11,12). To confirm earlier reports of a measurable GSH signal in the CNS (6,13), MEGA-PRESS (14), a difference-editing technique, was adapted for detection of the cysteinyl resonance of GSH.

Spectral editing for the strongly coupled resonances of GSH can result in complicated, modulated spectral patterns that are difficult to integrate and thus quantify. This difficulty was overcome by the novel application of LCModel analysis (15) to edited spectra. LCModel used the unique spectral pattern of GSH, measured *in vitro* with the editing method, to determine the contribution of GSH to the edited *in vivo* spectrum. Because the aspartyl resonances of NAA were coedited with GSH, and because they resonate nearby with comparable intensity, the model spectrum of NAA was also included. These coedited resonances of NAA also served as an internal concentration reference.

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## METHODS

Nine normal volunteers (six male, three female, average age 25 years), three of whom were studied twice, gave informed consent for this study, which was conducted according to procedures approved by the Institutional Review Board. All experiments were performed with a 4 T, 90 cm bore magnet (Oxford Magnet Technology, Oxford, UK) interfaced to a Varian INOVA spectrometer (Varian, Palo Alto, CA) equipped with gradients capable of switching to 40mT/m in 400  $\mu\text{s}$  (Sonata, Siemens, Erlangen, Germany) and a surface coil  $^1\text{H}$  quadrature transceiver (16). Subjects were positioned supine inside the magnet with the RF transceiver subjacent to their occipital lobe. The protocol for each volunteer began with localizer multislice RARE images (TR = 4.0 sec, TE = 60 ms, echo train

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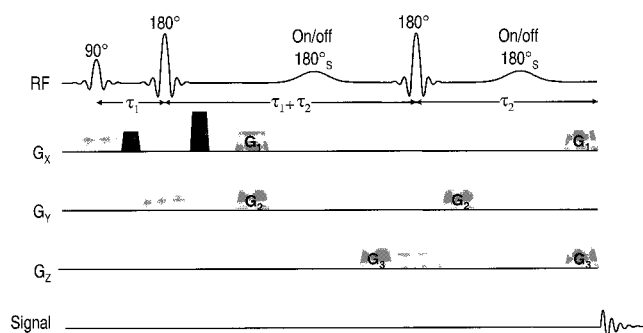


FIG. 1. MEGA-PRESS pulse sequence designed for localization and editing of cysteinyl  $\beta$  protons of GSH (2.95 ppm). Slice-selective pulses and light gradients accomplish localization. Darkest crusher gradients also account for rewind of excitation gradient. Main editing components are (single-banded 20 ms gauss with a bandwidth of 75 Hz) selective inversion pulses, applied at the chemical shift of the cysteinyl  $\alpha$  protons of GSH (4.56 ppm), which are switched on and off alternately. Labeled MEGA gradients ( $G_1$ ,  $G_2$ , and  $G_3$ ) are for suppression of unwanted signal (14).

length = 8,  $256 \times 128$  matrix, two averages, 2 mm slice thickness, five slices) for selection of a cubic volume of interest ( $3 \times 3 \times 3 \text{ cm}^3$ ) centered on the midsagittal plane in the occipital lobe. Shimming of all first- and second-order coils was achieved using FAST(EST)MAP (17), resulting in water linewidths of 8–14 Hz (average 9 Hz). Following adjustment of the spectrometer frequency, difference-editing using MEGA/BASING (14,18) was implemented as described below.

MEGA-PRESS difference editing (14) was applied (Fig. 1) to edit for the cysteine  $\beta$ - $\text{CH}_2$  resonances of GSH (2.95 ppm), which are J-coupled to the cysteine  $\alpha$ -CH resonance (4.56 ppm). The three slice selective pulses in conjunction with the lightest shaded gradients selected the standard localized PRESS coherences (Fig. 1). The darkest shaded crusher gradients also accounted for rewinding of the excitation gradient.  $\tau_1$  was minimized (7.5 ms) for optimal refocusing. The key editing components were the selective inversion pulses (single banded 20 ms Gaussian shape with a bandwidth of 75 Hz at half-height). When the selective inversion pulses were applied (“on”) at the  $^1\text{H}$  frequency of GSH cysteine  $\alpha$ -CH (4.56 ppm), evolution due to J-coupling at GSH cysteine  $\beta$ - $\text{CH}_2$  (2.95 ppm) was partially refocused at an echo time of  $1/2J$ , as opposed to the case when the selective inversion pulses were “off” (applied identically except at the resonance frequency of cysteine  $\alpha$ -CH plus 2000 Hz). Subtraction of “on”-scans from “off”-scans resulted in exclusive detection of resonances coupled to the region at 4.56 ppm. Therefore, the aspartyl resonances of NAA at 2.45 and 2.67 ppm were coedited with the cysteinyl resonance of GSH because they are coupled to the  $\alpha$ -CH at 4.38 ppm, which falls within the bandwidth of the editing pulse. Theoretical calculation of the optimal echo time was complicated by nonequivalence of the cysteine- $\beta$  protons of GSH. Therefore, the echo time was varied between 60 and 100 ms experimentally in phantoms and the maximum of the edited signal was found at 68 ms ( $2\tau_1 + 2\tau_2$ ). Additional water suppression using VAPOR and outer volume saturation based on a

