

Semiselective POCE NMR Spectroscopy

Pierre-Gilles Henry, Ronan Roussel, Françoise Vaufrey, Caroline Dautry, and Gilles Bloch*

A new scheme is proposed to edit separately glutamate C_3 and C_4 resonances of ^1H bound to ^{13}C , in order to resolve these two signals which overlap at intermediate magnetic fields (1.5 T–3 T), commonly available for human brain studies. The two edited spectra are obtained by combining the individual acquisitions from a four-scan measurement in two different ways. The four acquisitions correspond to the two steps of the classical POCE scheme combined with another two-scan module, where the relative phases of the C_3 and C_4 ^1H resonances are manipulated using zero quantum and double quantum coherence pathways. This new technique exhibits the same sensitivity as POCE and allows the ^{13}C labeling of C_3 and C_4 glutamate from $[1-^{13}\text{C}]\text{glucose}$ to be monitored separately in the rat brain at 3 T. Magn Reson Med 44:395–400, 2000. © 2000 Wiley-Liss, Inc.

Key words: POCE; glutamate; tricarboxylic acid cycle; brain; glucose

INTRODUCTION

Over the last decade, the *in vivo* NMR monitoring of glutamate ^{13}C labeling from intravenously infused $[1-^{13}\text{C}]\text{glucose}$ has become a prominent tool for the investigation of brain oxidative metabolism (1–7). The recent evidence that the tricarboxylic acid (TCA) cycle flux determined by this approach is stoichiometrically coupled to glutamate-glutamine cycling has enhanced the interest of *in vivo* NMR spectroscopy for evaluating glutamatergic neuronal activity (8,9). While direct ^{13}C detection offers a wealth of spectral information, enabling sophisticated metabolic modeling, it requires large sample volumes to attain sufficient temporal resolution (10–12). In order to investigate functionally-specialized cortical areas (or even deep brain structures), direct ^{13}C detection has been often abandoned to the benefit of reverse detection of ^1H bound to ^{13}C , which offers a greatly improved sensitivity at the expense of spectral resolution (4–7,13–15).

Among several reverse $\{^{13}\text{C}\}\text{-}^1\text{H}$ spectroscopy techniques, whose *in vivo* feasibility has been demonstrated (16–18), the techniques mostly used for robust neurobiological investigations are derived from the well-established Proton Observed Carbon Edited (POCE) approach based on difference spectroscopy (1,19). At very high field (e.g. 9.4 T in Ref. 6) reverse $\{^{13}\text{C}\}\text{-}^1\text{H}$ experiments provide information on ^{13}C labeling of several metabolites (glutamate, glutamine, aspartate, lactate, GABA). However, at intermediate fields mostly accessible for human studies (1.5 to 4 T), kinetic information is usually restricted to the labeling of the C_3 and C_4 positions of glutamate (3,15). Below 3 T, the resolution of the C_3 and C_4 ^1H signals

becomes difficult, partly because of their broad multiplet structures. Even if the time course of label incorporation into glutamate C_4 alone has already been used to estimate TCA cycle flux (4,5,7), it is more accurate to use both C_4 and C_3 kinetics (2). The time lag of C_3 labeling (occurring on the second turn of TCA cycle) behind C_4 labeling (occurring on the first turn) is indeed directly related to the cycle rate. Therefore, when both kinetics are available, an estimate of the TCA cycle rate can be obtained even in the absence of information on the time course of plasma glucose ^{13}C -enrichment.

The purpose of this study is to propose and evaluate a new editing scheme, called SPOCE (Semiselective POCE), designed to resolve the time courses of glutamate C_3 and C_4 ^1H resonances, even when both signals strongly overlap. The two kinetics are acquired simultaneously and without loss of sensitivity as compared to the standard POCE method. After a theoretical analysis of the editing sequence, *in vitro* and *in vivo* results demonstrating the efficiency of the new method are presented.

THEORY

The SPOCE sequence is depicted in Fig. 1. It is very similar to the POCE sequence, except that two ^{13}C editing pulses are used instead of one. Depending on the flip angle and the relative phase of these two pulses, non-selective or semiselective inversion of glutamate C_3 and C_4 resonances of ^1H bound to ^{13}C (referred to as satellites) can be obtained, as explained below. In the conventional POCE scheme, two sequences A and B are run with $(\theta_1, \theta_2) = (0^\circ, 0^\circ)$ and $(\theta_1, \theta_2) = (0^\circ, 180^\circ)$, respectively. The glutamate C_3 and C_4 satellites are inverted in sequence B and not inverted in sequence A, relative to the resonances of ^1H bound to ^{12}C (referred to as mother resonances). By taking the difference between the two spectra (A–B), both C_3 and C_4 satellites are co-edited with the same sign (defined as positive by reference to the mother resonances), and the mother resonances are canceled.

