Published in final edited form as:

J Magn Reson. 2012 January ; 214(1): 252–257. doi:10.1016/j.jmr.2011.11.012.

Spectral editing for in vivo ^{13}C magnetic resonance spectroscopy

Yun Xiang^{a,b} and Jun Shen^{a,*}

^aMolecular Imaging Branch, National Institute of Mental Health Intramural Research Program, National Institutes of Health, Bethesda, MD, United States

^bDepartment of Laboratory Medicine, Wuhan Medical and Health Center for Women and Children, Wuhan, P.R. China

Abstract

In vivo detection of carboxylic/amide carbons is a promising technique for studying cerebral metabolism and neurotransmission due to the very low RF power required for proton decoupling. In the carboxylic/amide region, however, there is severe spectral overlap between acetate C1 and glutamate C5, complicating studies that use acetate as an astroglia-specific substrate. There are no known in vivo MRS techniques that can spectrally resolve acetate C1 and glutamate C5 singlets. In this study, we propose to spectrally separate acetate C1 and glutamate C5 by a two-step J-editing technique after introducing homonuclear ^{13}C - ^{13}C scalar coupling between carboxylic/amide carbons and aliphatic carbons. By infusing $[1,2\text{-}^{13}\text{C}_2]\text{acetate}$ instead of $[1\text{-}^{13}\text{C}]\text{acetate}$ the acetate doublet can be spectrally edited because of the large separation between acetate C2 and glutamate C4 in the aliphatic region. This technique can be applied to studying acetate transport and metabolism in brain in the carboxylic/amide region without spectral interference.

Keywords

In vivo ^{13}C MRS; carboxylic/amide spectral region; acetate metabolism; spectral editing

1. Introduction

In vivo proton spectra usually contain many overlapping peaks resonating over a small chemical shift range [1]. Spectral overlap complicates unambiguous peak assignments and quantification [2,3]. As a result, spectral editing techniques have been developed that can simplify proton MRS spectra in order to limit the detection to specific metabolite signal(s) [4]. Many proton spectral editing methods such as two-step J editing [5], multiple quantum filtering [6] and homonuclear polarization transfer [7] rely on scalar couplings between metabolite signals of interest. Manipulation of echo times can also lead to spectral simplification by the effects of echo time on transverse relaxation and J coupling evolution. For proton-detected heteronuclear experiments (e.g., POCE), spectral editing can be achieved by separating protons coupled to magnetic heteronuclei from the rest of the proton signals [8,9].

In contrast to the ubiquity of various proton spectral editing techniques there have been no reports in the MRS literature of spectral editing in experiments with direct detection of heteronuclei. In many cases the spectral dispersion in heteronuclear spectra is sufficiently

*Correspondence to: Jun Shen, PhD Molecular Imaging Branch National Institute of Mental Health Bldg. 10, Rm. 2D51A 9000 Rockville Pike, Bethesda, MD 20892-1527 Tel: +1 301 451-3408 Fax: +1 301 480-2397 shenj@intr.nimh.nih.gov (J. Shen).

large although unwanted spectral overlap arises in many situations. For example, in proton-decoupled in vivo ^{13}C MRS of brain, GABA C2 at 35.2 ppm may overlap with glutamate C4 at 34.1 ppm in the aliphatic spectral region due to ^{13}C - ^{13}C homonuclear couplings [10]. There is also significant spectral overlap among the ^{13}C isotopomers of glutamate, glutamine and aspartate C2's in the aliphatic spectral region [11].

Recently, we have developed a new approach for in vivo ^{13}C MRS of brain that uses [2- ^{13}C] glucose to generate singlet signals in the carboxylic/amide spectral region [12,13]. We found that broadband proton decoupling for carboxylic/amide carbons can be achieved using very low RF power [14]. In the carboxylic/amide regions there is spectral overlap between glutamine C5 at 178.5 ppm and aspartate C4 at 178.4 ppm and between glutamate C5 at 182.0 ppm and acetate C1 at 182.15 ppm. We have shown that the spectral overlap between glutamine C5 and aspartate C4, which may become problematic at relatively low magnetic field (e.g., 3 Tesla), can be largely avoided by optimization of magnetic field homogeneity and/or spectral deconvolution [12, 13]. The spectral overlap between glutamate C5 at 182.0 ppm and acetate C1 at 182.15 ppm, however, cannot be resolved by existing techniques. When [1- ^{13}C] acetate is used care has to be taken to ensure that tissue concentration of acetate is sufficiently low so as not to contaminate the glutamate C5 signal [15].