series of hyperbolic secant RF pulses (19) were adapted for the human system (20) and incorporated prior to MEGA-PRESS (not shown). Each subspectrum (NEX = 1) was stored separately in memory, then frequency- and phase-corrected based on the single-shot NAA signal prior to summation. Processing was performed using spectrometer built-in software.

GSH concentration was quantified relative to coedited aspartyl NAA (assumed at 10 mM) by using LCModel analysis (15) applied to the edited spectra. Two basis spectra were acquired using the same MEGA-PRESS difference-editing scheme (Fig. 1) as in vivo from two separate phantoms (pH = 7.1,  $37^\circ\text{C}$ ) containing 5 mM NAA and 5 mM GSH, respectively. LCModel fitting was performed over the spectral range from 2.0 and 3.5 ppm. Global frequency referencing of the in vivo edited spectrum was based on both model spectra and was constrained to within  $\pm 0.2$  ppm. Subsequently, the frequencies of edited GSH and NAA were allowed to vary slightly ( $\pm 0.004$  ppm), independent of one another. The phase of the edited spectrum was adjusted based on the unedited subspectrum. Some flexibility in the zero-order phase ( $\pm 20^\circ$ ) was left to LCModel for the fit. The relative signal contributions were calibrated using an edited spectrum acquired from a phantom containing equimolar (10 mM) GSH and NAA. The accuracy of the ensuing quantitation was verified by analyzing spectra acquired from four phantoms (pH = 7.1,  $37^\circ\text{C}$ ), all containing 10 mM NAA and 8 mM creatine (Cr), but each containing a different concentration of GSH (1 mM, 2.5 mM, 5 mM, and 10 mM). Furthermore, to confirm the concentration of GSH, the GSH signal was also quantified relative to the integral of the Cr methyl peak at 3.03 ppm measured in the subspectra using built-in spectrometer software. This procedure was calibrated in a phantom containing 10 mM GSH, 8 mM Cr, and 10 mM NAA (pH = 7.1,  $37^\circ\text{C}$ ) and validated by analyzing spectra from three phantoms (pH = 7.1,  $37^\circ\text{C}$ ) all containing 10 mM NAA and 8 mM creatine (Cr), but each containing a different concentration of GSH (1 mM, 2.5 mM, 5 mM).

## RESULTS

Subspectra and the corresponding edited spectrum from one volunteer are shown in Fig. 2. Note the strong NAA signal in the subspectra, which was used for unambiguous determination of offset (NAA was referenced to 2.009 ppm) and phase. Figure 2d is a single-shot spectrum (i.e., NEX = 1), illustrating that the SNR for NAA was sufficient for frequency and phase correction of individual acquisitions prior to summation.

The cysteine  $\beta$ - $\text{CH}_2$  resonance of GSH was unequivocally and consistently detected at 2.95 ppm in all 12 in vivo studies as judged from the consistent spectral pattern at 2.95 ppm, mimicking that measured in an accordingly line-broadened phantom spectrum (Fig. 3).