In the new SPOCE scheme, two additional sequences C and D are run with $(\theta_1, \theta_2) = (90^\circ, 90^\circ)$ and $(\theta_1, \theta_2) = (90^\circ, -90^\circ)$, respectively. All ^{13}C hard pulses are applied at the C_4 frequency and $1/\tau$ is set to twice the frequency difference $\Delta\nu_{C_3-C_4}$ between the C_3 and C_4 ^{13}C resonances ($\Delta\nu_{C_3-C_4} = 201$ Hz for glutamate at 3 T). Using the product operator formalism for a weakly coupled IS system (I for ^1H , S for ^{13}C) with an heteronuclear coupling constant J , the effect of the different sequences (A, B, C, D) on C_3 and C_4 satellites can be analyzed. All pulses are assumed to have + x phase and the ^{13}C rotating frame is set at the C_4 frequency. Chemical shift effects for ^1H are refocused by the spin echo, so that their influence is not explicitly accounted for in the calculations. The evolution of the IS spin system

CEA, Service Hospitalier Frédéric Joliot, Département de Recherche Médicale, Orsay, France.

*Correspondence to: Dr. G. Bloch, CEA, SHFJ-DRM, 4 Place du Général Leclerc, 91406 Orsay Cedex, France. E-mail: bloch@shfj.cea.fr

Received 2 February 2000; revised 20 April 2000; accepted 21 April 2000.

© 2000 Wiley-Liss, Inc.

Table 1
Sign of the C_3 and C_4 Satellites Signals, Relatively to Their Mother Resonances, for the Four Sequences A, B, C, and D of the SPOCE Scheme Depicted in Fig. 1

	C_3 satellites	C_4 satellites
A : $(\theta_1, \theta_2) = (0^\circ, 0^\circ)$	+	+
B : $(\theta_1, \theta_2) = (0^\circ, 180^\circ)$	-	-
C : $(\theta_1, \theta_2) = (90^\circ, 90^\circ)$	+	-
D : $(\theta_1, \theta_2) = (90^\circ, -90^\circ)$	-	+

during sequence C, after the excitation pulse, is depicted in the following diagram:

$$\begin{aligned}
 \text{C4: } -I_y &\xrightarrow{1/2J} 2I_x S_z \xrightarrow{\theta_1} -2I_x S_y \xrightarrow{\tau + 180^\circ} \\
 &\quad -2I_x S_y \xrightarrow{\theta_2} -2I_x S_z \xrightarrow{1/2J} -I_y \\
 \text{C3: } -I_y &\xrightarrow{1/2J} 2I_x S_z \xrightarrow{\theta_1} -2I_x S_y \xrightarrow{\tau + 180^\circ} \\
 &\quad 2I_x S_y \xrightarrow{\theta_2} 2I_x S_z \xrightarrow{1/2J} I_y
 \end{aligned}$$

Of course, for the mother C_3 and C_4 resonances (no heteronuclear J coupling), the spin state at the end of sequence C is represented by I_y . Thus, in the case $(\theta_1, \theta_2) = (90^\circ, 90^\circ)$, C_4 satellites, but not C_3 satellites, are inverted relatively to their mother resonances. This simple result suggests that, although $1/\tau$ is of the same order of magnitude as J , sequence C works as if heteronuclear scalar coupling could be neglected during the evolution delay of the 90° - τ - 90° semiselective pulse on the ^{13}C channel. Whatever the relative values of $1/\tau$ and J , this naive interpretation is strictly true because the transverse terms of the density operator during τ are heteronuclear zero and double quantum coherences which do not evolve under scalar coupling.

The results of similar analyses, carried out for all four sequences of the SPOCE scheme, are summarized in Table 1. It appears from this table that the spectrum obtained through the combination A-B+C-D retains the full intensity of the C_3 satellites signal and suppresses the C_4 satellites signal, as well as both mother resonances. On the other hand, the combination A-B-C+D suppresses the C_3 satellites signal while editing the full C_4 satellites signal. Therefore, separate editing of C_3 and C_4 satellites is achieved from the same four spectral acquisitions, and no time is wasted as compared to the standard POCE method.