The severe spectral overlap between glutamate C5 and acetate C1 limits the usefulness of ^{13}C MRS based on the carboxylic/amide region and low RF power proton decoupling because acetate is a valuable specific substrate for cerebral astroglial metabolism [16–18]. Since glutamate C5 and acetate C1 only generate singlets when [1- ^{13}C] acetate is administered spectral editing techniques based on homonuclear scalar couplings cannot be used to resolve the two overlapping resonances. In this work, we devised a novel ^{13}C - ^{13}C spectral editing strategy for in vivo ^{13}C MRS of carboxylic/amide carbons. We introduced homonuclear ^{13}C - ^{13}C couplings by infusing [1,2- $^{13}\text{C}_2$]acetate in order to spectrally separate glutamate C5 from acetate C1. We demonstrated that the same approach can also be used to spectrally separate the glutamine C5 and aspartate C4 doublets. Essentially with the introduction of homonuclear ^{13}C - ^{13}C couplings the same J editing principles widely used in proton MRS were applied to ^{13}C MRS.

2. Materials and methods

2.1 Animal preparation

^{13}C -enriched D-glucose and sodium acetate were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Enrichment of all ^{13}C -labeled chemicals was 99%. Animals were used in accordance to protocols approved by the National Institute of Mental Health (NIMH) Intramural Research Program Animal Care and Use Committee, National Institutes of Health (NIH). All animals (male, body weight (BW) = 200–251 g) were fasted overnight. The rats were orally intubated and mechanically ventilated (SAR-830/AP, CWE, Inc., Ardmore, PA, USA) with a mixture of 70% N_2O , 30% O_2 and 1.5% isoflurane. The left femoral artery was cannulated for periodically sampling arterial blood to monitor blood gases (pO_2 , pCO_2), pH, and glucose concentration using a blood analyzer (Bayer Rapidlab 860, East Walpole, MA, USA), and for monitoring arterial blood pressure levels. The isolateral (left) vein was also cannulated for intravenous infusion of ^{13}C -labeled chemicals. [$^{13}\text{C}_6$]-D-glucose (0.75M) was infused to four animals with an initial bolus at an infusion rate of 75.5 mg/min/kg BW for ten minutes followed by an approximately constant infusion rate at 28.5 mg/min/kg BW for 4 hrs. Blood glucose level was maintained at ~16–19 mM. Six animals were given intravenous infusion of 0.9 M [1,2- $^{13}\text{C}_2$]acetate (pH = 7.0) with an initial bolus of 37.4 mg/min/kg BW for 10 min followed by an approximately constant infusion rate at 14.2 mg/min/kg BW for 4 hrs. Throughout all experiments, normal physiological conditions were maintained (pH: ~7.4, pCO_2 : ~35mmHg and pO_2 : > 100

mmHg, blood pressure: 60~90/75~120 mmHg, heart rate: 320~480 bpm), by real-time regulation of ventilation (SurgicalVet, SIMS BCI, Inc., Waukesha, WI, USA). An external pump for heat exchange by circulating warm water (Bay-Voltex, Modesto, CA, USA) was used to maintain body temperature at 37.5 ± 0.5 °C.

2.2 MR hardware

All experiments were performed on a Bruker AVANCE spectrometer (Bruker Biospin, Billerica, MA) interfaced to an 11.7 Tesla 89-mm bore vertical magnet (MagneX Scientific, Abingdon, UK). The spectrometer is equipped with a 57-mm i.d. gradient (Mini 0.5, Bruker Biospin, Billerica, MA, with a maximum gradient strength of 3.0 G/mm and a rise time of 100 μ s) for studying young adult rats in vivo. A home-built RF surface coils/head holder system mounted on a half-cylindrical plastic cradle was used [19]. The ^{13}C and ^1H RF coils were coplanar and made of single-sided printed circuit board. The inner loop of the RF assembly is the ^{13}C coil with an inner diameter and conductor width of 10.8 mm and 4.3 mm, respectively. The outer loop is the ^1H coil with an inner diameter and conductor width of 23.6 mm and 5.4 mm, respectively. No noise injection was found in the ^{13}C channel due to proton decoupling. To provide shielding against ambient RF noise, the main body of the RF probe/animal handling system was built using an aluminum tube with an outer diameter of 56.6 mm. The lower end of the integrated RF surface coils/head holder system was an aluminum interface box for connecting RF cables, ventilation tubes, fiber-optic rectal thermal probe, and catheters. The RF probe/animal handling system also provides rat head fixation and body support. It allows maintenance of normal physiology despite of increased cardiac load and blood draining associated with vertical positioning. The animal handling system was mechanically interfaced to the magnet using an adaptor ring which allows vertical and angular position adjustments. Commercial broadband low-pass and high-pass filters (Bruker Biospin, Billerica, MA) were inserted in front of the preamplifiers. The loaded isolation between ^1H and ^{13}C coils (S21), which have a large frequency separation of 375 MHz, is -30.6 dB at 125MHz and -31.2 dB at 500 MHz, respectively.

2.3 MRS

After the experimental animals were placed in the scanner anatomical images were acquired using the three-slice (coronal, horizontal, and sagittal) scout Rapid Acquisition with Relaxation Enhancement (RARE) imaging method (field of view: 2.5cm $\times$ 2.5 cm, slice thickness: 1.0 mm, TR/TE: 200/15 ms, rare factor: 8, data matrix size: 128 $\times$ 128). The gradient isocenter was 0–1 mm posterior to bregma based on separate calibrations. The ^{13}C editing pulse sequence is schematically illustrated in Fig. 1. An $8.5 \times 6 \times 8.5$ mm³ (434 μL) spectroscopy voxel was placed at the gradient isocenter along the brain midline. The rat brain was shimmed as described previously using the FLATNESS methods [20]. A train of non-selective hard pulses with a nominal flip angle of 180° spaced at 100 ms apart was used for generation of broadband $^1\text{H} \rightarrow ^{13}\text{C}$ heteronuclear Overhauser enhancement. Direct three-dimensional spatial localization of ^{13}C spins in the carboxylic/amide region uses a 0.75 ms adiabatic half-passage pulse followed by three pairs of hyperbolic secant pulses (one pair per dimension, 2-ms per pulse with phase factor = 5 and truncation level = 1%) as described previously [21]. The ^{13}C 180° pulses also refocus the long-range heteronuclear ^1H - ^{13}C couplings during the spin echoes. No additional outer volume suppression schemes were found necessary. TR/TE: 20000/44~48 ms. Spectral width was set to 10 kHz with a sampling time of 204.8 ms. For each data block 32 scans were acquired. The ^{13}C carrier frequency was placed around 180.4 ppm. A single spectrally selective editing pulse (180° hyperbolic secant pulse, phase factor: 3 and truncation level: 1%, pulse length: 10~12 ms, bandwidth: 1012~842 Hz) was placed either at 24.5 ppm for separating glutamate C5 from acetate C1 (10 ms) or at 31.7 ppm for separating Gln C5 (178.5 ppm) from aspartate C4 (178.4 ppm, 12 ms). The proton decoupler frequency was at 4.82 ppm. Because the

carboxylic/amide carbons are coupled to protons via very weak long-range ^1H - ^{13}C scalar couplings, they can be effectively decoupled at a very low RF power using stochastic decoupling [22]. The ^1H decoupling used a pseudo noise decoupling scheme with constant γB_2 amplitude ($\gamma B_2 < \sim 274$ Hz calibrated at the gradient isocenter), randomly inverted phases [22,23] and a repetition unit of 0.2–0.4 ms. The ^{13}C signals in the carboxylic/amide region were analyzed using Bruker Biospin XWINNMR software (Bruker Biospin, Billerica, MA). Prior to Fourier transform, the time-domain data were zero-padded to 16 K followed by a line broadening of 10 Hz.