LCModel analysis of edited in vivo spectra was highly successful, as illustrated in Fig. 4. The fit precisely replicated the edited in vivo spectrum (Fig. 4). NAA was clearly demarcated from GSH (Fig. 4), both of which have a complex spectral pattern. The fit residual was at the level of noise, consistent with the visual similarity of the in vivo spectral pattern to that in phantoms (Figs. 3, 4). The GSH

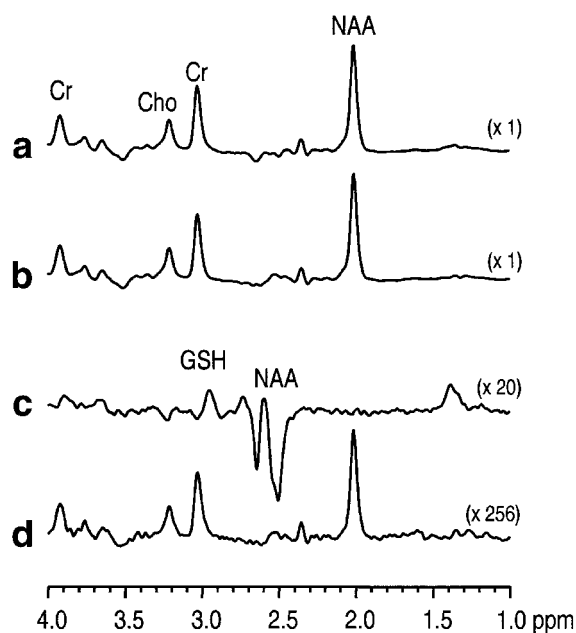


FIG. 2. In vivo  $^1\text{H}$  NMR spectroscopy of GSH using MEGA-PRESS. **a**: In vivo subspectrum acquired in the presence of the frequency-selective editing pulse and **(b)** in the absence of the editing pulses (TR = 4.5 sec, TE = 68 ms, NEX = 256 each). **c**: Difference spectrum resulting from subtraction of **a** from **b**. **d**: Subspectrum from the same dataset (NEX = 1).

concentration determined using the ratio of fitted GSH to fitted NAA (set to 10  $\mu\text{mol/g}$ ) as described in Subjects and Methods was  $1.3 \pm 0.2 \mu\text{mol/g}$  (mean  $\pm$  SD,  $n = 12$ ). The Cramer-Rao bounds for GSH were 9% and 2% for NAA on average, and never higher than 10% for GSH. The influence of changes in editing efficiency due to changes in frequency, which were monitored during each scan, was minimal (negligible on average, 7% at most).

Spectra acquired from the series of phantoms containing 1, 2.5, 5, and 10 mM GSH plus 8 mM Cr and 10 mM NAA are illustrated in Fig. 5, along with typical in vivo spectra. From the comparison of the in vivo spectrum with the phantom spectra it was apparent that the in vivo GSH concentration was in the range of 1–2  $\mu\text{mol/g}$ .

Quantification of GSH with respect to Cr measured in the subspectra resulted in a concentration of  $1.0 \pm 0.2 \mu\text{mol/g}$  (mean  $\pm$  SD,  $n = 12$ ) relative to 8  $\mu\text{mol/g}$  Cr. Additional simplistic attempts at estimating GSH concentration relative to Cr using integration techniques were undertaken in order to affirm accurate quantification and provided estimates in the range of 1–2 mM with higher standard deviation.

## DISCUSSION

The similarity of line shape, position, phase, and spectral pattern between in vivo and in vitro (phantom) spectra demonstrates the unequivocal detection of the cysteine  $\beta\text{-CH}_2$  resonance of GSH at 2.95 ppm (Figs. 3, 5). The estimated GSH concentration,  $1.3 \pm 0.2 \mu\text{mol/g}$ , is in good agreement with the estimated concentration from the first edited detection of GSH in human brain (9,10). The

present study is in excellent agreement with preliminary estimates of human GSH concentration obtained using in vivo  $^1\text{H}$  NMR spectra (7 T) and LCModel at short echo times, reporting  $1.6 \pm 0.1 \mu\text{mol/g}$  (13). Studies have reported GSH concentrations in human brain using biochemical methods with the following values:  $1.2 \pm 0.2 \mu\text{mol/g}$  (21), 1–10 mM (22), and  $\sim 1\text{--}3$  mM (5). Our study is in excellent agreement with the lower end of these concentrations, previous NMR measurements, and natural abundance  $^{13}\text{C}$  NMR estimations (unpubl. obs.). It is noteworthy that the coefficient of variation in the present study (15%) using MEGA-PRESS at 4 T is smaller than the 40% scatter of the GSH/water ratio by double quantum coherence filtering at 1.5 T (9). LCModel analysis and difference editing with MEGA-PRESS at 4 T has greatly reduced the variability compared to prior results obtained from edited detection of GSH. The quantification of GSH in edited spectra depends on the relative transverse relaxation rates between GSH, Cr, and NAA aspartyl resonances, which were assumed to be identical in this study. Nevertheless, the concentration obtained in this study is similar to that obtained from human short echo time (TE = 6 ms) spectra using LCModel analysis (13), and to short echo time animal studies (6), where  $T_2$  relaxation effects are expected to have a negligible effect on quantification.

