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. 13C 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 13C 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 1H MRS spectra [1] while 13C labeling has been conducted mainly ex vivo. Measurements of altered glutamate and glutamine levels (or their sum, Glx) in 1H 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 13C labeled precursor substrates, glucose and acetate. In these studies the 13C 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 13C or 1H-[13C] 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 13C 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 13C labeling were measured in vivo during an intravenous infusion of [1,6-13C2]glucose with 13C 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 1H 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:


