BISEP-based, Ultra-short TE ^{1}H–[^{13}C] NMR Spectroscopy of the Rat Brain at 14.1 T

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Introduction

The detection of ^{13}C label incorporation in conjunction with ^{13}C label administration is a powerful tool to explore neuron-glial metabolism. Indirect detection through ^{1}H can offer higher sensitivity but suffers from lower spectral resolution, such as the incomplete separation of labeling in C3 of Glutamate (Glu) and Glutamine (Gln)[1]. With increased magnet field strength, higher spectral resolution and signal-to-noise ratio can be achieved. Therefore, the aim of the present study was i) to demonstrate a novel ^{1}H-[^{13}C] NMR scheme, which combined the spin echo coherence based (SPECIAL) [2] localization with a 180 degree "B_1-insensitive spectral editing pulse" (BISEP) [3]; ii) to apply this new scheme at 14.1T with infusion of the glia-specific substrate - [2-^{13}C] acetate.

Materials and Methods

The proposed ^{1}H-[^{13}C] NMR sequence ('SPECIAL-BISEP') can be easily implemented by adding a 180^{o} BISEP pulse [3] prior to the localization part of the SPECIAL sequence (Fig. 1a). At the end of the 180^{o} BISEP pulse, ^{13}C coupled ^{1}H coherences were inverted (–Iz), while when using the 180^{o} BISEP pulse without adiabatic full passage (AFP) at ^{13}C, all coherences were returned to +Iz. The bandwidth of 180^{o} BISEP pulse was ~ 2.3kHz (in ^{1}H), which was enough to cover the ^{1}H spectral region. The AFP at ^{13}C was turned on and off on alternate scans to prepare the -/+Iz coherences before localization and the obtained difference spectum contained ^{13}C coupled ^{1}H resonances only. Ultra-short echo time of 2.8ms was used to attain full signal intensity. Adiabatic ^{13}C decoupling [1] was applied during the 145 ms acquisition time.

All experiments were carried out on a 14.1T/26cm magnet (Varian/Magnex) using a homebuilt 10mm (^{13}C)/13mm (^{1}H quad) surface coil. ^{1}H-[^{13}C] spectra were acquired from a volume of 60µl located in cerebral cortex, shimmed with FASTMAP. Following optimization and validation in phantom studies (not shown), *in vivo* performance was evaluated in rat brain in conjunction with 99% enriched [2-^{13}C] sodium acetate infusions for 2.5 hours. Five male Sprague-Dawley rats (fasted over night) were intubated and one femoral artery and both veins were cannulated for blood sampling, acetate and α-chloralose infusion. Blood plasma was used to measure acetate concentration and isotopic enrichment. Individual carbon edited proton spectra were acquired with 64 scans (32 scans with AFP on, 32 scans with AFP off) and TR was 4 s. All spectra were quantified with LCModel [4].

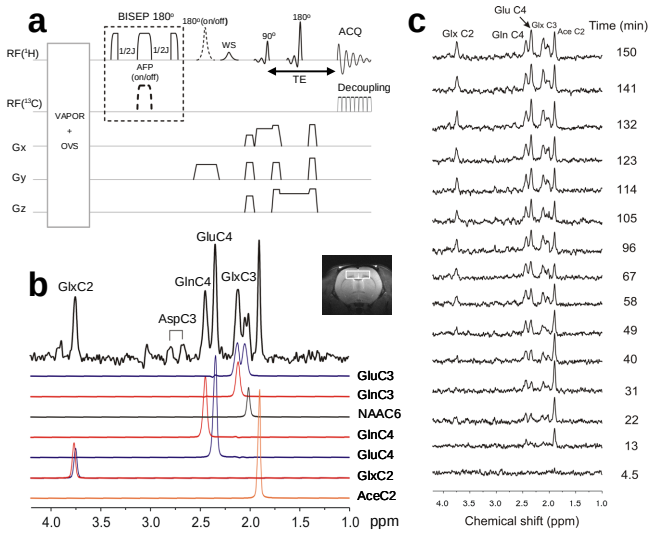

Figure 1. ^{1}H-^{13}C NMR spectroscopy of the rat brain at 14.1T. a). Sequence diagram of SPECIAL-BISEP (TE = 2.8 ms and 1/2J = 3.4 ms). The editing was achieved by applying with and w/o AFP in the ^{13}C channel in alternate scans. b). ^{1}H-^{13}C NMR spectrum summed from the last 51min of infusion of [2-^{13}C] Acetate (nt = 768) and the individual fits of the labeled metabolites. c). Stack plot of time resolved ^{13}C labeling into metabolites of rat brain *in vivo* during [2-^{13}C] Acetate infusion. Each spectrum was summed over ~ 9 min (nt = 128) scans.

