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Selective Proton-Observed, Carbon-Edited (selPOCE) MRS method for measurement of glutamate and glutamine ^{13}C -labeling in the human frontal cortex

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Abstract

Purpose— ^{13}C magnetic resonance spectroscopy (MRS) in combination with infusion of ^{13}C -labeled substrates has led to unique insights into human brain metabolism and neurotransmitter cycling. However, the low sensitivity of direct ^{13}C MRS and high radiofrequency (RF) power requirements has limited ^{13}C MRS studies to predominantly data acquisition in large volumes of the occipital cortex. The purpose of this study is to develop an MRS technique for localized detection of ^{13}C -labeling of glutamate and glutamine in the human frontal lobe.

Methods—We used an indirect (^1H - ^{13}C), proton-observed, carbon-edited MRS sequence (selPOCE) for detection of ^{13}C -labeled metabolites in relatively small volumes located in the frontal lobe at 4 Tesla. SelPOCE allows for selective and separate detection of glutamate and glutamine resonances, which significantly overlap at magnetic field strengths used for clinical MRI.

Results—Phantom data illustrate how selPOCE can be tuned to selectively detect ^{13}C labeling in different metabolites. 3D specific absorption rates (SAR) simulations of RF power deposition show that the selPOCE method operates comfortably within the global and local FDA SAR guidelines. *In vivo* selPOCE data are presented acquired from a 45 mL volume in the frontal lobe of healthy subjects. The *in vivo* data show the time-dependent ^{13}C -labeling of glutamate and glutamine during intravenous infusion of $[1-^{13}\text{C}]$ -glucose. Metrics describing spectral fitting quality of the glutamate and glutamine resonances are reported.

Conclusion—SelPOCE allows the detection of ^{13}C -labeling in glutamate and glutamine from a relatively small volume in the human frontal lobe at low RF power requirements.

Keywords

MRS; ^{13}C ; frontal lobe; human

Introduction

Detection of ^{13}C -labeling in brain metabolites using ^{13}C magnetic resonance spectroscopy (MRS) during administration of a ^{13}C -labeled substrate (e.g. [1- ^{13}C]-glucose) is a powerful, noninvasive method for quantifying rates of metabolism and glutamate/glutamine (Glu/Gln) neurotransmitter cycling in brain (1,2). In vivo ^{13}C MRS methods can be classified as direct and indirect detection techniques. Indirect MRS techniques (^1H -[^{13}C] MRS) make use of the ^1H - ^{13}C coupling to detect protons bound to ^{13}C atoms and are inherently more sensitive than direct ^{13}C MRS because of the larger gyromagnetic ratio of ^1H compared to ^{13}C . Indirect, proton-observed carbon-edited (POCE) MRS sequences have mostly been used for in vivo animal brain studies, and at relatively high magnetic field strengths (7 Tesla (T) and higher) (3–5). At high field strengths POCE benefits from good spectral resolution and therefore the peaks of Glu and Gln are minimally overlapping. Restrictions on the radiofrequency (RF) power are less stringent in animal studies compared to MRS applications in human brain. Broadband ^{13}C -decoupling can therefore be applied to further simplify spectral appearance and to improve sensitivity (6). For human applications the amount of RF power that can be used is limited by the specific absorption rates (SAR) outlined by the FDA to prevent tissue heating. Furthermore, at lower magnetic fields mostly available for human studies (< 7 T) the partial overlap of Glu and Gln in the proton spectrum compromises accurate quantification.

There are few reports of ^1H -[^{13}C] MRS applications in human brain, mostly limited to acquisitions in the occipital lobe (7–9), with some application in other brain regions emphasized on the detection of labeling in Glu (10,11). Due to concerns about RF heating and difficulties in obtaining adequate B_0 homogeneity, applications of ^{13}C MRS in the frontal lobe of human brain have relied on direct ^{13}C MRS and ^{13}C -labeling strategies that enrich the carboxylic carbons (12–15). The carboxylic carbons can be detected without localization schemes because peaks of natural abundance lipids do not overlap with the Glu and Gln peaks. Furthermore, decoupling the long range ^1H - ^{13}C couplings of the carboxylic carbons can be achieved with low RF power (14). This robust, direct ^{13}C MRS technique can also be applied at 7 T with (16) or without low power decoupling (12).