MATERIALS AND METHODS

In Vitro Sample and Animal Preparation

A 1.8-ml glass sphere was filled with a 500 mM solution of glucose in 99.9% D_2O . The ^{13}C -enrichment of glucose on carbon 1 was adjusted to about 50% using $[1-^{13}\text{C}]$ glucose (ISOTECH Inc.). A male Sprague-Dawley rat (300 g), fasted

overnight, was anesthetized with ketamine and xylazine. The left femoral vein was cannulated with PE 50 tubing for infusion of glucose. The scalp was removed and the skull was cleaned of all tissues between the temporal ridges. The animal was installed on a platform with its head maintained in place by a bite-bar and ear barrels. A thin layer of plastic wrap was placed over the skull to limit desiccation and avoid contact of moisture with the probe positioned just above the skull.

During the in vivo NMR experiment, the plasma glucose level of the rat was rapidly raised from euglycemia (1.1 g/l) to about 4 g/l by a 3.5 ml/kg bolus of 100%-enriched $[1-^{13}\text{C}]$ -glucose (ISOTECH Inc.) in a 20% (wt/vol) solution. The bolus was infused at an exponentially decaying rate over 9 min, and was followed by a continuous infusion at $3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$. This infusion profile had been previously optimized to rapidly achieve a stable hyperglycemic plateau.

NMR Acquisitions

All spectra were acquired on a Bruker AVANCE spectrometer interfaced to a 3 T whole-body magnet (Oxford). A gradient insert with a rise time of 170 μsec and a maximum gradient strength of 25 mT/m was used for spoiling and localization. The home-build RF probe consisted of a ^1H surface coil (12 mm diameter) for transmission and reception, and a larger ^{13}C concentric coil (30 mm diameter) for editing and decoupling pulses.

The pulse sequence in Fig. 1 was used to edit either glucose $C_{1\alpha}$ and $C_{1\beta}$ satellites, in vitro, or glutamate C_3 and C_4 satellites, in vivo. For glucose, the chemical shift difference between the ^{13}C resonances of $C_{1\alpha}$ (92.7 ppm) and $C_{1\beta}$ (96.6 ppm) is smaller than for glutamate C_3 (27.9 ppm) and C_4 (34.4 ppm). However, the heteronuclear coupling constants are higher for glucose (162–170 Hz) than for glutamate (127–130 Hz). Thus, for glucose, the experimental delays $1/J_{\text{CH}}$ and τ were set to 6.0 msec and 4.0 msec, respectively, resulting in a total echo time of 10.0 msec. For glutamate, the experimental delays $1/J_{\text{CH}}$ and τ were set to 8.0 msec and 2.5 msec, respectively, resulting in a similar total echo time of 10.5 msec. In both cases, the ^{13}C carrier was set on the low field resonance ($C_{1\beta}$ for glucose, C_4 for glutamate), and 2.5 msec gradient spoilers at 70% maximum strength (on x and z directions) were symmetrically applied in the spin echo to eliminate non-refocusing magnetization.

Localization of a $10 \times 6 \times 10 \text{ mm}^3$ (in x, y, and z directions) volume of interest (VOI) was achieved through outer volume suppression before the SPOCE sequence. Six hyperbolic secant pulses were used for $\sim 90^\circ$ excitation in a non-adiabatic manner to saturate the six slices adjacent to the VOI (20). The water linewidth in the VOI was optimized manually down to about 15 Hz in vivo. The

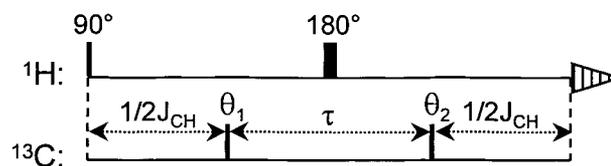


FIG. 1. Schematic representation of the pulse sequence used in the SPOCE scheme.

SWAMP method was used for adiabatic water suppression (21). The ^{13}C pulses were calibrated using a 1 ml sphere containing a solution of $[1-^{13}\text{C}]$ formate and positioned above the probe. For in vivo measurements, the ^{13}C frequency of glutamate C_4 was deduced from ^1H frequency of the creatine singlet at 3.0 ppm, based on previous in vitro calibration. The sensitivity of in vivo NMR experiments was improved through WALTZ-8 broadband decoupling, applied on the ^{13}C channel during ^1H acquisition.