Results and Discussion

In the proposed scheme a 180^{o} BISEP pulse was employed, not as a refocusing element, but to invert the ^{13}C coupled ^{1}H coherences on alternate scans prior to the localization (Fig. 1a). Since the BISEP editing pulse prepared the -/+Iz coherences before localization, such a ^{13}C editing block is independent of the subsequent localization part, which allows to insert it prior to not only SPECIAL but any localization sequence.

During *in vivo* application of SPECIAL-BISEP with [2-^{13}C] acetate infusion, ^{13}C labeling into metabolites (Fig. 1b) was observed as at 9.4 T [1]. However, compared to lower field strengths, the GlnC4 was clearly resolved from GluC4 and a more distinct structure of GluC3 (separate multiplets at 2.04ppm and 2.12ppm) was noted. The distinct multiplet signal of GluC3 compared to GlnC3 (Fig. 1b) implied the ability to measure individually GluC3 and GlnC3. To demonstrate the measurement of time resolved ^{13}C labeling at 14.1T by SPECIAL-BISEP, a stack plot of ^{1}H-[^{13}C] NMR spectra is shown in Fig.1c. The ^{13}C labeling into AcetateC2, GluC4 and GlnC4 was observed early and the labeling of GlnC3, GluC3, GluC2+GlnC2 steadily increased. The high sensitivity at 14.1T combined with full signal intensity ^{1}H-[^{13}C] localization sequence permitted the time course measurement of ^{13}C labeling with a time resolution of ~ 4.5 min (nt = 64) from a VOI of 60µl in the cortex, in particular the separate time courses of GluC3 and GlnC3(Fig. 2).

We conclude that the ^{1}H-[^{13}C] NMR sequence 'SPECIAL-BISEP' provides a versatile scheme of measuring ^{13}C labeling through ^{1}H with full signal sensitivity. The results demonstrate the feasibility of separate measurement of time-resolved GluC4, GlnC4, GlnC3 and GluC3 with high temporal resolution at 14.1T, which greatly enhanced the ability to study neuron-glial metabolism using ^{1}H observed ^{13}C edited NMR spectroscopy.

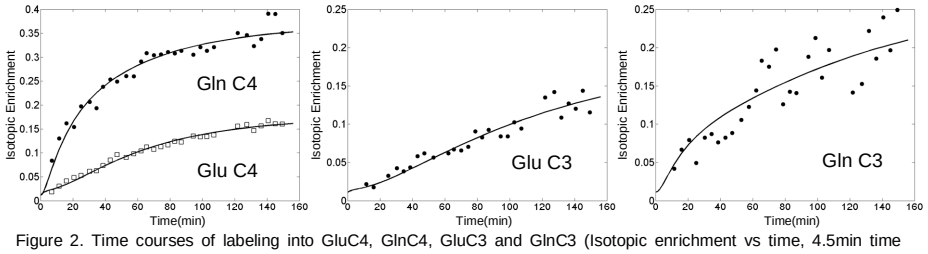

Figure 2. Time courses of labeling into GluC4, GlnC4, GluC3 and GlnC3 (Isotopic enrichment vs time, 4.5min time resolution).The solid lines show the best fit of a two compartment model (neuron-glia) [5] to the time courses.

References [1] J. Pfeuffer et al., Magn Reson Med. 41 (1999). [2] V. Mylnarik et al., Magn Reson Med. 56 (2006). [3] M. Garwood et al., J Magn Reson. 94 (1991). [4] S. Provencher, Magn Reson Med. 30 (1993). [5] R. Gruetter et al., Am J Physiol Endocrinol Metab. 281 (2001). **Acknowledgements** Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations, NIH grant R01NS042005 and SNF 3100A0-116220.