However, an MRS method that provides well-defined spatial localization of the ^{13}C -signal of both Glu and Gln, and can be used in the human frontal lobe is currently lacking.

We describe a fully localized, ^1H -[^{13}C] MRS technique, selective proton-observed, carbon-edited (selPOCE), for detection of ^{13}C -bound protons of aliphatic carbons while keeping SAR low by omitting ^{13}C -decoupling, that can be applied in the frontal lobe of human brain at 4 T. To allow quantification of the partially overlapping satellite peaks of Glu and Gln generated by ^1H - ^{13}C coupling, selective editing is implemented. We describe the MRS pulse sequence, and provide RF power requirements and SAR characteristics. The method's

functionality is illustrated in metabolite phantoms, and human in vivo data are shown, acquired from a 45 mL voxel located in the frontal lobe during infusion of [1-¹³C]-glucose.

Methods

General

Three healthy subjects were studied (age: 19-39 yrs, 2 male, 1 female). All procedures described were approved by the Yale University Institutional Review Board. Subjects fasted overnight and presented around 7:00 am at the Magnetic Resonance Research Center. Two intravenous lines were placed, for infusion of [1-¹³C]-glucose and blood sampling, respectively. MRS experiments were carried out on a 4 T whole-body magnet interfaced to a Bruker Avance III HD spectrometer, running on Paravision 6 (Bruker Instruments, Billerica, MA, USA). Around 8:00 am subjects were positioned supine on the scanner bed and the RF coil placed over the forehead. The RF coil setup consisted of two circular surface coils positioned 11.5 mm apart in parallel planes: an outer ¹³C transmit only coil (diameter 135 mm) and an inner ¹H transmit/receive coil (diameter 95 mm).

MRS acquisition

Gradient-echo MR images were acquired to confirm accurate positioning of the subject and voxel placement. Shimming of the voxel was performed using methods implemented by the manufacturer. First and second order shims settings were calculated automatically following the acquisition of a B₀ map, and first order shims were fine-tuned with an iterative shimming routine using the selPOCE method without any ¹³C RF pulses (transforming it into a traditional STEAM sequence) for localized detection of the water signal. RF power calibration was based on the reference power determined for a 90 degree flip angle acquired in a slice parallel to the ¹H surface coil and positioned to overlap with the voxel position. The selPOCE MRS method is a combination of POCE based on stimulated echoes (17) and 2D heteronuclear single-quantum coherence (HSQC) MRS (18). Figure 1 schematically illustrates the selPOCE sequence. The basic STEAM localization method (Fig. 1A) is based on 1.0 ms asymmetric Shinnar-Le Roux excitation pulses (6.1 kHz bandwidth)(19), allowing an echo-time TE of 7.8 ms. The mixing time (TM) period is modified to allow for (B, C) a conventional POCE experiment or (D, E) a selective POCE experiment. In the conventional POCE experiment, inversion of the ¹³C spins is achieved with a 5.0 ms adiabatic full passage pulse (HS4 modulation (20), 4.0 kHz bandwidth). The selective POCE scans (Fig. 1D/E) are similar to a 2D HSQC sequence in which the spins evolve on the carbon-13 axis following excitation by a 0.25 ms square pulse. In our application, the ¹³C transmitter frequency is on-resonance for the Glu-C4 position at 34.2 ppm. Proton-carbon scalar coupling is refocused by a 2.0 ms adiabatic full passage pulse (HS4 modulation (20), 10 kHz bandwidth). With the Glu-C4 on-resonance the 90(+x) – delay – 90(+x) combination (Fig. 1D) leads to an inversion of the [4-¹³C]-Glu-H4 signal, whereas the 90(+x) – delay – 90(-x) combination leads to an identity rotation (Fig. 1E). The Gln-C4 resonance at 31.7 ppm is 2.5 ppm or $\nu = 107$ Hz off-resonance compared to the Glu-C4 resonance. During TM the [4-¹³C]-Gln-H4 resonance acquires a phase of $2\pi \nu t$, which can be made equal to 180 degrees by setting $t = 1/2 \nu$. The pulse/delay combination shown in Fig. 1D leads to an identity rotation for [4-¹³C]-Gln-H4, whereas the combination in Fig. 1E leads to an