In order to minimize subtraction artifacts, single scans from SPOCE sequences A, B, C, and D were acquired in an interleaved mode using the pipeline filter capability of the Bruker AVANCE system. For in vivo kinetic studies, each block of four spectra consisted of two dummy scans and 128 interleaved scans recorded with $\text{TR} = 2.5$ s, leading to an accumulation time of 5.5 min. These 128 scans were summed into four separate FIDs of 32 scans each, corresponding to SPOCE sequences A, B, C, and D. Edited spectra were obtained by directly combining individual FIDs, before Fourier transform.

RESULTS

In Vitro Test

The SPOCE scheme was first evaluated in vitro, on a spherical phantom containing a solution of glucose ^{13}C -enriched on carbon 1. This phantom provided at 3 T two well-resolved sets of satellites, corresponding to the $C_{1\alpha}$ and $C_{1\beta}$ resonances and mimicking glutamate C_3 and C_4 resonances, respectively. Thus, with a straightforward adjustment of the delays of the acquisition sequence, this phantom enabled us to test the selectivity of the semiselective editing in optimal conditions in terms of signal-to-noise ratio and spectral resolution.

Figure 2 shows the spectra resulting from four different combinations of the four SPOCE sequences A, B, C, and D. Figure 2a corresponds to the standard POCE scheme, with the difference spectrum A-B, where both sets of satellites are co-edited with the same sign. By taking the difference C-D (Fig. 2b), $C_{1\alpha}$ and $C_{1\beta}$ satellites are also co-edited, but with opposite signs. The spectrum in Fig. 2c is the sum of the two previous difference spectra and shows the selective editing of the $C_{1\alpha}$ satellites (combination A-B+C-D). Similarly, the spectrum in Fig. 2d results from the subtraction of the two initial difference spectra and shows the selective editing of the $C_{1\beta}$ satellites (combination A-B-C+D). The excellent selectivity of the SPOCE scheme is reflected by the very low level of contamination of the edited satellites by the non-edited satellites both in Fig. 2c and 2d.

In Vivo Validation

The in vivo SPOCE editing of glutamate C_3 and C_4 resonances is illustrated in Fig. 3 using ^{13}C decoupling during acquisition. These spectra, acquired on a rat brain, correspond to 22 min of accumulation centered 70 min after the beginning of a $[1-^{13}\text{C}]$ glucose IV infusion. Similarly to Fig. 2, glutamate C_3 and C_4 resonances are co-edited with the same sign and with opposite signs in Fig. 3a and b, respectively. Figure 3c demonstrates the selective editing of glutamate C_3 resonance, at 2.10 ppm, while Fig. 3d demon-

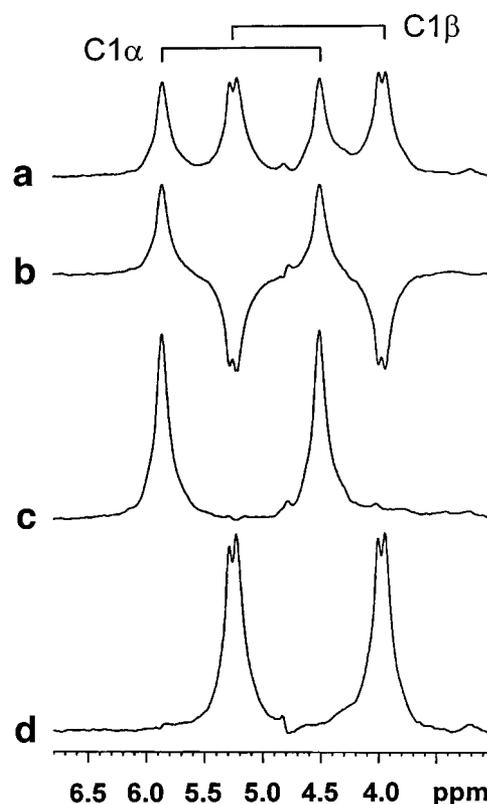


FIG. 2. Validation of the SPOCE scheme on a phantom of glucose ^{13}C -enriched on carbon 1. The four spectra correspond to the following combinations of the acquisition sequences listed in Table 1: **a**: A-B; **b**: C-D; **c**: A-B+C-D; **d**: A-B-C+D. The semiselective editing of the ^1H bound to ^{13}C at $C_{1\alpha}$ and $C_{1\beta}$ positions is demonstrated in **c** and **d**, respectively.

strates the selective editing of glutamate C_4 resonance, at 2.35 ppm. The edited spectra also display minor signals, which can be assigned to lactate/alanine, aspartate, and creatine/GABA, according to Ref. 6.

