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Measurement of glycine in gray and white matter in the human brain *in vivo* by ¹H-MRS at 7.0 T

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Abstract

The concentration of glycine (Gly) was measured in gray and white matter (GM and WM) in the human brain using single-voxel localized ¹H-MRS at 7 T. A point-resolved spectroscopy (PRESS) sequence with TE = 150 ms was used for measuring Gly levels in various regions of the frontal and occipital lobes in eleven healthy volunteers and one subject with a glioblastoma. The PRESS spectra were analyzed with LCModel using basis functions generated from density matrix simulations that included the effects of volume localized radio-frequency and gradient pulses. The fraction of gray and white matter within the voxels was obtained from T₁-weighted image segmentation. The metabolite concentrations within the voxels, estimated with respect to the GM + WM water concentrations, were fitted to a linear function of fractional GM content. The Gly concentrations in pure GM and WM were estimated to be 1.1 and 0.1 mM, with 95% confidence intervals 1.0 - 1.2 and 0.0 - 0.2, respectively.

INTRODUCTION

Glycine (Gly) functions as an inhibitory neurotransmitter by binding to glycine receptors that conduct chloride ions (1). It also modulates excitatory neurotransmission in its role as a coagonist at N-methyl-D-aspartate receptors (NMDA) (2,3). Alterations in Gly levels are implicated in several diseases of the central nervous system. Precise measurement of Gly *in vivo* is important for the investigation of hereditary neuromotor disorders, in which changes in glycinergic inhibitory transmission occur (3). High Gly diets and glycine transporter inhibitors are being tested for their therapeutic potential for treating schizophrenia, which may be associated with reduced glutamate transmission (4,5), and for treating mood

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disorders, in which modification of the NMDA receptor's function is associated with an antidepressant effect (6). Gly levels following oral administration of Gly as an adjuvant treatment for schizophrenia were tested with MRS (7). Additionally, recent *in-vivo* MRS studies (8-10) have indicated that Gly may be a biomarker for brain tumor malignancy since elevated Gly was detected in *in-vivo* MRS studies of high grade gliomas.

The two uncoupled protons of Gly give rise to a singlet at 3.55 ppm at neutral pH (11). Measurement of brain Gly *in vivo* is often elusive in standard ^1H MRS, primarily due to the extensive spectral overlap with the multiplet from abundant myo-inositol (mI), requiring modification of data acquisition and/or analysis strategies. Reported methods include 2D J-resolved MRS at 3 T (12), TE-averaging at 4 T (7), TE-optimized triple refocusing (13), and PRESS (point-resolved spectroscopy) (10) at 3 T, and optimized short-TE single-echo (14) and long-TE PRESS (15) approaches at 7 T. The range of normal Gly concentrations in the human brain, reported by these studies which mostly focused on gray matter dominant regions, is broad (0.5 - 2 mM). This could be due to differences in MRS methodology or to potential experimental errors in measuring the low-concentration metabolite. However, given that the concentrations of neurotransmitters such as GABA (16-18) and glutamate (Glu) (19-21) differ between gray matter (GM) and white matter (WM), the large variation of the reported Gly levels could be due to differences in the brain regions chosen for Gly measurements in the studies. We hypothesized that the concentration of the inhibitory neurotransmitter Gly may differ between GM and WM, and we carried out Gly measurements in various regions of normal human brains using a previously-reported Gly-optimized PRESS method at 7 T (15). The fractions of GM, WM and CSF within the PRESS-localized volume were obtained from T_1 -weighted image segmentation and used for estimating the Gly levels in pure GM and WM *via* linear regression.

MATERIALS and METHODS

Measurement of Gly in GM and WM in the human brain was carried out using a previously-reported Gly-optimized PRESS sequence (15) in a 7.0 T whole-body scanner (Philips Medical Systems, Cleveland, OH, USA). A quadrature birdcage head coil was used for radio-frequency (RF) transmission and a 16-channel phased-array coil was used for signal reception. Volume selective RF pulses included an 8.8-ms amplitude/frequency-modulated 90° RF pulse (BW = 4.7 kHz) and an 11.9-ms amplitude-modulated 180° RF pulse (BW = 1.4 kHz) at an RF field intensity (B_1) of 15 μT . The protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. Written informed consent was obtained prior to the scans.