For phantom experiments two phantoms were prepared. Phantom A contains non-labeled L-glutamic acid (0.6 M) and sodium acetate (0.6 M, pH = 7.4); Phantom B contains 0.1 M $[1,2-^{13}\text{C}_2]\text{acetate}$ (pH = 7.4). Phantom A was scanned without spectral editing with 192 averages. Phantom B was scanned using the pulse sequence shown in Fig. 1 with 16 averages.

3. Results

Fig. 2A shows a proton decoupled ^{13}C spectrum in the carboxylic/amide spectral region acquired from phantom A. Glutamate C5 (182.0 ppm), acetate C1 (182.15 ppm) and glutamate C1 (175.4 ppm) were detected. Due to the close proximity of glutamate C5 to acetate C1, complete separation of two was not feasible even at the high magnetic field strength of 11.7 Tesla. In Fig. 2B, the editing pulse sequence shown in Fig. 1 was used on phantom B. The 10 ms editing pulse was placed at the resonance frequency of acetate C2 (24.5 ppm). At the presence of the editing pulse, the homonuclear ^{13}C - ^{13}C coupling between acetate C1 and C2 (51 Hz) was defocused for a nominal period of $1/J_{\text{CC}}$. Due to the transient Bloch-Siegert effect generated by the editing pulse [24], a 28.8° zero order phase shift was observed for the inverted acetate C1 signal shown in the middle trace in Fig. 2B. After zero order phase correction, clean cancellation of the acetate C1 signal (Fig. 2B, upper trace) was achieved after addition of the bottom trace acquired with the editing pulse off to the middle trace acquired with the editing pulse on.

To ensure that the editing procedure does not affect glutamate C5, in vivo experiments with $[^{13}\text{C}_6]\text{-D-glucose}$ infusion were performed. When $[^{13}\text{C}_6]\text{-D-glucose}$ was administrated intravenously to the animal, glutamate C5 and C1, glutamine C5 and C1, aspartate C4 and C1 as well as ^{13}C labeled bicarbonate (HCO_3^-) were observed in the carboxylic/amide spectral region in the brain. In Fig. 2C, the editing pulse sequence and parameters used in Fig. 2B were applied with the 10 ms editing pulse placed at the resonance frequency of acetate C2 (24.5 ppm). The experiments were interleaved with the editing pulse turned on and off during alternating scans. Total number of scans was 32. There are no appreciable differences between the spectrum acquired without the editing pulse (a) and the spectrum acquired with the editing pulse except for a zero order phase shift. After zero order phase correction, subtracting the spectrum acquired with the editing pulse on (b) from (a) resulted in a clean baseline-type spectrum (c), indicating that glutamate C4 (34.1 ppm) and therefore the signal intensity of glutamate C5 are not affected by the editing pulse.

Fig. 3 (a) and (b) show the interleaved brain ^{13}C MRS spectra acquired from one animal during intravenous infusion of $[1,2-^{13}\text{C}_2]\text{acetate}$. Total number of scans was 32. Unlike uniformly ^{13}C -labeled glucose which produces only a doublet at glutamate C5 [15], infusion of $[1,2-^{13}\text{C}_2]\text{acetate}$ leads to both a doublet and a singlet at glutamate C5 due to pyruvate recycling [18]. Because of the close proximity of glutamate C5 to acetate C1 as shown in Fig. 2A the signal at 180.0 ppm appears as a triplet when the editing pulse is not turned on (see Fig. 3(a)). With the editing pulse on, the spectral pattern is complicated due to the inversion of the acetate doublet. The smaller acetate doublet signal was revealed in (c) after

subtracting (b) from (a). The pure glutamate triplet signal is obtained in (d) by adding (b) to (a) which cancels the acetate doublet as demonstrated in Fig. 2B. Note that the relative intensity of the glutamate C5 central peak in (d), which represents the glutamate C5 singlet, is much greater than that in (a) due to the removal of contribution to the outer two lines from acetate C1. Similar results were obtained from other animals infused with [1,2- $^{13}\text{C}_2$]acetate.