inversion of the $[4-^{13}\text{C}]\text{-Gln-H4}$ resonance. The difference between (B) – (C) gives the sum of $[4-^{13}\text{C}]\text{-Glu-H4}$ and $[4-^{13}\text{C}]\text{-Gln-H4}$, while (D) – (E) gives the difference between $[4-^{13}\text{C}]\text{-Glu-H4}$ and $[4-^{13}\text{C}]\text{-Gln-H4}$. Combining all four scans in the proper order provides MR spectra with separated $[4-^{13}\text{C}]\text{-Glu-H4}$ and $[4-^{13}\text{C}]\text{-Gln-H4}$ peaks.

Safety simulations

The RF power output was measured continuously at the amplifier and corrected for the hardware losses up to the RF coil connection. Finite difference time domain simulations were carried out in Sim4Life (Zürich MedTech, Zurich) to simulate SAR10g-distributions for the 4 T ^{13}C and ^1H -tuned single-turn surface coils on the human model Duke (21). The ^1H and ^{13}C surface coil models are positioned 1 cm and 2 cm away from the head, respectively. Both coil models are placed at an angle of 30° with respect to the coronal plane. Separate broadband simulations were carried out for each coil model. In both simulations, the unused coil was not included in the voxelated model. An isotropic voxel size of 1 mm was used to model the coil elements, resulting in a grid size of 41.10×10^6 cells for the ^1H -coil model and 40.98×10^6 cells for the ^{13}C -coil model. Two capacitors for tuning were included in the coil model, 2×37.6 pF for the ^{13}C coil and 2×3.44 pF for the ^1H coil.

Phantoms

Two different 50 mL solutions were used in phantoms to illustrate the selPOCE MRS method. One solution consisted of deionized water and ~ 60 mM of acetate (Ace), 15 mM $[2-^{13}\text{C}]\text{-Ace}$, 40 mM lactate (Lac) and 8 mM $[3-^{13}\text{C}]\text{-Lac}$. A second 50 mL solution consisted of deionized water and 100 mM Glu and 80 mM Gln. The solutions are put in a 50 mL container that is integrated in a ~ 4 L container that itself is filled with 50 mM KCL in deionized water. This setup allows for exchanging the 50 mL phantom solutions, while mimicking the electrical load on the RF coil of a human head with the 4 L KCL solution. The phantom studies used the same RF coil as used for the in vivo studies, with the 50 mL phantom placed directly under and perpendicular to the coil.

MRS processing

Processing of MR spectra was performed with in-house built software written in Matlab (The Mathworks, MA) using routines similar to those previously described (22). Briefly, the spectral fitting is based on the principle of LCmodel (23) in which the spectrum is approximated as a linear sum of the individual components. The basis set was simulated in Matlab using the density matrix formalism taking RF pulses and gradients of the pulse sequence into account. The basis set for the spectral region $[0.5 \dots 3.5]$ ppm included $[3-^{13}\text{C}]\text{-Glu-H3}$, $[3-^{13}\text{C}]\text{-Gln-H3}$, $[4-^{13}\text{C}]\text{-Glu-H4}$, $[4-^{13}\text{C}]\text{-Gln-H4}$, $[3-^{13}\text{C}]\text{-Lac-H3}$ (lactate) and $[2-^{13}\text{C}]\text{-NAA-H2}$ (N-acetyl aspartate) on top of a second-order, polynomial baseline. The concentration of ^{13}C -labeled Glu and Gln was calculated using the natural abundance signal of NAA before administration of $[1-^{13}\text{C}]\text{-glucose}$ as a 10 mM internal concentration reference. In order to demonstrate the value of the selPOCE scans (Fig. 1D/E) in addition to the standard POCE scans (Fig. 1B/C) the acquired data were processed in two different ways to obtain estimates for ^{13}C -labeled Glu-H4 and Gln-H4. The processing options consisted of processing the conventional POCE difference data, and processing the

separated [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 data derived from the selPOCE acquisition simultaneously. During fitting of the selPOCE data the [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 resonances were amplitude, line width and frequency were linked for the simultaneous fitting of the two datasets. The accuracy of the obtained [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 concentrations under these two processing options was evaluated using the Cramer-Rao Lower Bounds (CRLBs, (24).

