Title: Measurements of Glutamate/GABA/Glutamine Cycling in the Mouse Brain

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Glutamate is the major excitatory neurotransmitter in the mammalian CNS and serves as precursor to γ-aminobutyrate (GABA) the major inhibitory neurotransmitter. Glutamate and GABA released at nerve terminals are taken up and metabolized in astroglia to glutamine, which is released and transported into neurons for neurotransmitter re-synthesis, completing the glutamate-GABA/glutamine cycle. ^{13}C labeling combined with NMR permits the assessment of the neurotransmitter cycle fluxes when an appropriate metabolic model is used to extract the kinetic information. MRS is uniquely suited as the only currently available method to assess neurotransmitter cycling *in vivo*.

Over the last decade the development of the tools to measure glutamate and GABA neurotransmitter cycling *in vivo* have come from studies of rodents, primarily the rat. The use of mice pose considerable challenges for ^{13}C labeling studies *in vivo* with MRS; their small size, delicate physiology, propensity to movement, and limited vascular access add to the difficulties in shimming and sensitivity. Thus far *in vivo* MRS studies of the mouse brain have been limited to the measurement of metabolite concentrations in ^{1}H MRS spectra [1] while ^{13}C labeling has been conducted mainly *ex vivo*. Measurements of altered glutamate and glutamine levels (or their sum, Glx) in ^{1}H MRS spectra *in vivo* have been used to infer potential effects on glutamate/glutamine cycling in studies of pharmacologic and transgenic mouse models of neurological disease [2-4].

Measurements of neuron-glial trafficking of glutamate, GABA and glutamine in mice have mainly involved intraperitoneal infusions of the ^{13}C labeled precursor substrates, glucose and acetate. In these studies the ^{13}C labeled substrate was infused for a limited number of time points (one or two) followed by euthanasia and measurement of the tissue extract by high-resolution ^{13}C or ^{1}H-[^{13}C] MRS [5-8]. Characterization of the complete time course using intravenous infusion with several infusion times has expanded the potential kinetic information available [9]. However, to date the use of metabolic modeling has not been reported in ^{13}C labeling studies of mouse brain, but this is likely to change in the near future as metabolomic approaches, e.g., 'fluxomics', assume greater importance in studies of genetically altered mice. Recently, dynamic time courses of glutamate and glutamine ^{13}C labeling were measured *in vivo* during an intravenous infusion of [1,6-$^{13}C_2$]glucose with ^{13}C MRS at 7 Tesla from a 175 μL volume of the adult anesthetized mouse brain [10]. Further improvements in detection sensitivity and spectral resolution can be expected with indirect ^{1}H detection and emerging shimming methods.

The objectives of this lecture will be to provide an overview of the methods currently employed to measure glutamate/GABA/glutamine neuron-astrocyte trafficking in mouse brain *ex vivo* and *in vivo* and the challenges involved. Examples will be drawn from the literature and from the author's laboratory.

References:

[1] Tkác I, Henry PG, Andersen P, Keene CD, Low WC, Gruetter R. Highly resolved in vivo ^{1}H NMR spectroscopy of the mouse brain at 9.4 T. Magn Reson Med. 2004 52(3):478-84.

[2] Jenkins BG, Klivenyi P, Kustermann E, Andreassen OA, Ferrante RJ, Rosen BR, Beal MF. Nonlinear decrease over time in N-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. J Neurochem. 2000 74(5):2108-19.

[3] Moussa CE-H, Rusnal M, Hailu A, Sidhu A, Fricke ST. Alterations of striatal glutamate transmission in rotenone-treated mice: MRI/MRS in vivo studies. Exp Neurol. 2007 209:224-233.
[4] Tkac I, Dubinsky JM, Keene CD, Gruetter R, Low WC. Neurochemical changes in Huntington R6/2 mouse striatum detected by in vivo ^{1}H NMR spectroscopy. J Neurochem. 2007 100(5):1397-406.
[5] Hassel B, Sonnewald U, Fonnum F. Glial-neuronal interactions as studied by cerebral metabsolism of [2-^{13}C]acetate and [1-^{13}C]glucose: an ex vivo ^{13}C NMR spectroscopic study. J Neurochem. 1995 64:2773-82.
[6] Kondziella D, Hammer J, Sletvold O, Sonnewald U. The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: glial-neuronal metabsolic interactions. Neurochem Internatl 2003 43:629-37.
[7] Alexander JJ, Zwingmann C, Quigg R. MRL/lpr mice have alterations in brain metabsolism as shown with [^{1}H-^{13}C] NMR spectroscopy. Neurochem Internatl. 2005 47:143-151.
[8] Rodrigues TB, Granado N, Ortiz O, Cerdan S, Moratalla R. Metabolic interactions between glutamatergic and dopaminergic neurotransmitter systems are mediated through D_1 dopamine receptors. J Neurosci Res. 2007 85:3284-93.
[9] Chowdhury GM, Gupta M, Gibson KM, Patel AB, Behar KL. Altered cerebral glucose and acetate metabolism in succinic semialdehyde dehydrogenase-deficient mice: evidence for glial dysfunction and reduced glutamate/glutamine cycling.J Neurochem. 2007 103(5):2077-91.
[10] Nabuurs CI, Klomp DW, Veltien A, Kan HE, Heerschap A. Localized sensitivity enhanced in vivo ^{13}C MRS to detect glucose metabolism in the mouse brain. Magn Reson Med. 2008 59(3):626-30.