The results of spectral editing of glutamine C5 and aspartate C4 were illustrated in Fig. 4. The interleaved brain ^{13}C MRS spectra acquired from one animal during intravenous infusion of [$^{13}\text{C}_6$]-D-glucose were shown in (a) and (b). Total number of scans was 32. The 12 ms editing pulse was placed at glutamine C4 at 31.7 ppm. With the editing pulse off (a), the glutamine C5 doublet at 178.5 ppm partially overlaps with the aspartate C4 doublet at 178.4 ppm. With the editing pulse on (b), the glutamine C5 doublet is inverted while the aspartate C4 doublet is not affected because the aspartate C3 resonates at 37.1 ppm. Note that glutamate C5 is significantly affected by the editing pulse. Glutamate C4 resonates at 34.1 ppm. Primarily due to its couplings to the methylene protons part of the uncoupled glutamate C4 multiplets is spanned by the transition band of the editing pulse, leading to partial inversion as well as phase distortion of the glutamate C5 doublet signal. In the subtracted spectrum (c), the pure glutamine C5 doublet was detected. In the summed spectrum (d), only aspartate C4 doublet signal was seen. ^{13}C spectral editing leads to complete separation of glutamine C5 from aspartate C4 (at the expense of distorted glutamate C5 signal). Similar results were obtained from other animals infused with [U- $^{13}\text{C}_6$]glucose.

4. Discussion

The in vivo ^{13}C spectra of brain feature a large spectral separation between aliphatic carbons (~20–50 ppm) and carboxylic/amide carbons (~180 ppm). As a result, a typical spectrally nonselective pulse can act as a spectrally selective one for the homonuclear ^{13}C - ^{13}C scalar couplings between aliphatic and carboxylic/amide carbons even at relatively low field strength. For instance, we used 2 ms hyperbolic secant pulses ($\mu = 5$) with a bandwidth ($= 2\mu\ln(2/\text{truncation level})/(\pi \times \text{pulse duration})$) of 8437 Hz or 67 ppm at 11.7 Tesla for spatial localization. With the same pulse duration, one expects that the sech pulses ($\mu = 5$, truncation level = 1%) remain spectrally selective for carboxylic/amide carbons at or above 3 Tesla. Of course, longer (and therefore more selective) pulses would be needed for larger coils for human studies. In contrast to the wider spread of ^{13}C signals in the aliphatic region, the ^{13}C signals of interest in the carboxylic/amide region span a relatively narrow range of 178.4 (aspartate C4)~182.15 (acetate C1) ppm or 472 Hz at 11.7 Tesla. The localization error in the current experiment is therefore small (0.5 mm along x and z directions; 0.3 mm along the y direction). The sech pulses have no effect on the aliphatic carbon signals resonating at the 20~50 ppm range. The adiabatic spin echoes for spatial localization, therefore, also refocus homonuclear ^{13}C - ^{13}C scalar couplings in addition to spin evolution caused by chemical shifts and proton-carbon couplings. To selectively reintroduce net evolution by homonuclear ^{13}C - ^{13}C scalar couplings, a spectrally selective 180° soft adiabatic pulse was used to invert aliphatic carbon of interest. A density operator analysis shows that a carboxylic/amide carbon doublet undergoes a 180° phase shift (excluding the transient Bloch-Siegert effect) when the coupled aliphatic carbon is selectively inverted at the $0.5/{}^1J_{\text{CC}}$ time point prior to FID detection as shown in Fig. 1.

During the execution of the editing pulse, the transverse carboxylic/amide carbon magnetization evolves under the influence of both its resonance offset and the B_1 field of the editing pulse placed in the aliphatic spectral region. Because of the large separation between these two regions, the additional phase accumulation due to the transient Bloch-Siegert effect is simply $0.5 \times \langle \gamma B_1^2 \rangle T / \Delta\nu$, where $\langle \gamma B_1^2 \rangle$ and T are the average power and duration

of the editing pulse, respectively, and $\Delta\nu$ is the frequency separation between the aliphatic and carboxylic/amide spectral regions. The transient Bloch-Siegert phase shift caused by the editing pulse can be easily removed using a pair of identical editing pulses to sandwich a nonselective 180° pulse instead of using a single editing pulse. The twin editing pulses approach, however, is not necessary here. Because of the large separation between the aliphatic and carboxylic/amide spectral regions the transient Bloch-Siegert effect only causes a global zero order phase shift ($0.5 \cdot \langle \gamma B_1^2 \rangle T / \Delta\nu$) in the carboxylic/amide spectral region which can be easily corrected.

