Imaging glutamine synthesis rates in the hyperammonemic rat brain

C. Cudalbu[1], V. Mlynárik[1], B. Lanz[1], H. Frenkel[1], N. Costers[1,2], and R. Gruetter[1,3]

[1]Laboratory for Functional and Metabolic Imaging (LIFMET), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [2]Katholieke Universiteit Leuven, Leuven, Belgium, [3]Departments of Radiology, Universities of Lausanne and Geneva, Switzerland

Introduction: In hyperammonemic conditions, glutamine is generated in astrocytes from ammonia and glutamate in a reaction catalyzed by glutamine synthetase. Recent in vitro observations suggested that during hyperammonemia, alterations in brain metabolites other than glutamine can occur (i.e. glutamate, myo-inositol, taurine, lactate) (1,2). In addition, it has been reported in vitro that alterations in ammonia, glutamine and glutamine synthetase may be different between cortex and the rest of the brain (3). These findings are encouraging for studying brain regions separately during hyperammonemia. The in vivo spatial distribution of brain metabolites can be measured using short-echo-time (TE) proton spectroscopic imaging (SI). Therefore, the aim of the study was to image for the first time the in vivo effect of hyperammonemia per se on 12 brain metabolites (i.e. Gln, Glu, tCr, tCho, Ins, Tau, Lac, NAA+NAAG, Lac, etc) in different brain regions using short TE ^{1}H SI . In addition, we also imaged the net glutamine synthesis rates during hyperammonemia in the rat brain.

Experimental: All the experiments were performed on a 9.4T system (Varian/Magnex Scientific) using a home-built 14 mm diameter quadrature ^{1}H coil as a transceiver. Five SD rats (300-350g) were fasted overnight. The femoral artery and vein were catheterized for blood sampling as well as ammonium chloride and α-chloralose infusion. Ammonium chloride was infused continuously at a stable rate (4.5mmol/h/kg) for up to 6h as described in (4,5). Metabolic maps were obtained using an excitation by a SPECIAL spectroscopy sequence (TR/TE = 2500/2.8 ms) followed by phase encoding in the coronal plane (6). Reference water signals were measured using the same protocol without water suppression and with TR=1500 ms. Field homogeneity over the excited region was adjusted by FASTMAP (7). The region of interest used for constructing metabolic maps consisted of 5×10 voxels with a nominal voxel size of 0.75×0.75×2 mm^3 (1.1 µL). The *k*-space data were filtered with an optimized Hanning function in two spatial domains. Concentrations of metabolites corrected for different T_1 of metabolites and water were calculated by LCModel (8) for individual voxels using water as a reference. The linear fit of the time-evolution of the Gln gives the net glutamine synthesis flux as well as the initial Gln concentration, assuming a negligible Gln efflux (4).

Results: Figure 1 displays the overall quality of the signals acquired in a voxel of 1.1 µL using the SPECIAL spectroscopy sequence followed by phase encoding in the coronal plane. The maps of Gln, Glu, Ins, NAA+NAAG and Lac acquired at different time points during ammonia infusion and superimposed on the anatomical T_{2w} images are displayed in Fig. 2. The VOI included somatosensory and retrosplenial cortex, hippocampus and a small amount of thalamus. The increase in the Gln pool at different time points during infusion was apparent from the maps. Before the infusion, the Gln map did not show any substantial variability in different brain regions (3.2±0.4 mmol/kg$_{ww}$ in the cortex and 3.2±0.6 mmol/kg$_{ww}$ in the hippocampus). However, during infusion the Gln concentration increased more in cortex than in hippocampus (2.5h of infusion 9.3±2.5 mmol/kg$_{ww}$ in the cortex and 6.7±1.5 mmol/kg$_{ww}$ in the hippocampus with p=0.01; 5.5hh of infusion 16.2± 2.7 mmol/kg$_{ww}$ in the cortex and 11.5±1.2 mmol/kg$_{ww}$ in the hippocampus with p=0.03). The maps of Glu, Ins, Lac and NAA+NAAG did not show any visible difference between and after ammonia infusion and retained their specific spatial distribution patterns: a higher concentration of Glu (12-14 mmol/kg) in cortex, a higher concentration of Ins in hippocampus (above 8 mmol/kg) and an increased NAA+NAAG concentration in cortex (above 12 mmol/kg). Similarly, no substantial difference was observed in maps of the other brain metabolites (data not shown). The time courses of Gln concentrations in cortex and hippocampus were highly reproducible in all five rats and are shown in Fig 3a. From the linear fit of the time-evolution of Gln we obtained a net glutamine synthesis rate of 0.039±0.007µmol/min/g in the cortex and 0.024±0.007µmol/min/g in the hippocampus. Figure 3b shows the net glutamine synthesis rates imaged over the VOI and superimposed on the anatomical T_2w images. The net glutamine synthesis rate was significantly higher in the cortex than in the hippocampus (p=0.05).