Eleven healthy volunteers were enrolled in the study (6 male and 5 female; age 26 ± 5). *In vivo* data acquisition was performed similarly to our prior study (15). In brief, following a survey image acquisition, a 3D sagittal T_1 -weighted image (MPRAGE) was acquired with TR/TE/TI = 2500/3.7/1300 ms, flip angle = 8° , field of view = $240 \times 240 \times 150$ mm, and 150 slices (slice thickness = 1.0 mm). Single-voxel localized proton MR spectra were obtained from various brain regions including anterior cingulate cortex, medial prefrontal and WM-dominant cortices in the frontal lobe and three regions (GM dominant, WM dominant, and intermediate) in the occipital lobe for a total of 3 to 6 voxels in each subject. Voxel size varied between 9 and 27 mL. MRS data from all subjects were divided approximately equally between voxels from the frontal and occipital lobes (24 and 20 voxels, respectively). Water-suppressed PRESS acquisition parameters included TR = 2.5 s, TE = 150 ms (TE₁ = 100 ms, TE₂ = 50 ms), acquisition bandwidth = 5 kHz, and 4096 sampling points. The number of signal averages (NSA) was 64 to 384 depending on the voxel size and on the brain region. We used increased signal averaging (2-fold) in scans on WM-dominant regions. The carrier frequencies of the volume selective RF pulses were set to 3.58 ppm,

which is approximately half way between the Gly and mI prominent peak locations (3.55 and 3.62 ppm, respectively). An unsuppressed water signal was acquired from each voxel for eddy current compensation in the metabolite data (22), using the same gradient scheme as that of the metabolite acquisition. With the RF carrier frequencies set to 4.67 ppm, an unsuppressed short-TE (14 ms) water signal was acquired using TR = 2.5 s from each voxel for use as a reference for metabolite quantification. A vendor-supplied four-pulse variable-flip-angle scheme was used for water suppression. Of the 16 channels of the receive coil, the upper 8 channels were used for data acquisition from the frontal lobe and the lower 8 channels were used for acquisition from the occipital lobe (Subjects were positioned supine in the magnet). The raw data of individual channels are combined by first correcting the zeroth order phase difference between channels, normalizing the noise per channel, and performing a complex square-root-sum-of-squares summation of all channels. This was done with manufacturer supplied routines. The RF power requirement of the MRS runs with TR = 2.5 s was ~1.3W/Kg for both frontal and occipital lobes, about 40% the FDA SAR limit.

MRS data were analyzed with LCModel software (Ver 6.2) (23). Model spectra of 23 metabolites were used as the basis set, adding the spectra of D-glucose α and β -anomers to the basis set of our previous study (15). The basis spectra were calculated with density matrix simulations that incorporated the volume selective RF and gradient pulses, according to the methods described in the Supplementary Information of our recent paper (24). The spectral fitting range was set to 0.5 - 4.2 ppm. Cramér-Rao lower bounds (CRLB) and correlation coefficients, returned by LCModel, were used for determining the reliability of the metabolite estimates and the dependence between metabolite estimates, respectively. Correlation coefficients between a pair of metabolite estimates, which are indicative of the inter-dependence of the signal estimates, were obtained from LCModel output files. The compositions of GM, WM, and CSF within the MRS voxels were obtained from segmentation of the T₁-weighted images using Statistical Parametric Mapping software (SPM5) (25). The probabilities of the three components which were calculated by SPM5 for each pixel of the T₁-weighted image were averaged over the corresponding MRS voxel to obtain the fractional proportions f_{GM} , f_{WM} , and f_{CSF} . The metabolite concentration, C_{meta} , was calculated from the LCModel estimates of metabolite signals, S_{meta} , and the water signal, S_{water} , using the equation

$$C_{meta} = C_{water} \frac{S_{meta} \exp(-TE_{diff}/T_2)}{S_{water} (1 - f_{CSF})}, \quad [1]$$

