In Vivo Measurement of Carbon-13 Labeling of Glutamate and Glutamine in Human Brain Using Proton Magnetic Resonance Spectroscopy

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Abstract

A single-step spectral editing ^1H magnetic resonance spectroscopy (MRS) technique was used to measure fractional enrichments of glutamate and glutamine in the dorsal anterior cingulate cortex of five healthy volunteers after oral administration of $[\text{U-}^{13}\text{C}]\text{glucose}$. Strong pseudo singlets of glutamate and glutamine were induced at an echo time of 56 ms using an always-on editing pulse at 2.12 ppm. At 113 ± 9 minutes after oral administration of $[\text{U-}^{13}\text{C}]\text{glucose}$, fractional enrichment of glutamate was found to be $64 \pm 5\%$ with 1.7% within-subject coefficient of variation (CV) and fractional enrichment of glutamine was found to be $40 \pm 10\%$ with 11% within-subject CV. This study demonstrated that ^{13}C labeling of both glutamate and glutamine can be measured with the high sensitivity and spatial resolution of proton MRS using a proton-only MRS technique with standard commercial hardware. Furthermore, it is now feasible to measure ^{13}C labeling of glutamate and glutamine in limbic structures, which play major roles in behavioral and emotional responses and whose abnormalities are involved in many neuropsychiatric disorders.

Introduction

Noninvasive in vivo detection of ^{13}C labeling of glutamate (Glu) and glutamine (Gln) is a powerful tool for investigating Glu and Gln metabolism and neurotransmission in the brain ¹. Two types of ^{13}C magnetic resonance spectroscopy (MRS) techniques have been widely used: direct ^{13}C MRS and indirect $^1\text{H-}[^{13}\text{C}]$ MRS. Due to the much lower sensitivity of ^{13}C nuclei, direct ^{13}C MRS generally requires surface coils and very large tissue volume to achieve adequate signal-to-noise ratio (SNR). Hence, only the neocortex is accessible to direct ^{13}C MRS experiments. The indirect $^1\text{H-}[^{13}\text{C}]$ MRS techniques make use of the $^1\text{H-}^{13}\text{C}$ coupling and difference spectroscopy to detect signals from protons bound to ^{13}C ²⁻⁹. Because of the larger gyromagnetic ratio of ^1H compared to ^{13}C , these indirect ^{13}C detection techniques have higher sensitivity compared to the direct ^{13}C detection techniques. To perform either direct ^{13}C or indirect $^1\text{H-}[^{13}\text{C}]$ MRS, broadband magnetic resonance imaging (MRI) machines equipped with heteronuclear capabilities are required. The RF coil assembly needs to be high-efficiency non-volume ^{13}C and ^1H coils, which is a non-standard device and not commercially available, to enhance sensitivity and make heteronuclear nuclear Overhauser enhancement/decoupling feasible. Because of these technical barriers, ^{13}C MRS of human brain has been largely confined to only a few research groups with very limited clinical applications.

Attempts have been made to circumvent the hardware limitation of ^{13}C MRS by measuring the changes in short echo time (TE) ^1H MRS spectra caused by incorporation of ^{13}C labels into brain amino acids ¹⁰. However, it has been difficult to reliably separate Glu and Gln in the crowded ^1H MRS spectra. With the incorporation of ^{13}C labels, the short TE ^1H MRS spectra become even more complex. Due to these difficulties, quantification of ^{13}C labeling of both Glu and Gln using ^1H MRS in the human brain has not been reported.

Recently, our laboratory developed a 7 T single-step spectral editing method which can reliably measure Glu and Gln at TE = 56 ms¹¹. By placing an editing pulse at 2.12 ppm, which targets the H3 protons of Glu and Gln, the H4 protons of Glu and Gln form intense pseudo singlets at TE = 56 ms. The editing pulse here is used to alter the J-evolution of the strongly coupled spins such that Glu H4 and Gln H4 form intense pseudo singlet peaks at a relatively short TE of 56 ms. Without using an editing pulse, Glu H4 and Gln H4 form pseudo singlet peaks at a much longer TE of 100–110 ms¹². We found that the Gln H4 pseudo singlet induced by the editing pulse at TE = 56 ms is at least 61% more intense than the Gln H4 pseudo singlet formed naturally at TE = 106 ms¹¹.