Determination of the area under the cysteine  $\beta\text{-CH}_2$  GSH resonance at 2.95 ppm using standard integration was complicated by the proximity of coedited resonances, po-

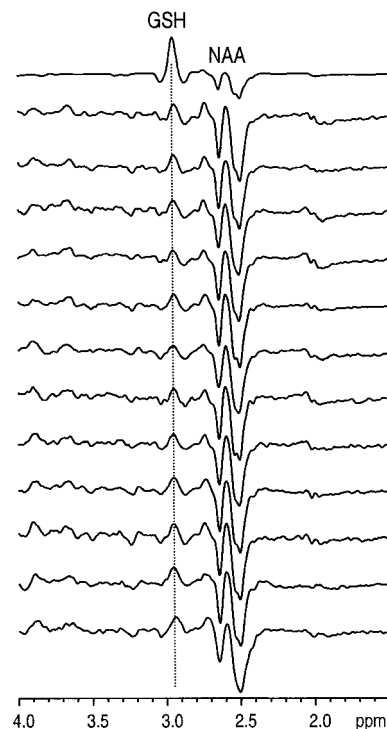


FIG. 3. Consistent detection of GSH in difference-edited MEGA-PRESS spectra. Twelve edited in vivo spectra (TR = 4.5 sec, TE = 68 ms, NEX = 256 or 512) and one edited spectrum from a phantom (top) containing equimolar NAA and GSH (TR = 4.5 sec, TE = 68 ms, NEX = 512) with a concentration of 5 mM. The dashed line indicates the chemical shift (2.95 ppm) of the edited GSH multiplet.

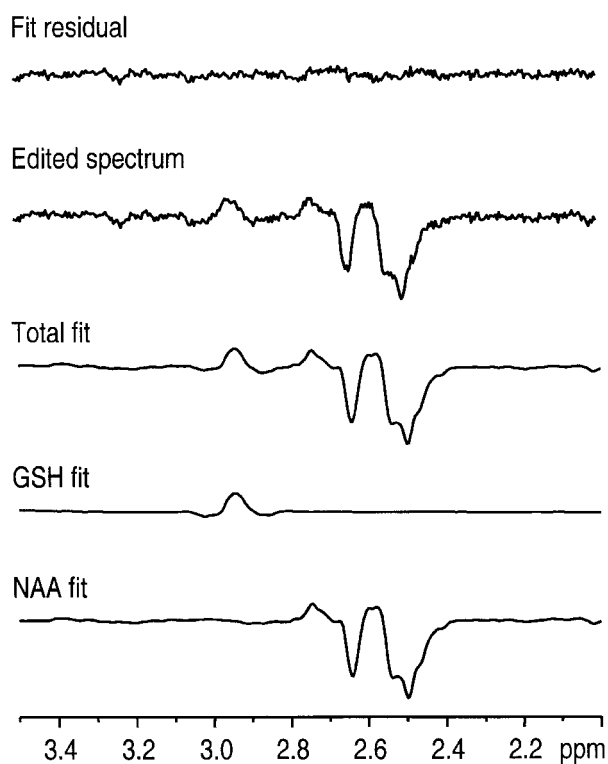


FIG. 4. LCMoel analysis of a representative edited spectrum (TR = 4.5 sec, TE = 68 ms, NEX = 512). Top: fit residual. Bottom: LCMoel analysis including: total fit, GSH component of fit, and NAA component of fit.

tential subtraction artifacts, and the heavily modulated pattern of the edited GSH resonance. These complications persisted despite the inherent benefits of measurement at high field strength (4 T) where the impact of subtraction artifacts at Cr and coedited NAA were reduced due to the increased spectral separation between these metabolites. Instead, LCMoel analysis provided an unbiased and unambiguous approach for separating the GSH resonance from both coedited NAA and the small subtraction artifact near the intense Cr resonance. This novel application of

LCMoel differed from prior applications in that a very small number (two) of metabolites were included in the basis set. LCMoel performed extremely well despite such limited input. This was attributed, in part, to the unique spectral pattern resulting from J-modulation at the long echo times characteristic of edited spectra. Implementation of LCMoel resulted in increased precision of quantification. Although the coedited NAA resonances from the aspartyl methylene were much less intense than the subspectral Cr resonance, it was interesting to note that the standard deviation of the ratio of GSH to NAA using LCMoel was approximately 8% less than that obtained from measuring GSH relative to Cr by manual integration.