where the tissue water concentration, C_{water} , was calculated using $(0.81 \times f_{GM} + 0.71 \times f_{WM}) \times 55.5$ M which incorporates published water concentrations in GM and WM (26). Here TE_{diff} was the echo time difference between the metabolite and water signal acquisitions ($150 - 14 = 136$ ms). The effects of T₂ relaxation on Gly and mI signals were corrected using a mean value (130 ms) of published NAA and Cr T₂s at 7 T (27). To obtain the Gly and mI concentrations in pure GM and WM, the metabolite estimates from various brain regions were fitted to a linear function of fractional GM matter content, namely $f_{GM}/(f_{GM} + f_{WM})$. Prism 5 (GraphPad Software, Inc.) was used for the linear regression.

In addition, a clinical application of the Gly-optimized PRESS was undertaken in a subject with a glioblastoma, as determined by histological analysis of biopsies according to the World Health Organization (WHO) criteria. Following the survey scans, T₂-weighted fluid-attenuated inversion recovery (T₂w FLAIR) images were acquired to identify tumor regions. A $2 \times 2 \times 2$ cm³ voxel was positioned at the center of the tumor mass for MRS data acquisition. Metabolite concentrations were estimated using a short-TE (14 ms) water signal

as reference, assuming equal compositions of GM and WM contents and adjusting for the metabolite T_2 relaxation effects, similarly to a prior tumor MRS study (24).

RESULTS

LCModel outputs of Gly and mI showed a dependency on the baseline spline function parameter, dkntmn (node spacing parameter), as indicated in a prior study (28). As shown in Fig. 1, in the case of a spectrum from the medial prefrontal cortex, in which Gly and mI appear as a well-defined large dual-peak composite signal, the estimates and CRLBs of Gly and mI were more or less independent of dkntmn. However, in the case of a spectrum from a right frontal WM-dominant region, the Gly and mI signals were small compared to neighboring signals and the Gly estimate and CRLB varied widely for dkntmn less than 0.1. For dkntmn < 0.1, the Gly estimate was low and the CRLB was large, indicating that the Gly signal was not well resolved. The Gly estimate progressively increased and CRLB decreased with increasing dkntmn, and both became stable at dkntmn $\approx$ 0.2 and above. The mI estimate and CRLB remained constant as the baseline spline parameter varied over the range tested. For data from the medial prefrontal lobe, the correlation coefficient decreased from 0.1 down to -0.1 as dkntmn increased and was close to 0.0 when dkntmn was in the vicinity of 0.2. The data from the right frontal lobe exhibited a negative correlation between Gly and mI for dkntmn in the range of 0 - 0.5. In general, the Gly and mI estimates and CRLBs were essentially independent of the baseline spline function parameter in data which had large Gly and mI signals, but when the Gly and mI signals were relatively small the estimates and CRLBs varied when dkntmn was small but became stable above dkntmn $\sim$ 0.2.

Figure 2 displays *in-vivo* PRESS spectra from six brain regions of a healthy volunteer, each normalized with respect to the GM+WM water signal, and results of LCModel analysis which were obtained with dkntmn set to the value of 0.2. The LCModel results closely matched the *in-vivo* spectra and the residuals did not exhibit considerable chemical-shift dependence. The spectra all showed a dual-peak composite signal pattern between 3.5 and 3.7 ppm, in which the peaks at 3.55 and 3.62 ppm are primarily attributed to Gly and mI, respectively. The composite signal intensity was different between brain regions. Gly and mI signals were readily discernible in the spectrum from the anterior cingulate cortex (Fig. 2a), with CRLBs of 3% and 2%, respectively. In spectra from the medial frontal and medial occipital lobes (Fig. 2b,d), Gly and mI signals were observed to be large with relatively low CRLB, whilst the signals were small in spectra from low GM content regions in both the frontal and occipital lobes (Fig. 2c,f). When the voxel was placed in a region with an intermediate fraction of GM, the Gly and mI peaks exhibited intermediate intensities (Fig. 2e). Voxel displacement due to the finite bandwidth (1.4 kHz) of the 180° RF pulses was extensive for resonances far from the RF carrier frequency (3.58 ppm). For example, the voxel of the NAA singlet resonance was displaced by $\sim$ 30% with respect to the slice thickness, as shown in Fig 2.