In this work, we demonstrate the feasibility of measuring ¹³C fractional enrichments of Glu and Gln using the single-step spectral editing ¹H MRS technique (TE = 56 ms) with a commercial proton-only head coil at 7 T. Since this is a proton-only technique, no broadband scanner or any custom-made hardware is necessary. In addition, because of the much higher sensitivity of proton MRS, we will also demonstrate, for the first time, detection of ¹³C labeling of Glu and Gln from an area in the limbic system. Specifically, we will show that ¹³C labeling of Glu and Gln can be measured with high precision from the dorsal anterior cingulate cortex (dACC), a limbic structure involved in cognition and motor control but is beyond the reach of conventional ¹³C MRS that relies on surface coils. It is hoped that the demonstration of measuring ¹³C-labeling of Glu and Gln with the high sensitivity and spatial resolution of proton MRS using commercial scanners and RF coils will greatly facilitate the adoption of ¹³C MRS strategies for probing energy metabolism and glutamatergic neurotransmission in clinical research.

Results

Figure 1 shows the basis spectra of Glu, [4-¹³C]Glu, [4,5-¹³C]Glu, Gln, [4-¹³C]Gln, [4,5-¹³C]Gln, Asp, [3-¹³C]Asp, [3,4-¹³C]Asp, GABA, and [2-¹³C]GABA. As shown in Fig. 1, the spectra of Glu and Gln at TE = 56 ms with the editing pulse placed at 2.12 ppm are dominated by their respective H4 and H2 pseudo singlets. The spectrum of [4,5-¹³C]Glu is highly similar to that of [4-¹³C]Glu. This high similarity is expected because the ¹³C satellites of Glu H4 in the proton channel are resulted from the large one-bond scalar coupling between the H4 pseudo singlet and the ¹³C label at C4 while the ¹³C label at C5 only contributes to additional line-broadening of the ¹³C satellite signals via weak long-range scalar interactions. The effects of additional downstream ¹³C isotopomers on the ¹³C satellite signals of Glu H4 are similar. As both carbons of the acetyl CoA are ¹³C-labeled after administration of uniformly labeled glucose ¹³, [4,5-¹³C]Glu was chosen to represent the ¹³C satellite signals of Glu H4. Similarly, [4,5-¹³C]Gln and [3,4-¹³C]Asp were chosen to represent the ¹³C satellite signals of Gln H4 and Asp H3, respectively. The basis spectrum of [2-¹³C]GABA was not included in the basis set because the differences between the basis spectra of [2-¹³C]GABA and GABA are vanishingly small compared to the resonance signals of Glu H4, Gln H4, and their ¹³C satellites, as shown in Fig. 1. Due to magnetization transfer within the strongly coupled spin system, the ¹³C satellite spectra of Glu H4 and Gln H4 at TE = 56 ms are highly asymmetrical and dominated by a single downfield peak. It can be shown using density matrix

simulation that the two ^{13}C satellites of Glu H4 and Gln H4 at TE = 56 ms become symmetrical at much higher field strength (data not shown).

Time-course ^1H spectra from the dACC of the five subjects are displayed in Fig. 2. The spectra are highly consistent with the spectral patterns predicted by the numerical simulations. The Glu H4 peak at 2.34 ppm dropped dramatically after oral administration of $[\text{U-}^{13}\text{C}]$ glucose and, correspondingly, the peak at 2.56 ppm significantly increased due to the rise of the downfield ^{13}C satellite signals of Glu H4. Figure 3 displays the time-course spectra of subject 1 and corresponding fitted spectra of Glu, Gln and their ^{13}C satellites. In the fitted Gln time-course spectra, the drop in peak amplitude of Gln H4 after oral administration of $[\text{U-}^{13}\text{C}]$ glucose can be clearly seen. The spectra and corresponding fits for the pre- ^{13}C MRS scan and the last post- ^{13}C scan of subject 1 are displayed in Fig. 4. The spectral model fits the in vivo spectra very well. The spline baseline obtained by fitting the pre- ^{13}C spectrum is labelled as baseline_1 . The total baseline for the post- ^{13}C spectrum is the sum of baseline_1 and a much weaker baseline_2 which was determined when fitting the post- ^{13}C spectrum.