[1- ^{13}C]-Glucose infusion

A primed, variable rate infusion of [1- ^{13}C]-glucose (1.1M, 99% enriched, Cambridge Isotopes, Cambridge, MA, USA) was performed to rapidly reach plasma glucose concentrations of ~10 mM. Towards the end of the infusion (90 to 120 min), the plasma glucose level was allowed to naturally drop to ~7 mM. Venous blood was sampled every 5 min, spun down and plasma glucose concentration was measured using a YSI 2700 STAT Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Remaining plasma was stored at -80°C until used for measurement of glucose ^{13}C fractional enrichment.

Plasma glucose ^{13}C fractional enrichment

Plasma (100 μL) was diluted with 500 μL phosphate buffer (100mM) containing 10% D_2O and 2 mM formic acid as chemical shift reference. Pulse-acquire ^1H MR spectra were collected on a 500MHz MR spectrometer (Bruker Avance, Bruker, Billerica, MA, USA). The fractional enrichment of [1- ^{13}C]-glucose was derived from the integrals of the downfield satellite (5.39 ppm) and the central peak (5.22 ppm), quantified using in-house build software written in Matlab (MathWorks, Natick, Massachusetts, USA).

Results

Safety simulations

Figure 2A/B shows the ^1H (blue) and ^{13}C (purple) surface coil placement with respect to the human model 'Duke'. Figs. 2C and F show sagittal B_1^+ distribution maps (1 W input power) for the ^1H and ^{13}C surface coils, respectively. The input power as time-averaged over all four POCE/selPOCE scans (see Fig. 1) was measured at 0.51 W and 0.31 W for the ^1H and ^{13}C channels, respectively. This resulted in maximum intensity projection (MIP) SAR maps as shown in Fig. 2D/E for ^1H and Fig. 2G/H for ^{13}C . The maximum SAR power deposition in any 10 gram tissue volume was 1.24 W/kg for ^1H and 0.85 W/kg for ^{13}C . The SAR distributions indicate that SAR limits of 3W/kg were not surpassed at the average power settings used. In a separate simulation (not shown) it was demonstrated that the presence of the ^1H surface coil had no significant effect on the B_1^+ and SAR distribution maps of the ^{13}C surface coil.

Phantom studies

The underlying principles of the selPOCE are experimentally demonstrated on the phantom solutions containing unlabeled and ^{13}C -labeled acetate and lactate. In the conventional STEAM spectrum (Fig. 3A) all signals appear positive, whereas the application of a broadband carbon-13 inversion pulses leads to a phase inversion of all resonances with a one-bond ^1H - ^{13}C scalar coupling (Fig. 3B). The difference between Fig. 3A and B provides

the conventional POCE spectrum (Fig. 3E) in which all signals without a one-bond ^1H - ^{13}C scalar coupling have been eliminated. Besides the ^1H chemical shift difference between $[2\text{-}^{13}\text{C}]\text{-Ace-H2}$ and $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ the POCE difference spectrum does not provide any intrinsic discrimination between the two compounds. This poses a problem when the ^1H chemical shift difference is very small, leading to spectral overlap. Such is the case for Gln and Glu which will be discussed in Figs. 4-7. Fig. 3C and D show that the phase of $[2\text{-}^{13}\text{C}]\text{-ace-H2}$ and $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ can be selectively manipulated with the HSQC pulse sequence elements shown in Figs. 1D and E. With the ^{13}C resonance of $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ being on-resonance and the TM set to 3.8 ms, the ^1H resonances of $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ (Fig. 3C) and $[2\text{-}^{13}\text{C}]\text{-Ace-H2}$ (Fig. 3D) are selectively phase-inverted based on the 3.0 ppm ^{13}C chemical shift difference. The difference between Fig. 3C and D also provides a POCE spectrum (Fig. 3F) of signals with one-bond ^1H - ^{13}C coupling in which the two compounds are phase-inverted relative to each other. Combining the two POCE difference spectra (Fig. 3E and F) by addition and subtraction provides pure $[2\text{-}^{13}\text{C}]\text{-Ace-H2}$ and $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ spectra, respectively. Fig. 4 shows data from the phantom containing 100 mM Glu and 80 mM Gln and illustrates how selPOCE can be applied for the selective detection of $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$ and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$. The POCE (Fig. 4B) difference spectrum shows the 1.1% natural abundance signals of $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$ and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$. Despite the excellent magnetic field homogeneity it is difficult to visually separate the two compounds due to the small 0.11 ppm ^1H chemical shift difference (18.8 Hz at 4 T). However, utilizing the 2.5 ppm ^{13}C chemical shift difference (107 Hz at 4 T) allows robust separation of the $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$ and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$ resonances in the ^1H MR spectra (Fig. 4C-F). In addition to Glu-C4 and Gln-C4 resonances, the ^{13}C MR spectrum also contains signals from Glu-C2 and C3 and Gln-C2 and C3. The HSQC element of Fig. 1D/E can be seen as achieving inversion with a sinusoidal dependence on the ^{13}C chemical shift. With the transmitter offset and TM length being optimized to achieve maximum separation of $[4\text{-}^{13}\text{C}]\text{-Glu H4}$ and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$, the other resonances will experience a partial inversion. This will lead to the appearance of $[3\text{-}^{13}\text{C}]\text{-Glu-H3}$ and $[3\text{-}^{13}\text{C}]\text{-Gln-H3}$, and $[2\text{-}^{13}\text{C}]\text{-Glu-H2}$ and $[2\text{-}^{13}\text{C}]\text{-Gln-H2}$ resonances in the final ^1H MR spectra (Fig. 4D and E). However, as the H2 and H3 resonances do not overlap with the H4 resonances of interest this effect is largely inconsequential as long the additional resonances are included in the spectral fitting routine.

