# In Situ 3D MR Metabolic Imaging of Microwave-Irradiated Rodent Brain: A New Tool for Metabolomics Research

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**Introduction –** The rapid elevation in rat brain temperature achieveable with focused beam microwave irradiation (FBMI) leads to a permanent inactivation of enzymes, thereby minimizing enzyme-dependent post mortem metabolic changes. An additional characteristic of FBMI is that the tissue is close to the *in vivo* condition, both metabolically and structurally and remains so for at least 12 hours. These features create an opportunity to develop MRI and MRS on microwave-irradiated samples into a technique with a resolution, coverage and sensitivity superior to any experiment performed directly *in vivo*. Furthermore, when combined with pre-FBMI infusion of $^{13}$C-labeled substrates the technique can generate maps of metabolic fluxes, like the TCA cycle flux at an unprecedented spatial resolution.

**Methods -** All experiments were performed on a 11.74 T Magnex magnet equipped with Magnex gradients (395 mT/m in 180 µs) interfaced to a Bruker console. For all *in situ* experiments RF transception was achieved with a four-turn, 25 mm diameter solenoidal coil of 45 mm length. Heteronuclear spectral editing and broadband decoupling was performed with a 28 mm diameter Helmholtz coil tuned to the carbon-13 (125.7 MHz). Ten male Sprague-Dawley rats (206 ± 11 g) were euthanized by microwave irradiation (4.5 kW in 1.2 s). Following microwave fixation all extracranial tissues were removed after which the skull and brain were immersed in Fluorinert FC-43 to minimize magnetic susceptibility artifacts created at air-brain interfaces.

**Results –**Fig. 1A shows a localized $^{1}$H NMR spectrum from rat brain, 60 min following microwave irradiation. The spectrum closely resembles an *in vivo* $^{1}$H brain spectrum (Fig. 1B). Low levels of lactate and GABA, high levels of phosphocreatine and common ratios of NAA, total creatine and total choline are all indicative of minimal post mortem changes in metabolite levels. A $^{1}$H NMR spectrum of halothane-euthanized rat brain is shown in Fig. 1C for comparison. The $^{1}$H NMR spectra from microwave-irradiated rat brain show remarkably little change in the first 12-16 hours (data not shown). Figure 2 shows one of the metabolic applications whereby the combination of intravenous infusion of $^{13}$C-labeled substrates and microwave irradiation can be used to obtain absolute metabolic fluxes. Figure 2B shows $^{1}$H (top) and $^{1}$H-[$^{13}$C]-NMR spectra from a 1.0 µL volume extracted from a multi-slice 20 × 20 MRSI data set acquired in circa 6 hours following 2 hours of intravenous infusion of [1,6-$^{13}C_2$]-glucose and subsequent microwave irradiation. The position of the volume is indicated in the MR image shown in Fig. 2A. Despite the small voxel size of 1.0 µL, the sensitivity is adequate to detect the formation of [4-$^{13}$C]-glutamate, [4-$^{13}$C]-glutamine and other $^{13}$C-labeled products. The high spatial resolution also allows the generation of metabolic maps as shown in Figs. 2E and F for [4-$^{13}$C]-glutamate and [4-$^{13}$C]-glutamine. Alternatively spectra from multiple voxels can be co-added to obtain high S/N data as shown in Fig. 2D for $^{1}$H and $^{1}$H-[$^{13}$C]-NMR spectra of the hippocampus as indicated in Fig. 2C. By repeating the studies shown in Fig. 2 for different infusion times (on different animals), the full dynamic turnover time course can be obtained at a spatial resolution and sensitivity that is several times higher than direct *in vivo* detection.

**Conclusions –** FBMI in combination with *in situ* MRI and MRS is a novel method to obtain high-resolution, high-sensitivity structural and metabolic information from rodent brain with 3D brain coverage. Intravenous infusion of $^{13}$C-labeled substrates can be used to provide cell-specific dynamic rate information on mitochondrial energetics and neuron-glia substrate trafficking at unprecedented spatial resolution. The ability to anatomically register the metabolic images with *in situ* immunohistochemistry of expressed proteins, phosphoproteins, and neuropeptides, which are preserved by FBMI and readily measured after the metabolic image is obtained, will add new dimensions to the versatility and information rich potential of this new metabolomics approach.

This research was supported by NIH grant R21/R33-CA118503 and R01-DK027121.

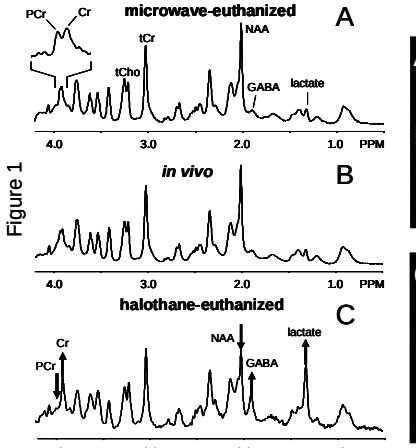


Figure 1

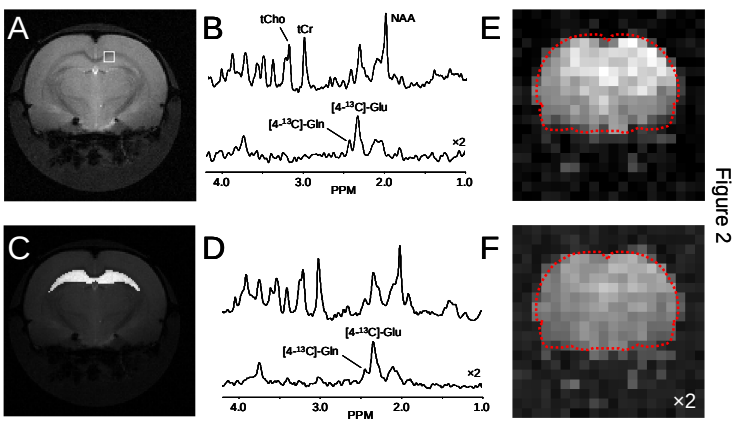


Figure 2