Metabolite ratios ($/[\text{tCr}]$) in the dACC of the five subjects quantified from the pre- ^{13}C spectrum of each subject are given in Table 1. The results are highly consistent with our earlier ^1H -only MRS study of the same brain region using the same pulse sequence ¹¹. Using the 12.6 mL ($3.5 \times 1.8 \times 2 \text{ cm}^3$) voxel size and 10 min scan time, the CRLB values for Glu and Gln were found to be $1.6 \pm 0.2\%$ for Glu and $3.2 \pm 0.4\%$ for Gln, indicating excellent precision. Table 2 gives the fractional enrichments of Glu H4 and Gln H4 for the five subjects computed from the last two post- ^{13}C spectra of each subject. The last two post- ^{13}C spectra were acquired at 113 ± 9 min after oral administration of $[\text{U-}^{13}\text{C}]$ glucose and each scan lasted 5 min. The fractional enrichments were found to be $64 \pm 5\%$ with 1.7% within-subject coefficient of variation (CV) for Glu and $40 \pm 10\%$ with 11% within-subject CV for Gln.

Table 1

Metabolite ratios ($/[tCr]$) in the dACC of five healthy volunteers quantified from the pre- ^{13}C spectrum of each subject. Voxel size = $3.5 \times 1.8 \times 2 \text{ cm}^3$; TR = 2.2 s; TE = 56 ms; editing pulse frequency = 2.12 ppm; editing pulse flip angle = 180° ; spectral width = 4000 Hz; number of data points = 1024; number of averages = 264; and total scan time = 10 min.

	Metabolite ratio ($/[tCr]$)	CRLB (%)
NAA	1.33 ± 0.09	1.2 ± 0.3
Glu	1.16 ± 0.08	1.6 ± 0.2
Gln	0.33 ± 0.07	3.2 ± 0.4
GSH	0.30 ± 0.02	3.0 ± 0.4
Asp	0.37 ± 0.07	8.0 ± 3.6
tCr	1	0.7 ± 0.2
tCho	0.25 ± 0.02	0.8 ± 0.2

Table 2

Fractional enrichments of Glu H4 and Gln H4 in the dACC of the five healthy volunteers computed from the last two post- ^{13}C spectra of each subject, which were acquired at 113 ± 9 min after oral administration of $[U-^{13}C]$ glucose. For each post- ^{13}C MRS scan, the number of averages was 132 and the total scan time was 5 min.

	Fractional enrichment (%)	Within-subject CV (%)
Glu H4	64 ± 5	1.7
Gln H4	40 ± 10	11

Figure 5 displays the plots of fractional enrichments of Glu H4 and Gln H4 vs. time after oral administration of $[U-^{13}C]$ glucose for all five healthy volunteers. The behavior of the time course of ^{13}C -labeled Glu and Gln shown in Fig. 5 is consistent with them attaining or approaching maximum ^{13}C labeling as expected from the time scale of cerebral Glu and Gln turnover¹. Similar behavior was also observed in our previous direct ^{13}C MRS experiments in the carboxylic/amide spectral region following oral administration of $[U-^{13}C]$ glucose¹⁴. In that experiment, there was little variation in the signal intensities of Glu C5 and Gln C4 + Asp C3 between spectra acquired at the 101–109 min and 113–

121 min intervals after oral [U-¹³C]glucose administration, indicating an approximate isotopic steady state being reached.