Serial spectra obtained through the SPOCE scheme on a rat brain are shown in Fig. 4 with a time resolution of 5.5 min. The two series of spectra, acquired simultaneously during the same $[1-^{13}\text{C}]$ glucose infusion, clearly reflect the difference in kinetics between the ^{13}C labeling of glutamate C_4 and that of C_3 . A three-parameter mono-exponential fit of the peak height versus time (Fig. 5) provided a time constant of 33 min for glutamate C_4 and 62 min for C_3 .

DISCUSSION

The proposed SPOCE approach allows two resonances of ^1H bound to distinct ^{13}C to be edited separately, even if they overlap, provided that the two ^{13}C resonances are sufficiently resolved. The method was validated in vitro by selectively editing the $C_{1\alpha}$ and $C_{1\beta}$ satellites of glucose, ^{13}C -enriched on carbon 1. Separate editing of glutamate C_3 and C_4 satellites also was achieved in vivo, on the rat brain, in order to monitor the incorporation of ^{13}C label during an IV infusion of $[1-^{13}\text{C}]$ glucose. With the SPOCE scheme, the individual kinetics of label incorporation on the two glutamate positions C_3 and C_4 could be measured

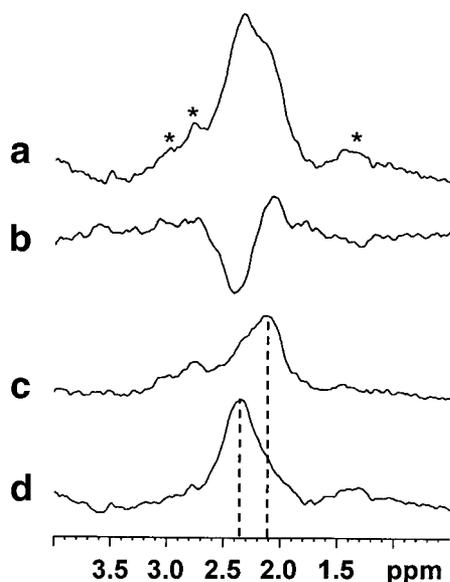


FIG. 3. Validation of the SPOCE scheme on the brain of an anesthetized rat receiving an IV infusion of $[1-^{13}\text{C}]$ glucose. The four ^{13}C -decoupled spectra correspond to the following combinations of the acquisition sequences listed in Table 1: **a**: A-B; **b**: C-D; **c**: A-B+C-D; **d**: A-B-C+D. The only data processing used was a line broadening of 5 Hz. The data were obtained from a 22 min accumulation centered 70 min after the beginning of the glucose infusion. The semiselective editing of ^1H bound to ^{13}C at C_3 and C_4 positions is demonstrated in **c** and **d**, respectively. Minor resonances from lactate/alanine (1.3 ppm), aspartate (2.8 ppm), and creatine/GABA (3.0 ppm), assigned according to Ref. 6, are highlighted with a star in **a**.

within the same acquisition, even at intermediate field strength (3 T in this study) where the two ^1H resonances strongly overlap.

The four-scan SPOCE measurement can be regarded as the combination of two distinct modules of difference spectroscopy, named SQ (for single quantum) and ZDQ (for zero and double quantum). In SQ, corresponding to the classical POCE scheme (sequences A and B in Table 1)

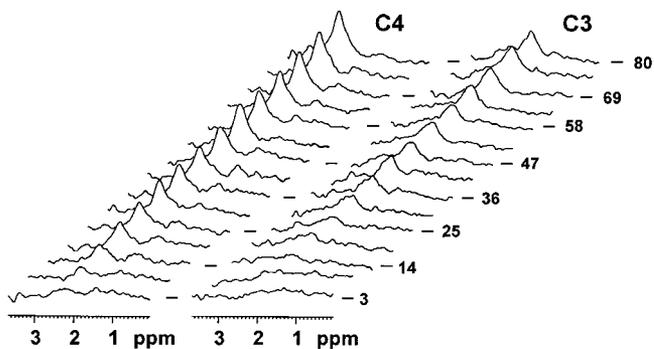


FIG. 4. Stacked plot of pairs of SPOCE spectra recorded from the brain of an anesthetized rat receiving an IV infusion of $[1-^{13}\text{C}]$ glucose and showing the ^{13}C labeling of glutamate C_3 and C_4 . The midpoint time (in min from the beginning of the glucose infusion) for the acquisition of each other pair of spectra is indicated to the right. The only data processing used was a line broadening of 10 Hz.