For the 44 voxels of the 11 subjects of the present study, the fractional GM content spanned 0.21 - 0.83. The CRLBs of Gly and mI showed similar correlation to the GM content; namely, small CRLBs ($\sim$ 5% and $\sim$ 4%, respectively) were achieved at large GM content, but there were marked increases in CRLBs at fractional GM contents less than 0.3, as seen in Fig. 3a,b. The CRLBs were less than 20% in all spectra. The correlation coefficients between Gly and mI were all within $\pm$ 0.3 (Fig. 3c). The Gly and mI concentrations, estimated with respect to the GM+WM brain water signal, are presented as a function of the fractional GM content in Figs. 3d and 3e, respectively. The linear correlation of the Gly and mI concentrations vs. fractional GM content was statistically significant ($p < 0.001$ for both Gly and mI) with the coefficient of determination R^2 equal to 0.69 and 0.56, respectively. The metabolite concentrations in GM and WM were estimated from the intercepts of the

linear regression line to the ordinate axes, while making the assumption that the metabolite levels in CSF are negligible (29). The Gly concentrations in GM and WM were estimated to be 1.1 and 0.1 mM (95% confidence intervals 1.0 - 1.2 and 0.0 - 0.2), respectively. The mI concentrations in GM and WM were estimated to be 6.9 and 1.6 mM (95% confidence intervals 6.2 - 7.8 and 0.8 - 2.4), respectively. The Gly and mI concentrations were observed to be about the same between frontal and occipital lobes.

Figure 4 presents an application of the Gly-optimized PRESS in a brain tumor. A spectrum from an untreated subject with a glioblastoma (WHO grade 4) showed an abnormal spectral pattern between 3.5 and 3.65 ppm, indicating a substantially elevated Gly concentration. The concentrations of Gly and mI were estimated to be 8.9 and 4.3 mM, respectively, with reference to a water concentration of 42.2 M, assuming equal compositions of GM and WM within the tumor mass (24). Compared to the normal-brain case of Fig. 2e, in which GM and WM fractions were approximately equal, Gly in the tumor was found to be increased by greater than 10 fold, while mI remained at its normal level. The CRLB of Gly was 1%, the same as that of Cr whose CH₃ signal was similar to the Gly peak. Although the mI estimate in the tumor was similar to the normal-brain value, the mI CRLB in the tumor data was somewhat larger (7%) compared to the normal brain data, likely due to potential interferences of the large Gly signal with resolving the relatively small mI signal at 3.62 ppm. For Cr, NAA and Cho, the signals exhibited a well-known tumor spectral pattern, namely, increased Cho and decreased Cr and NAA. The concentrations were estimated to be 5.8, 5.1 and 4.5 mM, with CRLBs at 1, 1 and 0%, respectively.

DISCUSSION

The current paper reports the first measurement of the difference in Gly concentrations in GM and WM of healthy human brains, as measured by optimized ¹H-MRS at 7 T. With excellent suppression of the mI multiplet at the Gly resonance, the Gly singlet was clearly differentiable from the mI background signals. The signal of the mI C4- and C6-protons at ~3.61 ppm was retained, allowing measurement of both Gly and mI with minimal interference. This result indicates that the Gly concentration is markedly higher in GM than in WM (~10 fold). The mI levels were also observed to be higher in GM compared to the levels in WM (~4 fold). Our observation of mI regional difference is in agreement with prior spectroscopic imaging studies (19,30), but the concentration difference is slightly larger in our study than in these prior studies (4 fold vs. 2-3 fold).