Discussion

Here, we demonstrated the feasibility of using a single-step spectral editing sequence with TE = 56 ms to measure fractional enrichments of Glu and Gln in the dACC of healthy volunteers after oral administration of [U-¹³C]glucose. Compared to the existing indirect ¹H-[¹³C] MRS techniques that use ¹H and ¹³C surface coils, this ¹H MRS method can acquire MRS data from a voxel away from the neocortex using a standard ¹H head coil. This method relies on using both the pre-¹³C and post-¹³C MRS data to compute fractional enrichments of Glu and Gln. Because the subjects exited from the scanner after the pre-¹³C scan for oral administration of glucose and reentered the scanner for acquisition of the post-¹³C spectra, the pre-¹³C and post-¹³C spectra generally had small differences in metabolite linewidths and lineshape, as well as in the spectral baseline. In our previous work (TE = 106 ms), the frequency shift, zeroth-order phase, and line-broadening of the pre-¹³C spectrum were adjusted to fit a post-¹³C spectrum before generating the difference spectrum¹⁵. The ¹³C-labelled Glu and Gln concentrations were obtained by fitting the difference spectrum. Due to the subtraction of the pre-¹³C and post-¹³C spectra, the noise level in the difference spectrum was amplified. In the difference spectrum, the unlabeled Gln H4 peak at 2.44 ppm was very weak with its ¹³C satellite signals barely discernible. Therefore, the ¹³C labelling of Gln could not be very reliably measured using the previous ¹H MRS technique with TE = 106 ms. In this work, a new post-processing method was developed. The metabolite concentration ratios ($/ [tCr] + 3[tCho]$) of Ace, NAA, NAAG, GABA, GSH, Asp, tCr, tCho, Tau, ml, and sl obtained by fitting the pre-¹³C spectrum were used as constraints when fitting the post-¹³C spectra. Meanwhile, the spline baseline obtained from the pre-¹³C spectrum was also used in fitting the post-¹³C spectrum, along with an additional much weaker baseline. This approach of using the prior information from the pre-¹³C spectrum in the fitting of each post-¹³C spectrum avoids spectrum subtraction and hence the corresponding noise amplification. Additionally, the Gln signals acquired using the current sequence (TE = 56 ms) were at least 61% higher than the Gln signals obtained by the previous sequence (TE = 106 ms)¹¹. Therefore, a much higher precision for measuring the fractional enrichment of Gln was achieved in this study, which is evidenced by its 11% within-subject CV.

The turnover of NAA and GSH in brain is known to be orders of magnitude slower than that of Glu and Gln. ¹³C labeling of NAA and GSH in our experiment therefore was ignored. Furthermore, this study used [4,5-¹³C]Glu to represent all ¹³C isotopomers of Glu with a ¹³C label at C4. This approximation is justified because the much greater one-bond ¹H-¹³C scalar coupling (¹J_{HC} = 127 Hz) splits Glu H4 into an uneven doublet (see Fig. 1) whereas ¹³C labels at other positions lead to line-broadening of the satellite signals due to the much smaller long-range ¹H-¹³C scalar couplings.

Our in vivo data in Fig. 5 clearly show the sizable effect of Gln isotopic dilution which leads to a smaller Gln fractional enrichment than that of Glu ¹⁶. Gln dilution is primarily resulted from exchange between astroglial Gln and unlabeled Gln in blood across the blood–brain barrier ¹⁷ as well as oxidation of short- and medium-chain free fatty acids and branched chain amino acids preferentially in astroglia ^{18,19}. The existence of Gln dilution sensitizes the Gln turnover to the effect of intercompartmental Glu-Gln cycling flux ^{16,20}. Using the standard small pool approximation in kinetic analysis, the Glu-Gln cycling flux V_{cyc} at isotopic steady state following administration of [U-¹³C]glucose is given by Eq. (8) in Ref. ²⁰:

$$V_{cyc} = V_{Gln(dil.)} f_{Gln}^{ss} / (f_{Glu}^{ss} - f_{Gln}^{ss})$$

where $V_{Gln(dil.)}$ is the Gln dilution flux, and f_{Glu}^{ss} and f_{Gln}^{ss} represent fractional enrichments of Glu H4 and Gln H4 at isotopic steady state, respectively. Using the mean literature value of $V_{Gln(dil.)}$ (0.18 $\mu\text{mol/g/min}$, averaged from the reported range of 0.14–0.22 $\mu\text{mol/g/min}$, Shen et al. ¹⁶ and references therein) and our end point fractional enrichments of Glu H4 and Gln H4, V_{cyc} in the dACC of the healthy subjects was estimated to be $0.32 \pm 0.13 \mu\text{mol/g/min}$ (mean $\pm$ SD, $n = 5$). Interestingly, the estimated V_{cyc} in the dACC of the five subjects closely matches most of the literature V_{cyc} values which were measured from brain regions dominated by the neocortex ¹⁶. The high activities of Glu neurotransmission in the dACC is consistent with its purported glutamatergic role in many brain functions and neuropsychiatric disorders.

Although the current study used a 7 T scanner to resolve Glu and Gln H4 protons in the ¹H MRS spectra, spectral resolution of Glu and Gln H4 signals at 3 T is also achievable ²¹. In principle, it is possible to use a similar strategy to measure ¹³C labeling of Glu H4 and possibly Gln H4 using ¹H MRS on the prevalent 3 T scanners. Research along this direction is currently in progress in our laboratory.

Previous studies have demonstrated quantification of the Glu-Gln neurotransmitter cycling flux between neurons and astroglia using direct ¹³C MRS by measuring fractional enrichments of Glu C4 and Gln C4 at isotopic steady state following administration of ¹³C-labeled acetate or by measuring dynamic turnover of Glu and Gln following administration of ¹³C-labeled glucose, lactate, or b-hydroxybutyrate ¹. Therefore, it is possible to use the ¹H-only MRS technique demonstrated in this study to quantify the Glu-Gln neurotransmitter cycling flux with much higher spatial resolution and from brain regions inaccessible to surface coils (e.g., from limbic structures which play a major role in many neuropsychiatric disorders).

In summary, a recently developed single-step spectral editing technique that induces intense Glu and Gln H4 singlets at TE = 56 ms was used to measure fractional enrichments of Glu and Gln in the dACC of five healthy volunteers after oral administration of [U-¹³C]glucose. A new post-processing method was developed, in which the metabolite ratios and spline baseline obtained from fitting the pre-¹³C spectrum were used in the fitting of the post-¹³C spectra to compute the fractional enrichments of Glu H4 and Gln H4. At 113 ± 9 min after oral administration of [U-¹³C]glucose, the fractional enrichment of Glu H4 was found to be $64 \pm 5\%$ with 1.7% within-subject CV and the fractional enrichment of Gln H4 was found to be

40 ± 10% with 11% within-subject CV. This technique offers the ability to measure Glu neurotransmission in the human brain with the high sensitivity and spatial resolution of ^1H MRS using standard commercial equipment. Brain regions inaccessible to surface coils can now be investigated using the method described in this study.

Methods

Five healthy volunteers (two females, three males; age = 34 ± 12 years) were recruited for the study. The study was approved by the Institutional Review Board (IRB) of the National Institute of Mental Health (NIMH). The administration of ^{13}C enriched glucose solution was approved by IRB-NIMH and the National Institutes of Health (NIH) Clinical Center Pharmacy Department. Written informed consent was obtained from every participant before enrollment, and the study was performed according to the Declaration of Helsinki. All experimental protocols and methods were performed in accordance with the guidelines and regulations of NIH MRI Research Facility. Experiments were carried out on a Siemens Magnetom 7 T scanner equipped with a 32-channel receiver head coil. The volunteers underwent overnight fasting before the MRS study.

