# In vivo detection of brain Krebs cycle intermediate by hyperpolarized MR

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**Introduction**
The tricarboxylic acid (TCA) cycle plays a central role in brain energy regulation and metabolism. Yet brain TCA cycle intermediates have never been directly detected *in vivo*. Here we present the first direct *in vivo* observation of a TCA cycle intermediate in intact brain, namely 2-oxoglutarate (2OG), a key biomolecule connecting metabolism to neuronal activity which hitherto considered undetectable. This observation became feasible via hyperpolarized MR protocol, while following hyperpolarized $^{13}$C acetate metabolism in the rat brain *in vivo*.

**Methods**
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 [1, 2]. Sprague-Dawley rats (350 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$^{13}$C spectrum observed in the brain 16 s after dissolution.

**Results**
To selectively measure the $^{13}$C signal from the brain, a localization pulse sequence was used in conjunction with a surface coil. A $^{13}$C spectrum observed in the brain 16 s after dissolution delineates two peaks separated by 0.15 ppm which were detected in all animals (n=3) the sum of them is presented in Fig.1. The more intense signal (182.2 ppm) was unambiguously assigned to the carboxyl $^{13}$C of the injected acetate molecule. The less intense resonance at 182.05 ppm likely originated from a metabolic product of acetate. The chemical shift of 182.05 ppm is consistent with that of the 5-$^{13}$C resonance of 5-$^{13}$C 2OG, which is synthesized from 1-$^{13}$C acetate through the incorporation of 1-$^{13}$C acetyl-CoA into the TCA cycle.
To provide even further evidence for the metabolite assignment, a custom-tailored adiabatic polarization transfer sequence was developed and applied. Prior to detection, the enhanced carboxyl $^{13}$C polarization was transferred to the aliphatic $^{13}$C spins which exhibit large chemical shift dispersion. To ensure that the same stage of the metabolic process was probed, the time interval between dissolution and data acquisition was set as in the previous measurement to 16 s. The spectra recorded from all animals (n=3) at the aliphatic region after the polarization transfer is presented in Fig. 2. The two resonances observed were assigned to acetate (24.5 ppm) and 2OG (36.0 ppm), which is consistent with the carboxyl spectrum (Fig. 1).

**Conclusion**
From the results described above, we conclude that the metabolite observed in the brain following the injection of hyperpolarized acetate is indeed 2OG. We conclude from the unprecedented observation of 2OG and the lack of Glu signal that the reactions leading to $^{13}$C-label incorporation into Glu are operating, in the glial compartment *in vivo*, at a rate much lower than that of transaminase thus implying that transport across the inner mitochondria membrane is rate limiting. More generally, the present study illustrates the potential of hyperpolarized acetate as a precursor to non-invasively directly probe glial TCA cycle activity and thus detect pathological alterations hitherto inaccessible through direct observation.

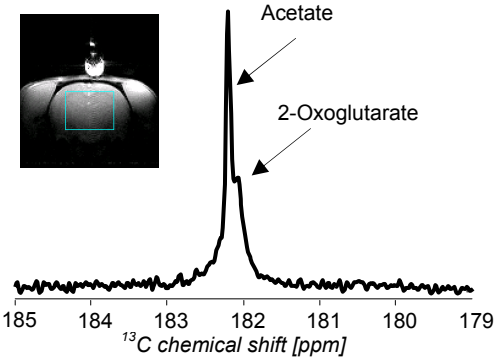


**Figure 1:** *In vivo* localized $^{13}$C spectrum measured 4 s after the completion of the infusion of hyperpolarized 1-$^{13}$C acetate (sum over 3 single scan experiments performed in 3 different animals). We selectively detected the $^{13}$C signal in the area delimited in blue on the $^{1}$H image.

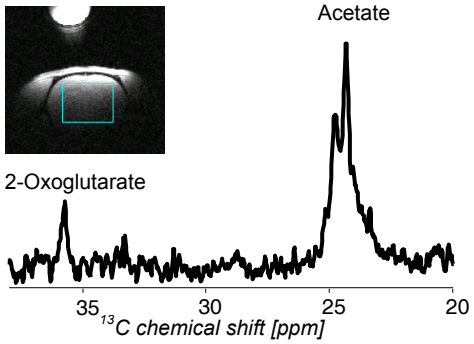


**Figure 2:** *In vivo* localized $^{13}$C spectrum measured 4 s after the completion of the infusion of 1,2-$^{13}C_2$ acetate and following carbon-carbon polarization transfer (sum over 3 single scan experiments performed in 3 different animals). We selectively detected the $^{13}$C signal in the area delimited in blue on the $^{1}$H image.

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**Reference:** [1] Comment A., van den Brandt B. *et al.,* Concepts Magn. Reson. **31B** (2007). [2] Jannin S., Comment A. *et al.*, J. Chem. Phys. **128** (2008)