In vivo MRS

Compared to the phantom data of Glu and Gln (Fig. 4), the faster in vivo T_2^* relaxation make the Glu and Gln overlap even more problematic as shown in the ^1H MR spectrum acquired in the frontal lobe of a healthy volunteer (Fig. 5). Despite the high magnetic field homogeneity, (typical full-width half maximum linewidth values for water: 9.5 ± 1.2 Hz), Glu and Gln peaks in the ^1H MR spectrum acquired in a 45 mL ($5 \times 3 \times 3$ cm³) voxel positioned in the frontal lobe (Fig. 5A) are overlapping significantly (Fig. 5B). SelPOCE spectra collected in 12 min from the frontal lobe voxel during infusion of $[1\text{-}^{13}\text{C}]\text{-glucose}$ are shown in Fig. 5C-F. Similar to the results obtained in the Glu-Gln phantom, subtraction of selPOCE sub-spectra generates difference spectra of all ^{13}C -bound protons (Fig. 5C), as well as individual MR spectra of $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$ (Fig 5E) and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$ (Fig 5F), respectively. In addition, the overlapping ^1H resonances of $[3\text{-}^{13}\text{C}]\text{-Glu-H3}$ and $[3\text{-}^{13}\text{C}]\text{-Gln-H3}$, combined as unresolved $[3\text{-}^{13}\text{C}]\text{-Glx-H3}$ can be observed. If labeling occurs in $[3\text{-}^{13}\text{C}]\text{-}$

Asp-H3 and [3- ^{13}C]-Lac-H3 these peaks can be unambiguously quantified from the conventional POCE difference spectrum because of minimal overlap with other resonances. Fig. 6 shows the quantification of POCE spectra using various combinations of data inclusion. Analysis of the conventional POCE spectrum alone (Fig. 6A) provides an adequate mathematical fit of [4- ^{13}C]-Glu-H4, [4- ^{13}C]-Gln-H4, [3- ^{13}C]-Glx-H3 and [3- ^{13}C]-Lac-H3 and resulted in reasonable estimates of [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 concentrations (Fig. 7A). However, the high level of spectral overlap leads to high CRLBs (Fig. 7B) for Glu (5.8%) and especially Gln (28.4%). Processing of the separated [4- ^{13}C]-Glu-H4 (Fig. 6B) and [4- ^{13}C]-Gln-H4 (Fig. 6C) sub-spectra simultaneously leads to improved CRLBs (2.3 % for Glu and 7.0 % for Gln) compared to the fitting of the conventional POCE spectrum (Fig. 7B). Note that the [3- ^{13}C]-Glx-H3 and [3- ^{13}C]-Lac-H3 peaks are included in the overall spectral fit but have not been quantified.