In contrast to the alkyl region which contains strong signals from subcutaneous lipids there is no spectral interference from lipids in the carboxylic/amide region of brain ^{13}C spectra. The crucial advantage of detecting the carboxylic/amide signals is that only very low RF power is needed to achieve full decoupling when stochastic decoupling schemes are used. Compared with aliphatic carbons carboxylic/amide carbons are located at an end of the carbon skeleton of a molecule. ^{13}C signals of carboxylic/amide carbons can only form a singlet (doublet) when its neighboring carbon is ^{12}C (^{13}C), leading to significant spectral simplification. This feature also guarantees the maximum editing efficiency since there is no coherence leakage from carboxylic/amide carbons to other J-coupled signals (e.g., glutamate C3 or aspartate C2).

In the studies of cerebral metabolism, acetate has been used as a marker of astroglial metabolism due to its exclusive uptake into astroglial cells [18,25,26]. As described earlier, when $[1-^{13}\text{C}]$ acetate is used there is severe spectral overlap between acetate C1 and glutamate C5 singlets that is beyond the capability of any known spectroscopy techniques. The solution found in this study is to introduce ^{13}C - ^{13}C homonuclear scalar couplings by the use of $[1,2-^{13}\text{C}_2]$ acetate. We reasoned that the large separation between acetate C2 and glutamate C4 in the aliphatic region can be utilized to separate the overlapping carboxylic/amide carbons. The results obtained in this study demonstrate that the spectral overlap between acetate C1 and glutamate C5 indeed can be successfully overcome using two-step J-editing. ^{13}C spectral editing therefore opens the possibility of studying acetate transport and metabolism in brain using in vivo ^{13}C MRS of carboxylic/amide carbons without spectral interference.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Mental Health. The authors thank Mr. Christopher Johnson for help.

References

- [1]. Qayyum A. MR spectroscopy of the liver: principles and clinical applications. *Radiographics*. 2009; 29:1653–1664. [PubMed: 19959513]
- [2]. Hiba B, Serduc R, Provent P, Farion R, Remy C, Ziegler A. 2D J-resolved spiral spectroscopic imaging at 7 T: application to mobile lipid mapping in a rat glioma. *Magnetic Resonance in Medicine*. 2004; 52:658–662. [PubMed: 15334587]
- [3]. Liimatainen T, Lehtimäki K, Ala-Korpela M, Hakumäki J. Identification of mobile cholesterol compounds in experimental gliomas by ^1H MRS in vivo: effects of ganciclovir-induced apoptosis on lipids. *FEBS Letters*. 2006; 580:4746–4750. [PubMed: 16893542]
- [4]. Jung WI. Localization flip angle based editing of lactate proton NMR spectra using PRESS, *Magnetic Resonance Materials in Physics. Biology and Medicine*. 1993; 1:5–9.
- [5]. Rothman DL, Petroff OA, Behar KL, Mattson RH. Localized ^1H NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:5662–5666. [PubMed: 8516315]