In the edited spectra (Fig. 3), additional intensities were observed, suggesting that more coherences were excited. For example, the resonance near 3.18 ppm can in principle indicate a subtraction artifact from the choline singlet coherence. However, the consistent, nondispersive spectral pattern suggests that this peak may arise from a coedited resonance, potentially homocarnosine, but the assignment of this resonance warrants further investigation. Further candidates for coediting include: phosphocholine and glycerophosphocholine, which couple to resonances close to the bandwidth of the frequency-selective editing pulses. The resonances at 1.24 and 1.43 ppm most likely arise from macromolecule (M2 and M3) coherences generated as a result of a partially excited connectivity at 4.23 and 4.3 ppm, respectively (23). Signal contributions from  $\gamma$ -CH<sub>2</sub> GABA near 3.0 ppm are unlikely, as the pulses designed for editing GSH do not excite resonances coupled to  $\gamma$ -CH<sub>2</sub> GABA.

The similarity between in vitro and in vivo GSH spectra suggests that macromolecule (MM) resonances did not contribute to the edited GSH signal. Furthermore, MMs resonate at a slightly different location, at 2.98 ppm (11), which is, at 4 T, 5 Hz downfield relative to GSH. Finally, the coupling partners of the macromolecule resonance at 3 ppm detected in the human brain resonate at 1.72 and 3.32 ppm (23), which are not within the bandwidth of the selective editing pulse used for the detection of GSH.

While correcting the FIDs on an individual basis for phase and frequency improved the subtraction errors in

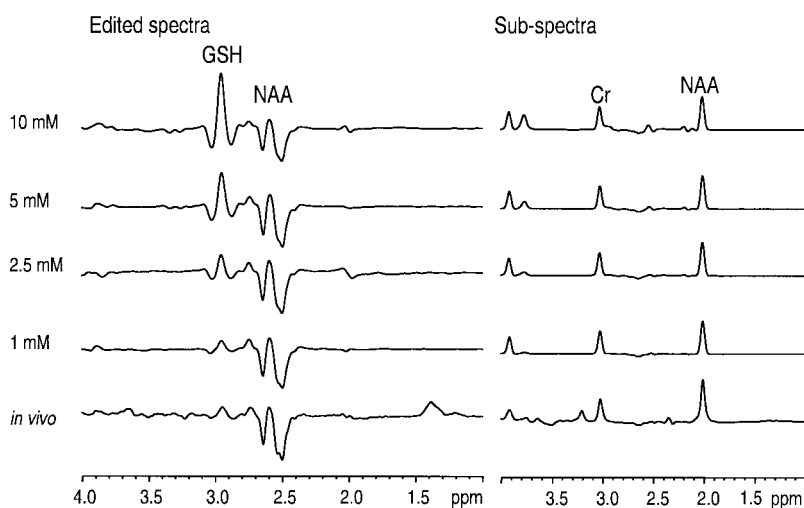


FIG. 5. Edited (difference) and corresponding sub-spectra (sum) acquired from: four phantoms, all containing 10 mM NAA and 8 mM Cr, but each containing a different concentration of GSH (1 mM, 2.5 mM, 5 mM, and 10 mM) (top), and a typical in vivo volume of interest (bottom). Relative signal contributions confirm an in vivo GSH concentration of  $\sim$ 1 mM.

the edited spectra, a persistent dispersive subtraction error was detected for the NAA methyl resonance. However, since the methyl NAA is well resolved from the region around 3 ppm, this subtraction error did not interfere with the detection of the edited GSH resonance at 2.95 ppm.

## CONCLUSIONS

In conclusion, the potential of edited  $^1\text{H}$  MRS for the detection of GSH was confirmed with difference editing, and the quality of in vivo GSH spectra was improved compared to previous studies, therefore establishing the unequivocal detection of GSH in the human brain. The frequency and phase information contained in the subspectra provided an unbiased reference for the frequency and phase of the edited GSH spectrum. The feasibility of LCModel analysis of edited spectra was demonstrated. Precision of quantitative signal contribution was increased through utilization of LCModel, adding greatly to the quantification of in vivo GSH concentration using spectral editing.