In each scan session, the subject was first scanned to acquire the pre- ^{13}C MRS data. T_1 -weighted magnetization prepared rapid gradient echo (MPRAGE) images were acquired with repetition time (TR) = 3 s, TE = 3.9 ms, matrix = $256 \times 256 \times 256$, and resolution = $1 \times 1 \times 1 \text{ mm}^3$. Based on the MPRAGE images, the MRS voxel with a size of $3.5 \times 1.8 \times 2 \text{ cm}^3$ was placed in the dACC of the subject. The first- and second-order B_0 shimming coefficients were adjusted, achieving water linewidths of $11.1 \pm 0.4 \text{ Hz}$. The pre- ^{13}C MRS scan was subsequently performed using the single-step spectral editing pulse sequence¹¹. The main component of the pulse sequence was a point resolved spectroscopy sequence (PRESS) with an always-on spectral editing pulse between the two 180° refocusing pulses. The editing pulse was a truncated Gaussian pulse with a duration of 10 ms, and it was applied at 2.12 ppm. The pulse sequence parameters were: TR = 2.2 s, TE = 56 ms, editing pulse frequency = 2.12 ppm, editing pulse flip angle = 180° , spectral width = 4000 Hz, number of data points = 1024, number of averages = 264, number of unsuppressed water signal averages = 2, and total scan time = 10 min.

After the pre- ^{13}C MRS scan was finished, the subject exited from the scanner and was orally administered 20% w/w 99% enriched $[\text{U-}^{13}\text{C}]$ glucose solution at a dosage of 0.75 g $[\text{U-}^{13}\text{C}]$ glucose per kg of body weight following procedures described in our previous study of carbonic anhydrase-catalyzed ^{13}C magnetization transfer¹⁴ and references therein. After a rest period, the subject reentered the scanner. The MPRAGE images were repeated, based on which the MRS voxel was placed at the same location and with the same size as the pre- ^{13}C MRS scan. Post- ^{13}C MRS scans were repeatedly performed, each lasted 5 min (number of averages = 132). B_0 shimming coefficients were adjusted before each MRS scan.

The pre- ^{13}C MRS data were processed first and the process was similar to that of the previous work¹¹. Briefly, the raw free induction decay (FID) data were reconstructed into the pre- ^{13}C spectrum after going

through the necessary steps that include multi-channel data combination ²², eddy current correction ²³, Bloch-Siegert phase shift correction ²⁴, frequency drift correction ²⁵, and Fourier transform. The reconstructed pre-¹³C spectrum was fitted in the range of 1.8–3.4 ppm by linear combination of numerically computed basis spectra of acetate (Ace), N-acetyl-aspartate (NAA), N-acetylaspartylglutamate (NAAG), γ-aminobutyric acid (GABA), Glu, Gln, glutathione (GSH), aspartate (Asp), total creatine (tCr), total choline (tCho), taurine (Tau), myo-inositol (ml), and scyllo-inositol (sl), as well as a spline baseline with 13 knots. The fitting program was developed in-house and was based on the Levenberg-Marquardt least square minimization algorithm. After the metabolite concentrations in arbitrary unit were obtained from the fitting, we computed the metabolite ratios, which were defined as the concentration of a metabolite divided by the sum of concentration of tCr and three times the concentration of tCho. The combined concentration, [tCr] + 3[tCho], weighs approximately equally the intensities of the tCr and tCho singlet peaks and is less prone to errors than using either [tCr] or [tCho] alone.