The time-dependent increase in ^{13}C -labeling of [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 as averaged over 12 min during infusion of [1- ^{13}C]-glucose can be seen in the serially acquired selPOCE difference spectra, depicted in Fig 8. The different combinations of selPOCE spectra reveal the ^{13}C -labeling of Glu-H4 and Gln-H4 in separate, yet simultaneous time courses. The appearance of ^{13}C -labeling shows the predictable labeling pattern of [1- ^{13}C]-glucose infusions, with Glu labeling preceding labeling of the (smaller) Gln pool.

Discussion

Two major limitations in the application of ^1H -[^{13}C] MRS to human brain are the poor ability to separate Glu and Gln and the requirement for decoupling to simplify the ^1H -[^{13}C] edited spectra. We described an MRS method implemented at 4 T that uses indirect detection of ^{13}C via ^1H and enables measuring the rate and degree of Glu and Gln ^{13}C -labeling in a well-defined volume in the frontal lobe of human brain. To operate within the FDA guidelines for RF power deposition, ^{13}C - ^1H decoupling was omitted. For robust detection of the overlapping [4- ^{13}C]-Glu-H4 and [4- ^{13}C]-Gln-H4 peaks heteronuclear editing was built into the MRS sequence. The MRS method was demonstrated by acquiring ^{13}C -labeling data from a 45 mL voxel in the frontal lobe of healthy volunteers during infusion of [1- ^{13}C]-glucose.

An important characteristic of the selPOCE method is the lack of ^{13}C -decoupling. This significantly reduces the required RF power and allows staying comfortably within the SAR limits defined by the FDA (3 W/kg). The lack of decoupling complicates the spectral appearance because the multiplet peaks from ^{13}C -bound protons of Glu and Gln are split into two. However, the predictable appearance of the MR signals can be accurately simulated using the density matrix formalism and used for spectral fitting of the selPOCE MR spectra, by means of the widespread method of linear combination of model spectra (LCModel) (23). The LCModel data illustrate high quality spectral fitting of the multiplet peaks in the selPOCE difference spectra, resulting in CRLB's of 5-10%. The anticipated loss in sensitivity due to the lack of ^{13}C - ^1H decoupling is thus adequately compensated by the selective detection of Glu and Gln in the separate scans. Another aspect of the sequence that contributes to its overall low RF power requirement is the use of stimulated echoes generated with 90 degree pulses instead of relying on spin echoes produced with 180 degree

pulses. The STEAM-based MRS sequence provided high quality localization as no lipid signal from the scalp was observed, all at a fraction of the FDA SAR limits.

For ^{13}C MRS studies with infusion of ^{13}C -labeled substrates of brain metabolism and neurotransmitter cycling it is crucial to quantify both Glu and Gln labeling. Glu and Gln are partially overlapping in the ^1H MR spectrum at 4 T and even more at 3 T. The presented selPOCE method achieves individual detection of Glu and Gln by using frequency selective pulses in the ^{13}C domain and timing the sequence to allow the coherence to evolve to result in an opposite phase in the ^1H domain. As demonstrated in the lactate/acetate phantom the technique can be applied to separate other metabolites than Glu and Gln in the ^1H domain as well, as long as their respective resonances in the ^{13}C domain are not overlapping, such as GABA and Glu.

The data presented were acquired on a 4 T clinical research scanner. At the common field strength of 3 T the overlap of Glu and Gln in the ^1H MR spectrum is even larger than at 4 T. However, the Glu and Gln peaks at 3 T are still well-separated on the ^{13}C frequency domain and therefore the selPOCE method could easily be used at 3 T as well. For applications at 7 T the benefit of selecting Glu and Gln is less an issue because the improved spectral resolution. Yet, the low RF power requirements of the selPOCE method would be the key aspect that allows for this technique to be implemented at 7 T, where SAR limits are significantly more difficult to adhere to. TE optimization and variation in a PRESS sequence has been used as a strategy to separate the partially overlapping resonances of Glu and Gln (11). The PRESS method will not have the 50% SNR penalty inherent to the STEAM sequence. However, some signal loss will be induced due to the typically longer TE in a PRESS sequence optimized for Glu and Gln separation. In addition, the refocusing pulses of the PRESS sequence will significantly increase the RF power requirements will simultaneously providing a smaller bandwidth and hence a larger chemical shift displacement error.