- [6]. Choi IY, Lee SP, Denney DR, Lynch SG. Lower levels of glutathione in the brains of secondary progressive multiple sclerosis patients measured by ^1H magnetic resonance chemical shift imaging at 3 T. *Multiple Sclerosis*. 2011; 17:289–296. [PubMed: 20921235]
- [7]. de Graaf RA, Rothman DL. Detection of gamma-aminobutyric acid (GABA) by longitudinal scalar order difference editing. *Journal of Magnetic Resonance*. 2001; 152:124–131. [PubMed: 11531371]
- [8]. de Graaf RA, Mason GF, Patel AB, Behar KL, Rothman DL. In vivo ^1H - ^{13}C -NMR spectroscopy of cerebral metabolism. *NMR in Biomedicine*. 2003; 46:339–357. [PubMed: 14679499]
- [9]. Yang J, Li CQ, Shen J. In vivo detection of cortical GABA turnover from intravenously infused $[1-^{13}\text{C}]\text{D-glucose}$. *Magnetic Resonance in Medicine*. 2005; 53:1258–1267. [PubMed: 15906278]
- [10]. Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo ^{13}C NMR. *Proceedings of the National Academy of Science of the United States of America*. 1999; 96:8235–8240.
- [11]. Xu S, Shen J. In vivo dynamic turnover of cerebral ^{13}C isotopomers from $[\text{U-}^{13}\text{C}]\text{glucose}$. *Journal of Magnetic Resonance*. 2006; 182:221–228. [PubMed: 16859940]
- [12]. Li S, Zhang Y, Wang S, Yang J, Araneta MF, Farris A, Johnson C, Fox S, Innis R, Shen J. In vivo ^{13}C magnetic resonance spectroscopy of human brain on a clinical 3 T scanner using $[2-^{13}\text{C}]\text{glucose}$ infusion and low-power stochastic decoupling. *Magnetic Resonance in Medicine*. 2009; 62:565–573. [PubMed: 19526500]
- [13]. Li S, Zhang Y, Wang S, Araneta MF, Johnson CS, Xiang Y, Innis RB, Shen J. ^{13}C MRS of occipital and frontal lobes at 3 T using a volume coil for stochastic proton decoupling. *NMR in Biomedicine*. 2010; 23:977–985. [PubMed: 20878974]
- [14]. Xiang Y, Shen J. Windowed stochastic proton decoupling for in vivo ^{13}C magnetic resonance spectroscopy with reduced RF power deposition. *Journal of Magnetic Resonance Imaging*. 2011 DOI: 10.1002/jmri.22667.
- [15]. Xiang Y, Shen J. Simultaneous detection of cerebral metabolism of different substrates by in vivo ^{13}C isotopomer MRS. *Journal of Neuroscience Methods*. 2011; 198:8–15. [PubMed: 21419161]
- [16]. Nicklas WJ, Clarke DD. Decarboxylation studies of glutamate, glutamine, and aspartate from brain labeled with $[1-^{14}\text{C}]\text{acetate}$, $[\text{U-}^{14}\text{C}]\text{aspartate}$, and $[\text{U-}^{14}\text{C}]\text{glutamate}$. *Journal of Neurochemistry*. 1969; 16:549–558. [PubMed: 5768211]
- [17]. Badar-Goffer RS, Bachelard HS, Morris PG. Cerebral metabolism of acetate and glucose studied by ^{13}C -n.m.r. spectroscopy. A technique for investigating metabolic compartmentation in the brain. *The Biochemical journal*. 1990; 266:133–139. [PubMed: 1968742]
- [18]. S. Kunnecke B, Seelig J. Cerebral metabolism of $[1,2-^{13}\text{C}_2]\text{acetate}$ as detected by in vivo and in vitro ^{13}C NMR. *The Journal of bioBiological Chemistry*. 1990; 265:12916–12926.
- [19]. Li S, Shen J. Integrated RF probe for in vivo multinuclear spectroscopy and functional imaging of rat brain using an 11.7 Tesla 89 mm bore vertical microimager. *Magma*. 2005; 18:119–127. [PubMed: 16007474]
- [20]. Chen Z, Li SS, Yang J, Letizia D, Shen J. Measurement and automatic correction of high-order B_0 inhomogeneity in the rat brain at 11.7 Tesla. *Magnetic Resonance Imaging*. 2004; 22:835–842. [PubMed: 15234452]
- [21]. Slotboom J, Bove'e WMMJ. Adiabatic slice-selective rf pulses and a single-shot adiabatic localization pulse sequence. *Concepts in Magnetic Resonance*. 1995; 7:193–217.
- [22]. Li S, Yang J, Shen J. Novel strategy for cerebral ^{13}C MRS using very low RF power for proton decoupling. *Magnetic Resonance in Medicine*. 2007; 57:265–271. [PubMed: 17260369]
- [23]. Ernst RR. Nuclear magnetic double resonance with an incoherent radio-frequency field. *Journal of Chemical Physics*. 1966; 45:3845–3861.
- [24]. Emsley L, Bodenhausen G. Phase shift induced by transient Bloch-Siegert effects in NMR. *Chemical Physics Letters*. 1990; 168:297–303.
- [25]. Waniewski RA, Martin DL. Preferential utilization of acetate by astrocytes is attributable to transport. *The Journal of Neuroscience*. 1998; 18:5225–5233. [PubMed: 9651205]

- [26]. Cruz NF, Lasater A, Zielke HR, Dienel GA. Activation of astrocytes in brain of conscious rats during acoustic stimulation: acetate utilization in working brain. *Journal of Neurochemistry*. 2005; 92:934–947. [PubMed: 15686496]

Figure 1.