Although direct comparison may not be possible due to the differences in MRS sequence and data analysis methods, the Gly concentration in pure GM from this study (1.1 mM) is in good agreement with a prior report from a small volume in the GM-rich occipital lobe (Gly/Cr = 0.14; voxel 8 mL, 7T, TE = 40 ms) (14), but is greater than what was reported in a recent 3T study (Gly/Cr = 0.1; voxel 8 mL, TE = 160 ms) (10), when a Cr concentration of 8 mM is assumed. The mI-to-Gly concentration ratio appears to be TE dependent. The mI/Gly ratio is higher in the present long-TE study than in the prior short-TE 7T study (6.3 vs. 4.4), implying that the Gly CH₂ signal may decay faster with increasing TE compared to the mI CH signals whose T₂ was measured to be quite long compared to the Cr methyl proton signal in human (31) and mouse (32) brain.

The coefficient of determination (R²), which indicates the goodness of the linear fit of the metabolite estimates vs. tissue type, was slightly greater in Gly than in mI. This result is somewhat in contrast with the overall lower CRLBs in mI than in Gly (Fig. 3a,b). The voxel displacement effects arising from the finite bandwidths of the volume localization RF pulses could cause errors in the mI estimation and the GM, WM, and CSF compartmentation. Myo-inositol has four resonances (4.06, 3.61, 3.52, and 3.27 ppm) and the signal recorded from

each resonance comes from a voxel that is shifted from the prescribed voxel in proportion to the spectral distance of the resonance from the RF carrier (3.58 ppm in this study). The actual spectral distances between mI peaks could therefore be different from the theoretical values, depending on B_0 inhomogeneity. When B_1 is inhomogeneous, the relative peak strengths of the mI resonances could be altered from the theoretical pattern because of the effects of average-flip-angle differences between the displaced voxels. The coherence proliferation of mI spins could also be modified due to non-uniform rotations of mI spins, thereby leading to alterations in individual multiplet patterns of the mI resonances. Furthermore, due to the effect of voxel displacement, the GM, WM and CSF contents may differ between the localized volumes of the mI resonances. Lastly, some errors in mI estimation could arise from the use of reduced mI signals for estimating the mI concentration. With the presence of noise and potential artifacts in the data due to subject movement, any small errors in mI signal estimates will propagate, leading to substantial errors in mI estimation. Together the precision of the mI data could be relatively lower compared to Gly in this study. The artifacts arising from the limited bandwidth of the volume-selective RF pulses in the present study can be minimized with spatial localization by phase encoding of spectroscopic imaging techniques, as demonstrated in several previous studies at 7 T (30,33,34), although fine adjustments of echo times may be needed for achieving acceptable Gly selectivity when different RF pulses are used.

We present linear regression results for Gly and mI only. For the voxels shown in Figs. 2b - 2f, the relative water signal strengths were 100, 69, 77, 70, and 55, respectively, due to the sensitivity inhomogeneity within the multi-channel coil used in this study. With the use of a volume selective RF pulse carrier set to 3.58 ppm in metabolite acquisitions and the use of water signal as reference, which was acquired with the RF carrier set to the water resonance (4.67 ppm), only the signals in the proximity of 3.58 ppm represent the concentration of the underlying metabolite properly. Analysis of the signals from other metabolites would require water data from the displaced voxels, which were not acquired in the present study.

Several metabolites have resonances in the proximity of the Gly resonance (11). These include threonine (Thr) (3.58 ppm; C2 proton), free choline (fCho) (3.51 ppm; C2 proton), and D-glucose α -anomer (Glc) (3.52 ppm; C2 proton). The resonances are all J coupled and thus the signals are modulated with echo time. For the TE chosen in this study, the Thr and fCho resonances are predicted to give multiplets with amplitude 21% and 3% relative to the Gly singlet for identical concentrations when relaxation effects are ignored, as shown in our prior 7 T study (15). In this study, the Thr estimates were essentially zero in all data with infinite CRLBs, largely because its signal at 1.31 ppm, the intensity of which is predicted to be about 2-fold greater than the signal at 3.58 ppm, was too small to be detected. The fCho estimates were negligible in the data from GM-rich regions, but the estimates in some data from WM-rich regions were comparable (up to 0.8 mM) to the Gly estimates. Since the fCho signal at the long TE was small (< 3%) compared to the Gly and mI signals, its interference with Gly and mI estimation may be negligible. Based on reports in the literature concerning fCho levels in normal brain (< 0.03 mmol/kg_{ww}) (11), the large fCho estimates in this study are most likely overestimates due to experimental errors. The Glc resonance is substantially degraded due to J evolution. The calculated amplitude of the Glc resonance between 3.4 - 3.7 ppm is ~3% of the Gly signal. Glucose levels were measured to be 0 - 1 mM with correlation coefficients to Gly and mI being between 0 - 0.3 and within ± 0.1 , respectively.