Using a simple set up of two coplanar surface coils for RF transmission (^1H and ^{13}C) and reception (^1H) provided high quality selPOCE data from a volume of 45 mL. The RF coil setup did not include extensive measures to decouple the ^1H and ^{13}C coils because no ^1H - ^{13}C decoupling is applied during acquisition. Various strategies could be implemented to improve the design of the RF setup. A volume resonator could be used for transmission (^1H and ^{13}C) providing homogenous flip angles of the RF pulses. This could be combined with an array of ^1H surface coils for reception, which would increase the signal-to-noise and also the area covered by the receive coils. These improvements would allow for selPOCE spectra acquisition from a smaller volume than used in the present study and provide more freedom for voxel positioning. Also a multi-voxel approach could be implemented, ideally combined with dynamic shim updating to acquire selPOCE spectra from different brain regions simultaneously (25). In addition, a close to doubling of the SNR of $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$ and $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$ could be achieved by using $[1,6\text{-}^{13}\text{C}_2]\text{-glucose}$. Metabolism of $[1,6\text{-}^{13}\text{C}_2]\text{-glucose}$ results in both glucose-derived triose molecules being ^{13}C -labeled instead of only one triose being labeled during metabolism of $[1\text{-}^{13}\text{C}]\text{-glucose}$. However, at this moment the cost of $[1,6\text{-}^{13}\text{C}_2]\text{-glucose}$ does present a hurdle for its application in human studies where an average dose of glucose is 50 grams is used.

In conclusion we have illustrated the functionality of the selPOCE MRS technique in frontal lobe of human brain during infusion of [1-¹³C]-glucose. This method solves the restrictions on performing ¹H-[¹³C] MRS in the frontal lobe due to not requiring ¹³C-¹H decoupling and cleanly separating the resonances of [4-¹³C]-Glu-H4 and [4-¹³C]-Gln-H4. SelPOCE retains much of the enhanced sensitivity of conventional POCE type sequences potentially allowing significant improvements in the spatial resolution for measuring neuroenergetics and neurotransmitter cycling over presently used direct detection ¹³C MRS methods.

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Figure 1. Schematic of selPOCE RF pulse sequence

(A) Basic selPOCE pulse sequence based on STEAM localization. (B-E) The traditional STEAM mixing time is used to play out various pulse sequence elements to provide distinction between (B, C) ^{13}C -labeled and unlabeled compounds and between (D, E) compounds with different ^{13}C chemical shifts. Protons attached to ^{12}C and ^{13}C are separated by subtracting signal acquired with a ^{13}C inversion pulse (C) from that acquired without any ^{13}C pulses (B). Compounds with a ν chemical shift difference (in Hz) can be separated through evolution over a time period $1/2 \nu$ followed by a ^{13}C excitation pulse along the (D) $+x$ or (E) $-x$ axis. More details can be found in the text.

Figure 2.

Finite difference time domain modeling of the transmit B_1+ and SAR distribution in the human brain. (A) Non-voxelated and (B) voxelated human head model 'Duke' showing the 95 mm diameter ^1H (blue) and 135 mm diameter ^{13}C (purple) surface coils. (C, F) Transmit B_1+ field distributions for (C) ^1H and (F) ^{13}C for 1 W of input power. (D, E) Maximum intensity projection (MIP) of ^1H SAR distributions for 0.51 W time-averaged ^1H input power. (G, H) MIP of ^{13}C SAR distributions for 0.31 W time-averaged ^{13}C input power.

Figure 3.

SelPOCE on a sample containing unlabeled and ^{13}C -labeled acetate (Ace) and lactate (Lac). (A, B) Spectra acquired without (A) and with (B) broadband ^{13}C inversion. (C, D) Spectra acquired with a HSQC element achieving phase inversion for (C) $[3-^{13}\text{C}]\text{-Lac-H3}$ and (D) $[2-^{13}\text{C}]\text{-Ace-H2}$. (E) = (A) – (B) provides the ^{13}C -coupled ^1H resonances, while simultaneously eliminating resonances without $^1\text{H}\text{-}^{13}\text{C}$ coupling. (F) = (C) – (D) also removes resonances without $^1\text{H}\text{-}^{13}\text{C}$ coupling. In addition, it provides phase discrimination between $[2-^{13}\text{C}]\text{-Ace-H2}$ and $[3-^{13}\text{C}]\text{-Lac-H3}$. (G) = (E) + (F) gives the pure $[2-^{13}\text{C}]\text{-Ace-H2}$ signal, whereas (H) = (E) – (F) provides the pure $[3-^{13}\text{C}]\text{-Lac-H3}$ signal.