Schematic diagram of the pulse sequence designed for in vivo ^{13}C editing. $^1\text{H} \rightarrow ^{13}\text{C}$ heteronuclear Overhauser enhancement (NOE) was generated by a train of non-selective hard pulses with a nominal flip angle of 180° spaced at 100 ms apart. The ^{13}C spins were excited by a 90° 0.75 ms adiabatic half-passage pulse followed by three pairs of slice selective hyperbolic secant pulses (one pair per dimension, 2-ms per pulse with phase factor = 5 and truncation level = 1%). For clarity, each pair of sech pulses was represented by a single 180° pulse in the diagram. A single spectrally selective editing pulse (180° hyperbolic secant pulse, phase factor = 3 and truncation level = 1%, pulse length = 10–12 ms, bandwidth = 1012–842 Hz) was placed either at 24.1 ppm for separating glutamate C5 from acetate C1 (10 ms) or at 31.7 ppm for separating Gln C5 (178.5 ppm) from aspartate C4 (178.4 ppm, 12 ms). The ^1H decoupling used a pseudo noise decoupling scheme with constant γB_2 amplitude ($\gamma B_2 < \sim 274$ Hz calibrated at the gradient isocenter, randomly inverted phase and a repetition unit of 0.2–0.4 ms).

Figure 2.

(A) In vitro ^{13}C NMR spectrum (170~186 ppm) from a phantom containing 0.6 M natural abundance L-glutamic acid and 0.6 M sodium acetate (pH = 7.4). NS = 192. Data processing parameters: si = 16K, lb = 5. Ace-C1 = acetate C1, Glu-C5 = glutamate C5, Glu-C1 = glutamate C1. (B) In vitro ^{13}C NMR spectrum (175~190 ppm) acquired from a phantom containing 0.1 M sodium $[1,2-^{13}\text{C}_2]$ acetate (pH = 7.4). NS = 16. Data processing parameters: si = 16K, lb = 10. Ace-C1 = acetate C1. (a): Spectrum acquired with the editing pulse turned off; (b): spectrum acquired with the editing pulse centered at acetate C2 (24.5 ppm); (c): addition of (a) and (b). (C) In vivo ^{13}C NMR spectra (155~186 ppm) from rat brain during systemic administration of $[^{13}\text{C}_6]$ -D-glucose (0.75 M). The spectra were acquired 2h after the start of infusion. (a): spectrum acquired without the editing pulse; (b): spectrum acquired with the editing pulse centered at acetate C2 (24.5 ppm); (c): addition of (a) and (b). Total NS = 64. Data processing parameters: si = 16K, lb = 10. Glu-C5 = glutamate C5, Gln-C5 = glutamine C5, Asp-C4 = aspartate C4.

Figure 3.

In vivo ^{13}C NMR spectra (155~186 ppm) acquired from one animal during intravenous infusion of sodium $[1,2-^{13}\text{C}_2]$ acetate (0.9 M). The spectra were acquired 2h after the start of infusion. (a) spectrum acquired without the editing pulse; (b) spectrum acquired with the editing pulse on acetate C2 (24.5 ppm); (c) subtraction of (b) from (a); (d) addition of (a) and (b). Data processing parameters: si = 16K, lb = 10. Glu-C5 = glutamate C5, Gln-C5 = glutamine C5, Ace-C1 = acetate C1.

Figure 4.

Interleaved in vivo ^{13}C NMR spectra (155~186 ppm) acquired from one animal infused intravenously with $[^{13}\text{C}_6]$ -D-glucose (0.75 M). The spectra were acquired 2h after the start of infusion. (a) spectrum acquired without the editing pulse; (b) spectrum acquired with the editing pulse on glutamine C4 (31.7 pp); (c) subtraction of (b) from (a); (d) addition of (a) and (b). NMR and data processing parameters were familiar to fig.2.. Data processing parameters: si = 16K, lb = 10., Glu-C5 = glutamate C5, Gln-C5 = glutamine C5, Asp-C4 = aspartate C4, Ace-C1 = acetate C1.