In vivo Detection of Rat Brain Metabolism using Hyperpolarized Acetate

M. Mishkovsky[1,2], A. Comment[1,2], and R. Gruetter[1,3]
[1]Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Department of Radiology, Université de Lausanne, Lausanne, Switzerland, [3]Departments of Radiology, Universités de Lausanne et Genève, Lausanne and Genève, Switzerland

Introduction
The advent of dynamic nuclear polarization (DNP) and the dissolution method into the field of nuclear magnetic resonance (NMR) opens up new perspectives that hitherto were not possible in MRS and MRI [1,2] by the ability to hyperpolarize a small amount of biological tracer and to detect its transformations *in vivo* and in real time [3]. In this study we demonstrate that ^{13}C acetate, which is readily taken up by the brain, is a metabolized hyperpolarized precursor for detecting *in vivo* brain metabolism.

Method
Carbon nuclear spins in frozen glassy solutions of 1-^{13}C and of $^{13}C_2$ labeled acetate were dynamically polarized using a custom-designed DNP polarizer operating at 5T and 1 ± 0.05K. Once ^{13}C spins reached maximal polarization the frozen mixtures were rapidly dissolved and transferred into an infusion pump capable of injecting 2.2 mL of hyperpolarized solution *in vivo* within 9 sec [4, 5]. Sprague-Dawley rats (450 g) were anesthetized using 1.5% isoflurane and their physiology was monitored during the experiments. The femoral vein was catheterized for injection of the hyperpolarized acetate into the animals. Measurements were carried out on a 9.4 T/ 31 cm actively shielded animal scanner (Varian/Magnex) using home-built quadrature ^{1}H surface coils and a 10mm diameter ^{13}C surface coil. In all experiments data acquisition started immediately after infusion, i.e., 15 s after the beginning of the dissolution. Proton decoupling was performed during all acquisitions.

Results and Discussion:
To confirm that acetate is rapidly transported through the blood brain barrier, a ^{13}C gradient echo multiple slice (GEMS) image was recorded following the infusion of hyperpolarized 1-^{13}C acetate (Fig. 1). The strong signal observed at the upper part of the brain is related to the central vessel from which the infused acetate enters the brain; the external ^{13}C-labeled reference, formic acid, can be seen on top of the head.
Localized ^{13}C spectra (n = 3) were measured using outer volume suppression (OVS) method, excluding the central vessel from the volume of interest. Data acquisition was started after the infusion of 1-^{13}C acetate (Fig. 2). Due to the high spectral resolution afforded using FASTMAP shimming protocol (6 -12 Hz line width), two carboxyl peaks separated by 0.15 ppm were detected. The more intense peak was assigned to the injected acetate molecule whereas the other signal is a consequence of brain metabolism. The same experiment was repeated (n = 3), but this time using hyperpolarized $^{13}C_2$ acetate. The spectral pattern is preserved and is now joined with the ^{13}C-^{13}C J-coupling splitting between the two labeled carbons (Fig. 3).
In order to assign the metabolic product observed, a set of localized and fully adiabatic polarization transferred experiments was carried out after infusion of $^{13}C_2$ labeled acetate (n = 3), leading to improved spectral resolution and additional spectroscopic information. In this experiment the acquisition was carried out at the aliphatic region and the observed resonances are tentatively assigned, with relation to the carboxyl spectrum, to acetate methyl group (24.07 ppm with 182.2 ppm) and methylmalonate (16.8 ppm with 182.05 ppm) (Fig. 4).

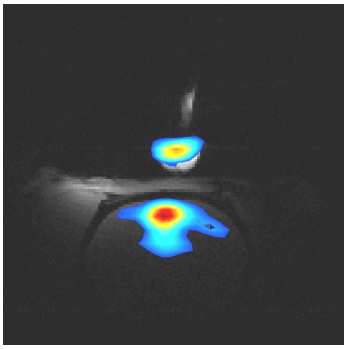

Fig.1: ^{13}C GEMS image of hyperpolarized 1-^{13}C acetate in the rat brain superimposed on ^{1}H scout image. The image presented is a sum of an array of three ^{13}C GEMS images measured just after the end of the infusion with 100 ms between successive measurements.

Conclusion:
This study demonstrates that metabolites related to brain metabolism are consistently observed *in vivo* after the infusion of hyperpolarized acetate. Additionally, it shows that dissolution DNP applications can be extended to doubly labeled precursors even when the hyperpolarized state survives only at one site. The additional labeling can be used to separate peaks in case of poor resolution and to earn additional spectral information which is required for full assignment.

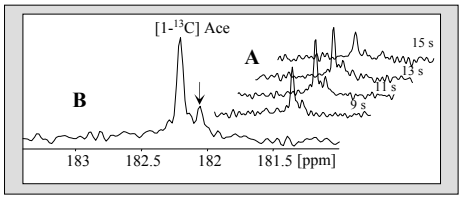

Fig.2: Array of localized rat brain ^{13}C detected on one of the rats (A) and their sum (B), measured after infusion of hyperpolarized 1-^{13}C acetate. Time is measured from the beginning of the infusion. Notice the two distinguished peaks at the carboxyl resonance area.

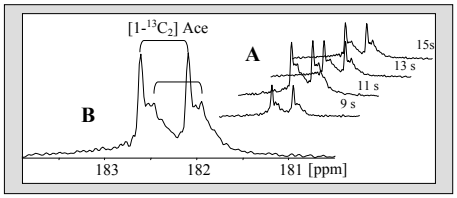

Fig.3: Array of localized rat brain ^{13}C detected on one of the rats (A) and their sum (B), measured after infusion of hyperpolarized $^{13}C_2$ acetate. Time is measured from the beginning of the infusion. The spectral pattern is preserved and accompanied by ^{13}C-^{13}C J-coupling splitting.

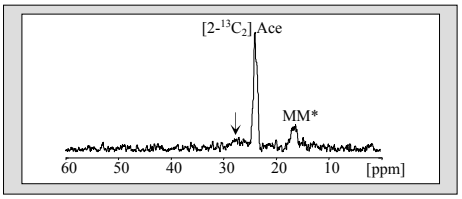

Fig.4: Localized rat brain ^{13}C spectrum following homonuclear polarization transfer, measured after infusion of hyperpolarized $^{13}C_2$ acetate on one of the rats. Note the triple gain, i.e. new information from the aliphatic region, better spectral separation and DNP enhancement.* - tentative assignment.

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