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## REFERENCES

1. Yudkoff M. Glutathione metabolism, In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, editors. *Basic neurochemistry*. Philadelphia: Lippincott, Williams & Wilkins; 1999. p 911.
2. Stryer L. Glutathione serves as a sulfhydryl buffer and amino acid transporter. *Biochemistry*. New York: W.H. Freeman; 1988. p 592.
3. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 2000;62:649–671.
4. Do KQ, Trabesinger AH, Kirsten-Kruger M, Lauer CJ, Dydak U, Hell D, Hosboer F, Boesiger P, Cuenod M. Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *Eur J Neurosci* 2000; 12:3271–3278.
5. Cooper AJL. Role of astrocytes in maintaining cerebral glutathione homeostasis and in protecting the brain against xenobiotics and oxidative stress, In: Shaw CA, editor. *Glutathione in the nervous system*. London: Taylor & Francis; 1998. p 91–115.
6. Pfeuffer J, Tkac I, Provencher SW, Gruetter R. Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time  $^1\text{H}$  NMR spectra of the rat brain. *J Magn Reson* 1999;141:104–120.
7. Tkac I, Keen CD, Pfeuffer J, Low WC, Gruetter R. Metabolic changes in quinolinic acid-lesioned rat striatum detected non-invasively by in vivo  $^1\text{H}$  NMR spectroscopy. *J Neurosci Res* 2001;66:891–898.
8. Cruz-Aguado R, Francis-Turner L, Diaz CM, Antunez I. Quinolinic acid lesion induces changes in rat striatal glutathione metabolism. *Neurochem Int* 2000;37:53–60.
9. Trabesinger AH, Weber OM, Duc CO, Boesiger P. Detection of glutathione in the human brain in vivo by means of double quantum coherence filtering. *Magn Reson Med* 1999;42:283–289.
10. Trabesinger AH, Boesiger P. Improved selectivity of double quantum coherence filtering for the detection of glutathione in the human brain in vivo. *Magn Reson Med* 2001;45:708–710.
11. Terpstra M, Ugurbil K, Gruetter R. Direct in vivo measurement of human cerebral GABA concentration using MEGA-editing at 7 Tesla. *Magn Reson Med* 2002;47:1009–1012.
12. Thiel T, Czisch M, Elbel GK, Hennig J. Phase coherent averaging in magnetic resonance spectroscopy using interleaved navigator scans: compensation of motion artifacts and magnetic field instabilities. *Magn Reson Med* 2002;47:1077–1082.
13. Tkac I, Kim J, Ugurbil K, Gruetter R. Quantification of 7 Tesla  $^1\text{H}$  NMR spectra of the human brain using LCModel and spin simulations. In: *Proc 9th Annual Meeting ISMRM*, 2001. Glasgow. p 214.
14. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed* 1998;11: 266–272.
15. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993;30:672–679.
16. Adriany G, Gruetter R. A half-volume coil for efficient proton decoupling in humans at 4 Tesla. *J Magn Reson* 1997;125:178–184.
17. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med* 2000;43:319–323.
18. Kelley DA, Wald LL, Star-Lack JM. Lactate detection at 3T: compensating J coupling effects with BASING. *J Magn Reson Imag* 1999;9:732–737.
19. Tkac I, Starcuk Z, Choi I-Y, Gruetter R. In vivo  $^1\text{H}$  NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med* 1999;41:649–656.
20. Tkac I, Anderson P, Adriany G, Merkle H, Ugurbil K. In vivo  $^1\text{H}$  NMR spectroscopy of the human brain at 7 T. *Magn Reson Med* 2001;46:451–456.
21. Kudo H, Mio T, Kokunai T, Tamaki N, Sumino K, Matsumoto S. Quantitative analysis of glutathione in human brain tumors. *J Neurosurg* 1990;72:610–615.
22. Kosower EM. Chemical properties of glutathione, In: Arias JM, Jacoby WB, editors. *Glutathione: metabolism and function*. New York: Raven Press; 1976. p 1–15.
23. Behar KL, Rothman DL, Spencer DD, Petroff OAG. Analysis of macromolecule resonances in  $^1\text{H}$  NMR spectra of human brain. *Magn Reson Med* 1994;32:294–301.