Figure 4.

SelPOCE spectra from a sample containing 100 mM Glu and 80 mM Gln in water. (A) ^1H MR spectrum acquired in the absence of ^{13}C RF pulses showing the strong overlap between Glu and Gln. At the high spectral resolution of the phantom, the Glu-H4 and Gln-H4 resonances are partially resolved. (B) Difference ^1H MR spectrum between data acquired with and without a global ^{13}C inversion pulse, corresponding to the ‘classical’ POCE experiment. The difference spectrum shows the ^1H signals bound to a ^{13}C nucleus, which only occurs for the 1.1% natural abundance of Glu and Gln. Note that even though ^1H - ^{12}C and ^1H - ^{13}C signals are effectively separated, $[4-^{13}\text{C}]$ -Glu-H4 and $[4-^{13}\text{C}]$ -Gln-H4 are still strongly overlapping. (C) Difference ^1H MR spectrum between data acquired with a HSQC module optimized for $[4-^{13}\text{C}]$ -Glu-H4 and $[4-^{13}\text{C}]$ -Gln-H4 phase modulation. Note that the $[4-^{13}\text{C}]$ -Gln-H4 is inverted relative to $[4-^{13}\text{C}]$ -Glu-H4. (D) = (B) + (C) displaying the $[4-^{13}\text{C}]$ -Glu-H4 signal and (E) = (B) – (C) displays the $[4-^{13}\text{C}]$ -Gln-H4 signal. (F) Overlay of the signals from (D, black) and (E, gray) demonstrating the good separation between Glu and Gln.

Figure 5. SelPOCE on human brain

(A) Axial and sagittal MR images with illustrations of RF coils and voxel size (45 mL) and position. (B) ^1H MR spectrum obtained without ^{13}C RF pulses. (C) Conventional POCE difference spectrum. $[4-^{13}\text{C}]\text{-Glu-H4}$ and $[4-^{13}\text{C}]\text{-Gln-H4}$ both have positive resonances, making their distinction difficult. (D) SelPOCE difference spectrum displaying positive $[4-^{13}\text{C}]\text{-Glu-H4}$ and negative $[4-^{13}\text{C}]\text{-Gln-H4}$ resonances. (E) = (C) + (D) cancels out the $[4-^{13}\text{C}]\text{-Gln-H4}$ signal, leaving a 'pure' $[4-^{13}\text{C}]\text{-Glu-H4}$ signal. (F) = (C) - (D) cancels out the $[4-^{13}\text{C}]\text{-Glu-H4}$ signal, leaving a 'pure' $[4-^{13}\text{C}]\text{-Gln-H4}$ signal.

Figure 6. LCModel fitting of POCE and selPOCE spectra

(A) Conventional POCE spectrum (gray, top) overlaid with a best spectral fit (black, top) containing contributions from $[4\text{-}^{13}\text{C}]\text{-Glu-H4}$, $[4\text{-}^{13}\text{C}]\text{-Gln-H4}$, $[3\text{-}^{13}\text{C}]\text{-Glx-H3}$, $[2\text{-}^{13}\text{C}]\text{-NAA-H2}$ and $[3\text{-}^{13}\text{C}]\text{-Lac-H3}$ (middle) and a fitting residual (bottom). (B) Fitting of the Glu and (C) Gln selPOCE sub-spectra calculated from selPOCE spectra. Color-coded components are the same as indicated in A.

Figure 7. ^{13}C concentration and CRLB's

(A) Concentration of ^{13}C -labeled Glu-H4 (black) and Gln-H4 (grey) calculated based on the fitted spectra of the conventional POCE acquisition and *simultaneous* fitting of the Glu and Gln sub-spectra calculated from selPOCE spectra, with (B) accompanying CRLB's for each fitting approach. Note the improved CRLB's achieved when fitting the selPOCE spectra compared to conventional POCE.