Lastly, the dependence of metabolite estimates on the LCModel baseline parameter in our study may be due to several experimental imperfections, including the effects of residual water and macromolecular baseline signals. Another major detrimental factor would include frequency drift artifacts primarily arising from subject movements although in the present

study the artifacts were reduced by acquiring data in multiple blocks and processing the multi-block data individually for frequency drift correction prior to LCModel analysis, as done in our prior study (15). In addition, some discrepancy between basis spectra and experimental data may be present in our long-TE study since basis spectra were generated using relatively large J coupling strengths (> 1 Hz). The time evolution of coherences due to smaller J coupling effects may be negligible at short TE.

In conclusion, we have measured the regional distribution of Gly concentrations in the human brain using Gly-optimized *in vivo* ^1H MRS at 7 T. Linear regression of Gly concentration against fractional gray matter content indicates much higher concentration in gray matter compared to white matter. This result suggests that evaluation of potential alterations in Gly concentrations in disease conditions may require comparison of the estimates from matched brain regions and/or interpretation of MRS data together with an estimate of the fractional gray and white matter contents within the volume of interest.

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REFERENCES

1. Lynch JW. Native glycine receptor subtypes and their physiological roles. *Neuropharmacology*. 2009; 56:303–309. [PubMed: 18721822]
2. Legendre P. The glycinergic inhibitory synapse. *Cell Mol Life Sci*. 2001; 58:760–793. [PubMed: 11437237]
3. Eulenburg V, Armsen W, Betz H, Gomeza J. Glycine transporters: essential regulators of neurotransmission. *Trends Biochem Sci*. 2005; 30:325–333. [PubMed: 15950877]
4. Tamminga CA, Lahti AC, Medoff DR, Gao XM, Holcomb HH. Evaluating glutamatergic transmission in schizophrenia. *Ann N Y Acad Sci*. 2003; 1003:113–118. [PubMed: 14684439]
5. Javitt DC. Glycine transport inhibitors and the treatment of schizophrenia. *Biol Psychiatry*. 2008; 63:6–8. [PubMed: 18082555]
6. Stewart CA, Reid IC. Antidepressant mechanisms: functional and molecular correlates of excitatory amino acid neurotransmission. *Mol Psychiatry*. 2002; 7(Suppl 1):S15–22. [PubMed: 11986991]
7. Kaufman MJ, Prescot AP, Ongur D, Evins AE, Barros TL, Medeiros CL, Covell J, Wang L, Fava M, Renshaw PF. Oral glycine administration increases brain glycine/creatine ratios in men: a proton magnetic resonance spectroscopy study. *Psychiatry Res*. 2009; 173:143–149. [PubMed: 19556112]
8. Hattingen E, Lanfermann H, Quick J, Franz K, Zanella FE, Pilatus U. ^1H MR spectroscopic imaging with short and long echo time to discriminate glycine in glial tumours. *MAGMA*. 2009; 22:33–41. [PubMed: 18830648]
9. Davies NP, Wilson M, Natarajan K, Sun Y, MacPherson L, Brundler MA, Arvanitis TN, Grundy RG, Peet AC. Non-invasive detection of glycine as a biomarker of malignancy in childhood brain tumours using in-vivo ^1H MRS at 1.5 tesla confirmed by ex-vivo high-resolution magic-angle spinning NMR. *NMR Biomed*. 2010; 23:80–87. [PubMed: 19795380]
10. Choi C, Ganji SK, DeBerardinis RD, Dimitrov IE, Pascual JM, Bachoo R, Mickey BE, Malloy CR, Maher EA. Measurement of glycine in the human brain in vivo by ^1H -MRS at 3 T: Application in brain tumors. *Magn Reson Med*. 2011; 66:609–618. [PubMed: 21394775]
11. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed*. 2000; 13:129–153. [PubMed: 10861994]
12. Schulte RF, Boesiger P. ProFit: two-dimensional prior-knowledge fitting of J-resolved spectra. *NMR Biomed*. 2006; 19:255–263. [PubMed: 16541464]
13. Choi C, Bhardwaj PP, Seres P, Kalra S, Tibbo PG, Coupland NJ. Measurement of glycine in human brain by triple refocusing ^1H -MRS in vivo at 3.0T. *Magn Reson Med*. 2008; 59:59–64. [PubMed: 18050319]