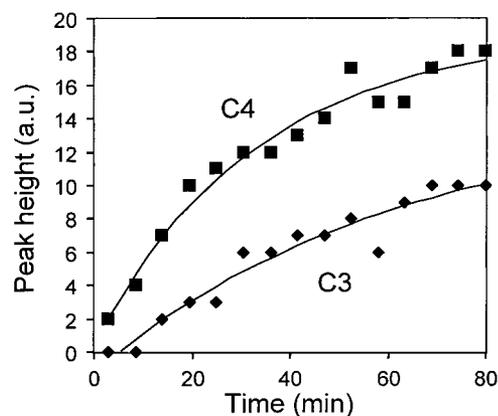


FIG. 5. Time course of ^{13}C labeling of glutamate C_3 and C_4 obtained through the SPOCE technique from the brain of an anesthetized rat (same experiment as in Fig. 4). The relative intensities of the resonances were determined by peak picking, after manual linear baseline correction. The solid lines represent the best fit of the data to a three-parameter mono-exponential equation.

and involving only single quantum coherences, glutamate C_3 and C_4 satellites are co-edited with the same sign, whatever the value of the delay τ (Fig. 1). For ZDQ (sequences C and D), the delay τ must be adjusted to a precise value ($1/2\Delta\nu_{C_3-C_4}$) in order to co-edit both C_3 and C_4 satellites with opposite signs. By adding or subtracting the difference spectra obtained from SQ and ZDQ, the C_3 or C_4 satellites are edited separately with the full intensity corresponding to the acquisition of the four individual spectra. Thus, while SPOCE is based on a four-scan measurement, cancellation of the mother resonances is obtained for each two-scan module (SQ or ZDQ). In addition to the interleaved acquisition mode, this feature contributes to the reduction of subtraction artifacts. It is noteworthy that sequence C or D is identical to the classical HMQC sequence (22) and involves a mixing of zero and double quantum coherences during the delay τ . Because the ^{13}C pulses are applied at the C_4 frequency, the C_4 satellites are always edited by ZDQ with maximum amplitude, whatever τ value. On the other hand, the amplitude of the C_3 satellites is modulated as $\cos(2\cdot\pi\cdot\tau\cdot\Delta\nu_{C_3-C_4})$, because the interference of zero and double quantum coherences varies with τ .

Because of this interference, the adjustment of the ^{13}C carrier frequency is critical for an optimal performance of the SPOCE ZDQ module. The misadjustment of this experimental parameter results in a cross-contamination between the glutamate C_3 and C_4 signals in edited ^1H spectra. In the worst case, where ^{13}C pulses are applied at the midpoint frequency between C_3 and C_4 ^{13}C resonances, the difference spectrum resulting from the ZDQ module does not contain any C_3 or C_4 signal, and the two SPOCE spectra which should correspond to the selective editing of C_3 and C_4 now simply contain the co-edited C_3 and C_4 resonances from the SQ module, with additional noise. The spectra presented in Fig. 3 and the difference in kinetics of ^{13}C labeling reflected in Fig. 5 indicate that no major cross-contamination occurred during the in vivo experiment. However, in order to double-check the correct setting of

the ^{13}C carrier frequency deduced from the creatine singlet ^1H frequency at 3.0 ppm, post mortem tests on the rat brain were performed at the end of the $[1-^{13}\text{C}]$ glucose infusion using the strong signal from $[3-^{13}\text{C}]$ lactate (data not shown).

The coherence pathways used during the delay τ for the ZDQ module do not allow a semiselective refocusing ^1H pulse to be used for water suppression, like most previous applications of POCE did. Because of this limitation, a simple 180° hard pulse was implemented in the ^1H spin echo and water suppression was optimized using adiabatic inversion pulses applied off-resonance (21). For future application in awake subjects, where subtraction error originating from unstable residual water signal may appear, further improvement of water suppression may be necessary. The ZDQ module of the SPOCE scheme also limits the total echo time of the sequence, through the constraint $\tau = 1/2\Delta\nu_{C_3-C_4}$. In the case of glutamate C_3 and C_4 (or of glucose $C_{1\alpha}$ and $C_{1\beta}$) at 3 T, the resulting value of 10.5 ms (or 10.0 ms, respectively) was short enough to limit the effect of homonuclear J-modulation on signal intensity, and long enough to be compatible with the current performances of gradients, even on a whole body system. At lower field, e.g. 1.5 T, where the SPOCE approach should be even more helpful, the total echo time of the editing sequence for glutamate would be increased to the perfectly acceptable value of 13.0 ms.