Discussion: In the present study, high resolution metabolic maps of rat brain in the coronal plane enabled to observe for the first time the in vivo spatial distribution of 12 metabolites in various brain structures under hyperammonemia. Contrary to other models of hyperammonemia associated with experimental acute liver failures (1,2), no changes in spatial distribution of metabolites were observed except for Gln. The Gln increase was higher in cortex than in hippocampus, which is in agreement with previous in vitro studies (3). In addition, we imaged for the first time the net glutamine synthesis rates in vivo in the rat brain, and showed that the net glutamine synthesis rates were significantly higher in the cortex than in the hippocampus.

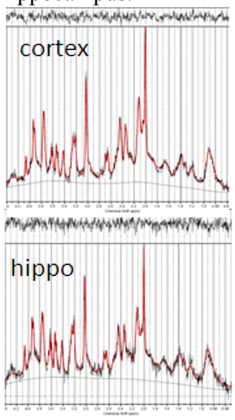
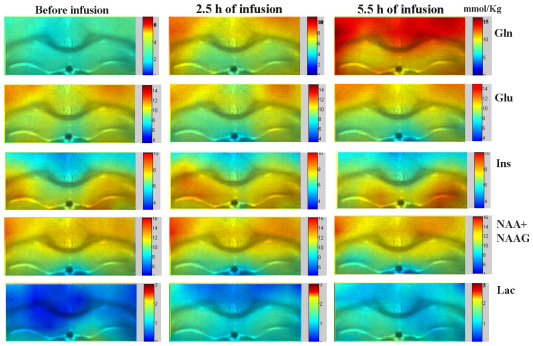


Fig. 1: Representative spectra of cortex and hippocampus acquired from 1 voxel (1.1 µL) in the rat brain at 5.5h of ammonia infusion. Note the Gln difference between these two spectra.



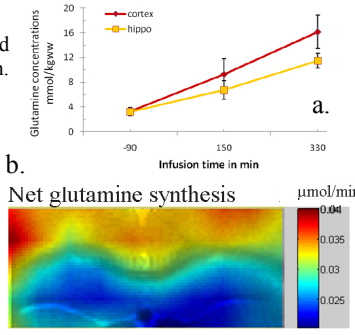


Fig.3: a) Gln conc (mean ± SD of 4 voxels) in cortex and hippocampus at different time points during ammonia infusion.
b) Net glutamine synthesis rates imaged over the VOI and superimposed on the anatomical T_{2w} images.

Fig. 2: Metabolic maps of Gln, Glu, Ins, NAA+NAAG, Lac superimposed on the anatomical T_{2w} images and acquired at different time points during ammonia infusion

Acknowledgments: Supported by CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations ;EU Grant No. MRTN-CT-2006-035801; and SNF grant No 3100A0-116220. **References:** [1] Zwingmann C, J Neurosci Res. 85:3429, 2007. [2] Rao KV et al, Metab Brain Dis. 16:67, 2001. [3] Dejong CH et al, J Neurochem, 59:1071, 1992. [4] Kanamori K et al., Biochem J 1993 ;293 :461. [5] Cudalbu C et al, Proc Intl Soc Mag Reson Med 17:1578, 2009. [6] Mlynárik V *et al.* Magn Reson Med. 59:52, 2008. [7]. Gruetter R. Magn Reson Med. 29:804, 1993. [8] Provencher SW. Magn Reson Med. 30:672, 1993.