14. Gambarota G, Mekle R, Xin L, Hergt M, van der Zwaag W, Krueger G, Gruetter R. In vivo measurement of glycine with short echo-time ^1H MRS in human brain at 7 T. *MAGMA*. 2009; 22:1–4. [PubMed: 18949497]
15. Choi C, Douglas D, Hawes H, Jindal A, Storey C, Dimitrov I. Measurement of glycine in human prefrontal brain by point-resolved spectroscopy at 7.0 tesla in vivo. *Magn Reson Med*. 2009; 62:1305–1310. [PubMed: 19780175]
16. Jensen JE, Frederick Bde B, Renshaw PF. Grey and white matter GABA level differences in the human brain using two-dimensional, J-resolved spectroscopic imaging. *NMR Biomed*. 2005; 18:570–576. [PubMed: 16273508]
17. Choi IY, Lee SP, Merkle H, Shen J. In vivo detection of gray and white matter differences in GABA concentration in the human brain. *Neuroimage*. 2006; 33:85–93. [PubMed: 16884929]
18. Choi C, Bhardwaj PP, Kalra S, Casault CA, Yasmin US, Allen PS, Coupland NJ. Measurement of GABA and contaminants in gray and white matter in human brain in vivo. *Magn Reson Med*. 2007; 58:27–33. [PubMed: 17659613]
19. 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]
20. Choi C, Coupland NJ, Bhardwaj PP, Kalra S, Casault CA, Reid K, Allen PS. T_2 measurement and quantification of glutamate in human brain in vivo. *Magn Reson Med*. 2006; 56:971–977. [PubMed: 17029225]
21. Pan JW, Avdievich N, Hetherington HP. J-refocused coherence transfer spectroscopic imaging at 7 T in human brain. *Magn Reson Med*. 2010; 64:1237–1246. [PubMed: 20648684]
22. Klose U. In vivo proton spectroscopy in presence of eddy currents. *Magn Reson Med*. 1990; 14:26–30. [PubMed: 2161984]
23. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. 1993; 30:672–679. [PubMed: 8139448]
24. Choi C, Ganji SK, DeBerardinis RJ, Hatanpaa KJ, Rakheja D, Kovacs Z, Yang XL, Mashimo T, Raisanen JK, Marin-Valencia I, Pascual JM, Madden CJ, Mickey BE, Malloy CM, Bachoo R, Maher EA. 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated glioma patients. *Nat Med*. 2012; 18:624–629. [PubMed: 22281806]
25. Friston, KJ.; Ashburner, JT.; Kiebel, SJ.; Nichols, TE.; Penny, WD. *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. Academic Press; London: 2006.
26. Norton WT, Poduslo SE, Suzuki K. Subacute sclerosing leukoencephalitis. II. Chemical studies including abnormal myelin and an abnormal ganglioside pattern. *J Neuropathol Exp Neurol*. 1966; 25:582–597. [PubMed: 5922554]
27. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, Merkle H, Ugurbil K, Chen W. Proton T_2 relaxation study of water, N-acetylaspartate, and creatine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T. *Magn Reson Med*. 2002; 47:629–633. [PubMed: 11948722]
28. Emir UE, Deelchand D, Henry PG, Terpstra M. Noninvasive quantification of T_2 and concentrations of ascorbate and glutathione in the human brain from the same double-edited spectra. *NMR Biomed*. 2011; 24:263–269. [PubMed: 20925125]
29. D’Aniello A, Fisher G, Migliaccio N, Cammisia G, D’Aniello E, Spinelli P. Amino acids and transaminases activity in ventricular CSF and in brain of normal and Alzheimer patients. *Neurosci Lett*. 2005; 388:49–53. [PubMed: 16039064]
30. Henning A, Fuchs A, Murdoch JB, Boesiger P. Slice-selective FID acquisition, localized by outer volume suppression (FIDLOVS) for ^1H -MRSI of the human brain at 7 T with minimal signal loss. *NMR Biomed*. 2009; 22:683–696. [PubMed: 19259944]
31. Ganji SK, Banerjee A, Patel AM, Zhao YD, Dimitrov IE, Browning JD, Brown ES, Maher EA, Choi C. T_2 measurement of J-coupled metabolites in the human brain at 3T. *NMR Biomed*. 2012; 25:523–529. [PubMed: 21845738]
32. Xin L, Gambarota G, Mlynarik V, Gruetter R. Proton T_2 relaxation time of J-coupled cerebral metabolites in rat brain at 9.4 T. *NMR Biomed*. 2008; 21:396–401. [PubMed: 17907262]