At magnetic field values beyond 3 T, where glutamate C_3 and C_4 ^1H resonances are sufficiently resolved, the SPOCE scheme should also prove useful in order to suppress the contamination of the glutamate C_4 signal by the glutamine C_4 resonance (6), e.g. for functional studies focused on ^{13}C incorporation at the C_4 position (4,5,7,15). It must be noted that this contamination, usually neglected because of the slower kinetics of glutamine ^{13}C labeling, is already reduced in the present in vivo data obtained at 3 T. The ^{13}C glutamine C_4 resonance is indeed located about the midpoint frequency between glutamate C_3 and C_4 ^{13}C resonances and it is hence poorly edited by the ZDQ module of the SPOCE scheme. However, the contamination from glutamine C_4 through the SQ module is still present. Similarly, glutamate C_3 ^1H signal is contaminated by glutamine C_3 resonance through the ZDQ module as well as through the SQ module, because the two corresponding ^{13}C resonances are very close. Thus, the separation of all glutamate and glutamine ^1H signals (especially for long turn-over studies) does not appear possible with the proposed SPOCE scheme. Even if high field systems (6) and two-dimensional NMR approaches (23) could offer solutions to this challenging problem, direct ^{13}C detection certainly remains an attractive alternative to study metabolites with turn-over much slower than that of glutamate.

Another possible source of contamination in the 2–2.5 ppm region of SPOCE-edited ^1H spectra is the N-acetylaspartate (NAA) methyl signal. The ^{13}C chemical shift of NAA methyl resonance is such that the corresponding ^1H resonance is predominantly co-edited with the glutamate C_4 signal through the SPOCE scheme. Because of its high concentration, natural abundance detection of NAA signal at 2.01 ppm has been achieved in human brain using a classical POCE scheme at 4 T (14). Due to lower sensitivity, no such signal could be observed in our spectra from

rat brain before $[1-^{13}\text{C}]$ glucose infusion (Fig. 4). In addition, because of its extremely slow turnover (24), NAA ^{13}C labeling was not observed on the time scale of the experiments reported here (Fig. 3d).

To conclude, the SPOCE scheme should advantageously replace classical POCE for all studies focused on brain glutamate C_4 and C_3 ^{13}C labeling, especially at magnetic field strength below 4 T. The more accurate kinetic information, as well as the simplified spectroscopic analysis, resulting from the separate editing of the C_4 and C_3 resonances will facilitate the measurement of the TCA cycle flux on intermediate magnetic field NMR systems, more readily available for applications in humans.