The post-¹³C spectra acquired after oral administration of [U-¹³C]glucose were reconstructed in the same way as the pre-¹³C spectrum. For the subsequent fitting process, additional basis spectra of ¹³C satellites of Glu H4, Gln H4 and Asp H3 were simulated. As shown in our previous work ²⁴, contributions from GABA is minimal at TE = 56 ms with the editing pulse placed at 2.12 ppm. Hence, ¹³C-labeled GABA was not included in the basis set. When fitting each post-¹³C spectrum, the metabolites ratios (/ [tCr] + 3[tCho]) of Ace, NAA, NAAG, GABA, GSH, Asp, tCr, tCho, Tau, ml, and sl were fixed to the pre-¹³C values. However, the linewidths and lineshape of the metabolites in a post-¹³C spectrum were allowed to be different from those of the pre-¹³C spectrum because subject repositioning and B₀ shimming caused changes in the linewidths and lineshape. The sum of metabolite ratios of Glu and its ¹³C satellites was constrained to be the same as the metabolite ratio of Glu obtained from the pre-¹³C spectrum. The same constraints were also applied to Gln and Asp. Because the spectral baseline in the post-¹³C spectrum was expected to be slightly different from the spectral baseline in the pre-¹³C spectrum due to subject repositioning and B₀ shimming, the spectral baseline in each of the post-¹³C spectrum was approximated by the sum of the spline baseline in the pre-¹³C spectrum and another much weaker spline baseline with 8 knots.

After the metabolite concentrations were obtained by fitting each post-¹³C spectrum, the ¹³C fractional enrichment of Glu H4 for the post-¹³C spectrum was computed as the ratio of the concentration of its ¹³C satellites to the total concentration of Glu. Meanwhile, the fractional enrichment of Gln H4 for each post-¹³C spectrum was similarly computed. The within-subject CV ²⁶ of the fractional enrichment values were estimated from the last two post-¹³C spectra for each subject, which were acquired at 113 ± 9 min after oral administration of [U-¹³C]glucose. The ¹³C enrichment of Glu H4 is expected to have approximately reached its maximum value at this stage ^{1,14}.

Declarations

Competing Interests:

The authors declare no competing interests.

Author Contributions

J.S. and L.A. conceived and designed the experiments and prepared the manuscript. L.A. developed the pulse sequence and data processing software. L.A. and S.L. performed the experiments. S.L., M.F.A., M.V., and C.S.J. provided medical support. All the authors read and approved the final manuscript.

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Data Availability

Data is available upon request.

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Author Contributions

J.S. and L.A. conceived and designed the experiments and prepared the manuscript. L.A. developed the pulse sequence and data processing software. L.A. and S.L. performed the experiments. S.L., M.F.A., M.V., and C.S.J. provided medical support. All the authors read and approved the final manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

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Figures

Figure 1

Numerically simulated spectra of Glu, $[4\text{-}^{13}\text{C}]\text{Glu}$, $[4,5\text{-}^{13}\text{C}]\text{Glu}$, Gln, $[4\text{-}^{13}\text{C}]\text{Gln}$, $[4,5\text{-}^{13}\text{C}]\text{Gln}$, Asp, $[3\text{-}^{13}\text{C}]\text{Asp}$, $[3,4\text{-}^{13}\text{C}]\text{Asp}$, GABA, and $[2\text{-}^{13}\text{C}]\text{GABA}$ at TE = 56 ms with an editing pulse placed at 2.12 ppm. The corresponding concentration ratios were set to 10: 10: 10: 3: 3: 3: 3: 3: 3: 1: 1. The spectra were broadened to a singlet width of 8 Hz.

Figure 2

Time-course ^1H spectra from the dorsal anterior cingulate cortex (dACC) of five healthy volunteers. No linebroadening was applied to the spectra. Voxel size = $3.5 \times 1.8 \times 2 \text{ cm}^3$; TR = 2.2 s; TE = 56 ms; editing pulse frequency = 2.12 ppm; editing pulse flip angle = 180° ; spectral width = 4000 Hz; number of data points = 1024; number of averages = 264 and total scan time = 10 min for the pre- ^{13}C spectra; number of averages = 132 and total scan time = 5 min for each individual post- ^{13}C spectrum.

Figure 3

Time-course spectra and corresponding fitted spectra of Glu, Gln, and their ¹³C satellites acquired from the dACC of subject 1.

Figure 5

Plots of fractional enrichments of Glu H4 and Gln H4 vs. time after oral administration of [U- ^{13}C]glucose for all five healthy volunteers.