33. Avdievich NI, Pan JW, Baehring JM, Spencer DD, Hetherington HP. Short echo spectroscopic imaging of the human brain at 7T using transceiver arrays. *Magn Reson Med*. 2009; 62:17–25. [PubMed: 19365851]
34. Boer VO, Siero JC, Hoogduin H, van Gorp JS, Luijten PR, Klomp DW. High-field MRS of the human brain at short TE and TR. *NMR Biomed*. 2011; 24:1081–1088. [PubMed: 21308826]

FIG 1.

Illustration of dependence of LCModel outputs on the baseline spline function parameter, $dkntmn$. For each of the *in-vivo* PRESS spectra from medial prefrontal (GM-dominant) and right frontal (WM-dominant) cortices, shown top to bottom are LCModel estimates of concentrations (semi-log scale), CRLBs of Gly and mI, and the correlation coefficients between Gly and mI. The number of signal averages was 64 and 256 for medial prefrontal and right frontal cortices, respectively. TR = 2.5 s, TE = 150 ms, voxel size = $23 \times 23 \times 23$ mm³.

FIG 2.

In-vivo PRESS spectra from six brain regions of a healthy volunteer are shown together with voxel positioning, LCMoel fits, residuals, baseline (red), and spectral components of Gly and mI. Spectra are scaled with respect to the brain GM+WM water signal. LCMoel estimates and CRLBs of Gly and mI are shown in brackets. The baseline spline function parameter in LCMoel analysis was set to 0.2. Voxels for 3.58 ppm are drawn in yellow and displaced voxels for 2 ppm (due to the effects of the finite bandwidths of volume localization RF pulses) are shown in blue. The ratios of GM and GM+WM contents for the 3.58 ppm voxels are shown for each voxel location. Voxel size was 30×20×15 mm³ in (a), and 23×23×23 mm³ for all other spectra. NSA = 256, 64, 256, 64, 128, and 256 for spectra in (a), (b), (c), (d), (e), and (f), respectively.

FIG 3.

(a,b) CRLBs of Gly and mI vs. fractional GM content. (c) Correlation coefficients between Gly and mI vs. fractional GM content. (d,e) Linear regression of Gly and mI levels normalized to the GM+WM water vs. fractional GM content for the 44 voxels positioned in frontal and occipital lobes of the brain. The dashed lines indicate 95% confidence intervals of the linear fits. Circles (brown) and squares (green) represent data from the frontal and occipital lobes, respectively. The baseline spline function parameter dkntmn in LCModel was set to 0.2.

FIG 4.

An *in-vivo* PRESS TE = 150 ms spectrum from a subject with a glioblastoma is shown together with voxel positioning in a T₂w FLAIR image and LCModel fitting results, in a similar fashion to Fig. 2. A vertical dotted line, drawn at 3.62 ppm, indicates an mI signal in the proximity of a large Gly singlet at 3.55 ppm. Data were acquired from a 20×20×20 mm³ voxel, with TR = 2.5 s and NSA = 128.