REFERENCES

- Fitzpatrick SM, Hetherington HP, Behar KL, Shulman RG. The flux from glucose to glutamate in the rat brain in vivo as determined by ^1H -observed, ^{13}C -edited NMR spectroscopy. *J Cereb Blood Flow Metab* 1990;10:170–179.
- Mason GF, Rothman DL, Behar KL, Shulman RG. NMR determination of the TCA cycle rate and α -ketoglutarate/glutamate exchange rate in rat brain. *J Cereb Blood Flow Metab* 1992;12:434–447.
- Rothman DL, Novotny EJ, Shulman GI, Howseman AM, Petroff OAC, Mason G, Nixon T, Hanstock CC, Prichard JW, Shulman RG. ^1H - $[^{13}\text{C}]$ NMR measurements of $[4-^{13}\text{C}]$ glutamate turnover in human brain. *Proc Natl Acad Sci USA* 1992;89:9603–9606.
- Hyder F, Chase JR, Behar KL, Mason GF, Siddeef M, Rothman DL, Shulman RG. Increased tricarboxylic acid cycle flux in the rat brain during forepaw stimulation detected with ^1H $[^{13}\text{C}]$ NMR. *Proc Natl Acad Sci USA* 1996;93:7612–7617.
- Hyder F, Rothman DL, Mason GF, Rangarajan A, Behar KL, Shulman RG. Oxidative glucose metabolism in rat brain during single forepaw stimulation: a spatially localized ^1H $[^{13}\text{C}]$ nuclear magnetic resonance study. *J Cereb Blood Flow Metab* 1997;17:1040–1047.
- Pfeuffer J, Tkac I, Choi IY, Merkle H, Ugurbil K, Garwood M, Gruetter R. Localized in vivo ^1H NMR detection of neurotransmitter labeling in rat brain during infusion of $[1-^{13}\text{C}]$ D-glucose. *Magn Reson Med* 1999; 41:1077–1083.
- Hyder F, Renken R, Rothman DL. In vivo carbon-edited detection with proton echo-planar spectroscopic imaging (ICED PEPSI): $[3,4-^{13}\text{CH}_2]$ glutamate/glutamine tomography in rat brain. *Magn Reson Med* 1999;42:997–1003.
- Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG. Stoichiometric coupling of brain metabolism and glutamatergic neuronal activity. *Proc Natl Acad Sci USA* 1998;95:316–321.
- 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. *Proc Natl Acad Sci USA* 1999;96:8235–8240.
- Gruetter R, Novotny EJ, Boulware SD, Mason GF, Rothman DL, Shulman GI, Prichard JW, Shulman RG. Localized ^{13}C NMR spectroscopy in the human brain of amino acid labeling from D- $[1-^{13}\text{C}]$ glucose. *J Neurochem* 1994;63:1377–1385.
- Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny EJ. Simultaneous determination of the rates of the TCA cycle, glucose utilization, α -ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J Cereb Blood Flow Metab* 1995;15:12–25.
- Gruetter R, Seaquist ER, Kim S, Ugurbil K. Localized in vivo ^{13}C -NMR of glutamate metabolism in the human brain: initial results at 4 Tesla. *Dev Neurosci* 1998;20:380–388.
- Pan JW, Mason GF, Vaughan JT, Chu WJ, Zhang Y, Hetherington HP. ^{13}C editing of glutamate in human brain using J-refocused coherence transfer spectroscopy at 4.1 T. *Magn Reson Med* 1997;37:355–358.
- Chen W, Adriany G, Zhu X-H, Gruetter R, Ugurbil K. Detecting natural abundance carbon signal of NAA metabolite within 12-cm 2 localized volume of human brain using ^1H - $[^{13}\text{C}]$ NMR spectroscopy. *Magn Reson Med* 1998;40:180–184.
- Mason GF, Pan JW, Chu WJ, Newcomer BR, Zhang Y, Orr R, Hetherington HP. Measurement of the tricarboxylic acid cycle rate in human grey and white matter in vivo by ^1H - $[^{13}\text{C}]$ magnetic resonance spectroscopy at 4.1T. *J Cereb Blood Flow Metab* 1999;19:1179–1188.

16. van Zijl PCM, Chesnick AS, DesPres D, Moonen CTW, Ruiz-Cabello J, van Gelderen P. In vivo proton spectroscopy and spectroscopic imaging of {1-¹³C}-glucose and its metabolic products. *Magn Reson Med* 1993; 30:544–551.
17. Inubushi T, Morikawa S, Kito K, Arai T. ¹H-detected in vivo ¹³C NMR spectroscopy and imaging at 2T magnetic field: efficient monitoring of ¹³C-labeled metabolites in the rat brain derived from 1-¹³C-glucose. *Biochem Biophys Res Commun* 1993;191:866–872.
18. de Graaf RA, Luo Y, Terpstra M, Garwood M. Spectral editing with adiabatic pulses. *J Magn Reson B* 1995;109:184–193.
19. Rothman DL, Behar KL, Hetherington HP, den Hollander JA, Bendall MR, Petroff OAC, Shulman RG. ¹H-observe/¹³C-decouple spectroscopic measurements of lactate and glutamate in the rat brain in vivo. *Proc Natl Acad Sci USA* 1985;82:1633–1637.
20. Tkac I, Starcuk Z, Choi I-Y, Gruetter R. In vivo ¹H NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med* 1999;41:649–656.
21. de Graaf RA, Nicolay K. Adiabatic water suppression using frequency selective excitation. *Magn Reson Med* 1998;40:690–696.
22. Ruiz-Cabello J, Vuister GW, Moonen CTW, van Gelderen P, Cohen JS, van Zijl PCM. Gradient-enhanced heteronuclear correlation spectroscopy. Theory and experimental aspects. *J Magn Reson* 1992;100:282–302.
23. Watanabe H, Ishihara Y, Okamoto K, Oshio K, Kanamatsu T, Tsukada Y. 3D localized ¹H-¹³C heteronuclear single-quantum coherence correlation spectroscopy in vivo. *Magn Reson Med* 2000;43:200–210.
24. Choi I-Y, Gruetter R. Dynamic detection of NAA/GSH turnover: a 3D-localized in vivo ¹³C NMR study of the rat brain. In: *Proceedings of the 8th Annual Meeting of ISMRM, Denver, 2000.* p 598.