# In vivo and longitudinal assessment of brain metabolism in Hepatic Encephalopathy using 1H MRS

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**Introduction:** Hepatic encephalopathy (HE) is a serious neurological complication of acute or chronic liver disease (ALF, CLD), which is characterized by an array of cognitive and motor deficits leading to altered mental status, coma and death [1-3]. It is generally accepted that ammonia is a neurotoxin implicated in the pathogenesis of HE and that astrocytes represent the principal target due to the unique astrocytic expression of glutamine synthetase, which synthesizes glutamine (Gln) from glutamate (Glu) and ammonia [1,3]. The pathogenic mechanisms leading to astrocytes swelling and brain edema are complex, and sometimes controversial (amino acids disturbances; alterations in neurotransmission; cerebral energy deficit; alteration of oxidative stress, mitochondrial permeability transition; impairment of axonal and dendritic growth during brain development; signaling transduction pathways; alterations in channels and transporters activity) [1,3,4].

Previous studies on CLD in humans were performed at low Bo and only few metabolites were reported (increase of Gln+Glu, and sometimes decrease in brain osmolytes (Ins, tCho)). Bile duct ligation (BDL) in rats is a frequently used animal model in CLD [5-6]. From our knowledge, only one previous *in vivo* study imaged longitudinally brain metabolism in a BDL rat model. They reported that the continuous increase of Gln can be compensated by a continuous decrease of other osmolytes with minimal brain edema and with no regional difference in Gln increase [6].$^{1}$H MRS performed at high magnetic field and short echo time (TE) allows the measurement of about 20 brain metabolites. In this context, the aim of the present study was to asses *in vivo* and longitudinally several pathogenic mechanisms involved in HE (i.e. osmoregulation, neurotransmitter metabolism, oxidative stress, energy dysfunction, cell swelling) using $^{1}$H MRS and histology.

**Methods:**

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. Eight Wistar rats (300-350g) were bile duct ligated (BDL) [7] and scanned longitudinaly before and after BDL for 8 week (once per week). The ultra-short-echo time SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 160 scans) [8] was used to localize a VOI of 2×2.8×2mm$^{3}$ in the hippocampus. First and second order shims were adjusted using FASTMAP (linewidth of 9-12Hz). Concentrations of metabolites were calculated by LCModel using water as internal reference. Histological assessment of brain tissue was performed 8 weeks after ligation by immunofluorescence against the astrocytic marker glial fibrillary acidic protein (GFAP).

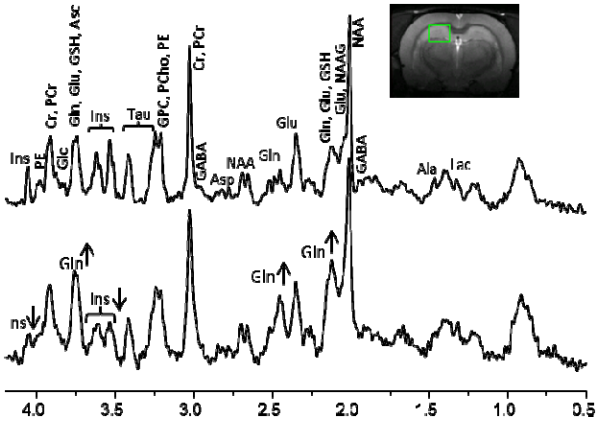


Fig. 1: *In vivo* brain spectra acquired in a rat model of CLD at 9.4T. From top to bottom spectrum acquired before BDL and 8 weeks after BDL.

**Results and Discussion:**

Fig. 1 shows typical spectra acquired in the hippocampus of BDL rats before and 8 weeks after ligation. In the present study we focused on the hippocampus due to its role in memory (a neurologic symptom in HE). $^{1}$H spectra exhibited excellent SNR (between 22 to 25 for 10 min of acquisition) allowing easy separation of Gln from Glu. Notable differences in metabolite signals were apparent already from the spectra (i.e. increase Gln, decrease Ins, Tau).

The neurochemical profile (mean ± SEM) measured before and 4, 6, 8 weeks after BDL is shown in Fig 2A. Gln started to increase already at 1 week after BDL and continued to increase over time (more than 200% at 8 weeks). As a compensatory effect for the osmotic imbalance created by the Gln increase, other brain osmolytes started to decrease: Ins being the first one (~ 35%), followed by Tau and tCho with ~ 20 and 40%, respectively. Cr showed a trend of decrease over time reaching ~20% at 8 weeks. Cr is a metabolite involved in energy metabolism but recently its involvement in osmoregulation and neuroprotection was reported [3]. Among the brain neurotransmitters, Glu, Asp and GABA were decrease over time (~15-30%, Fig 2A). We also noticed a trend of decrease of Asc and GSH (~15%) which are considered to be important brain antioxidants. This might be due to the induction of oxidative stress by ammonia [1,3-5]. Even though the sum of the main brain osmolytes (Gln, Ins, tCho, Tau) was constant over time, meaning that to compensate for the Gln increase these metabolites decreased proportionately, astrocytes swelling was still noticeable as shown by GFAP (Fig 2B) and in agreement with brain edema measured by DTI [6].

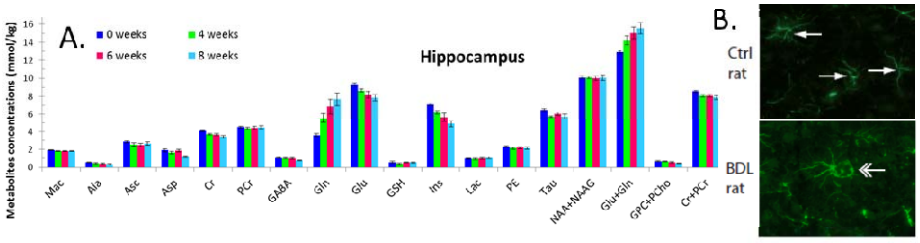


Fig. 2:A) Neurochemical profile (mean ± SEM) in the hippocampus of BDL rats before (0 week), and 4, 6 and 8 weeks after ligation. For visibility reasons only these time points are shown; B) Astrocytes labeled by immunofluorescence against the GFAP protein; 16 µm thick cryosections of brain of control and BDL adult rats. Typical swelled astrocytes (double arrows), as compared to the normal ones (simple arrows) illustrated for the control rats.

**In conclusion**, we characterized for the first time the *in vivo* and longitudinal progression of HE in a model of CLD using $^{1}$H MRS and histology. We were able to monitor changes in the brain osmolytes, neurotransmitters, antioxidants and cell swelling, something never previously assessed in such details *in vivo* in CLD. The use of *in vivo* longitudinal MRS and MRI measurements is mandatory promising tool for the assessment of HE progression in liver disease, especially prior to the onset of irreversible symptoms.

**References** [1] Norenberg MD et al, Metab Brain Dis 2009; [2] Caudle SE et al, J Pediatr. 2010 ; [3] Braissant O, Mol Genet Metab. 2010; [4] Butterworth RF, J Inher Metab Dis 21, 1998 ; [5] Bosoi C et al, Free radical Biol&Med 52, 2012; [6] Cudalbu C et al, Proc Intl Soc Mag Reson Med 2012; [7] Biecker E et al, J Pharmacol Exp Ther. 2005; [8] Mlynárik V et al. Magn Reson Med. 2006.

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