Characterization of Metabolism in Rat Olfactory Bulb by *In Vivo* ^{1}H and ^{1}H-[^{13}C] NMR

G. M. Chowdhury[1], K. L. Behar[1], D. L. Rothman[1], and R. A. de Graaf[1]

[1]MRRC, Yale University, New Haven, CT, United States

INTRODUCTION: MRI/S at high magnetic fields have generated new insights into brain structure, function, and metabolism. Although extensive data related to high resolution MRS of rodent cortex are available, MRS studies on the olfactory bulb (OB) are limited due to the small size of this structure and its proximity to bone and nasal cavities, which distorts field homogeneity leading to losses in sensitivity and resolution. In the present study, we measured metabolism in the rat OB at 9.4 T using a combination of ^{1}H MRS (metabolite quantification), J-edited ^{1}H MRS (GABA quantification), and ^{1}H-[^{13}C] MRS during infusions of [1,6-$^{13}C_2$]glucose (neuronal metabolism) and [2-^{13}C]acetate (astroglial metabolism).

METHOD: SD rats (160-190g, fasted overnight) were anesthetized, tracheotomized, and ventilated (69% N_2O/30% O_2/1% halothane), immobilized, and femoral artery and veins cannulated. Rats received continuous infusions of either [1,6-$^{13}C_2$]glucose or [2-^{13}C]acetate intravenously for 2.5 hrs. *In vivo* experiments were performed at 9.4 T (Bruker Instruments) using a surface coil positioned over the OB. Shimming was optimized using FASTMAP, typically resulting in water line widths of 14-16 Hz over the 2.5x2.5x2.5 mm^3 = 15.6 uL volume-of-interest. GABA detection was achieved through J-difference editing (1). ^{1}H and ^{1}H-[^{13}C] MRS (2) spectra were acquired using a LASER-based localization method with adiabatic broadband decoupling during acquisition (TR/TE = 4000/12.5 ms). Blood samples were taken periodically for the analysis of plasma glucose concentration and percent ^{13}C enrichment. *In vivo* peak intensities were quantified using LCModel (2).

RESULTS AND DISCUSSION: Fig. 1A shows the spectroscopic volume-of-interest (15.6 uL) in the rat OB superposed on a MRI. Figs. 1B/C show representative ^{1}H MR (TE = 68 ms) and GABA-edited ^{1}H MR spectra, the latter revealing the H2 and H4 methylene protons at 2.29 and 3.0 ppm, respectively. Fig. 1D shows a ^{1}H MR spectrum (TE = 12.5 ms) 120 mins following the onset of [2-^{13}C]acetate infusion, while Figs. 1E/F show ^{1}H-[^{13}C] NMR difference spectra following 120 mins of (E) [2-^{13}C]acetate and (F) [1,6-$^{13}C_2$]glucose infusion, respectively. The excellent spectral resolution allows the visual separation of [4-^{13}C]-glutamate and [4-^{13}C]-glutamine, whereas many other resonances can be quantified through LCmodel. Furthermore, despite the small volume, GABA is readily detected. Here we introduce high resolution MRS of rat OB with excellent spectral resolution (metabolite linewidth, tCr : 5-6 Hz, NAA : 4-5.5 Hz). Despite the small size of the rat OB and the corresponding NMR volume (15.6 uL), the combination of a high magnetic field, small surface coil (14 mm OD) and adiabatic pulses allows the detection of ^{13}C level turnover in glutamate and glutamine (Glu + Gln) following ^{13}C glucose or ^{13}C acetate infusions. Following LCmodel quantification, these ^{13}C turnover measurements can be converted to metabolic flux rates of important metabolic pathways, like the TCA cycle. The high sensitivity also allowed the reproducible observation of absolute GABA concentration (4.17 ± 0.51 mM (mean ± SD)). The presented results will lead to a complete metabolic model of metabolism in rat OB and will form the basis for extended studies in mice, as well as during regional functional activation and in models of disease.

ACKNOWLEDGEMENTS: This study was supported by NIH grant DK27121.

REFERENCES : 1. Rothman et al PNAS (1993) 90:5662, 2. de Graaf et al (2003) MRM 49:37.