# Short-echo-time 1H MRS studies of alcohol exposure on the mouse brain over-expressing glutamate dehydrogenase

**W-T. Wang[1], S-P. Lee[2], E. Michaelis[3], and I-Y. Choi[1,4]**

[1]Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS, United States, [2]1Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS, United States, [3]Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS, United States, [4]Department of Neurology, Molecular & Integrative Physiology, Hoglund Brain Imaging Center, Kasnas City, KS, United States

## INTRODUCTION

Chronic alcohol abuse leads to cell injury in various tissues, including liver, heart, and brain. In the brain as well as other tissues, ethanol (EtOH) is metabolized to oxidizing species, increases generation of reactive oxygen species and decreases glutathione (GSH) levels. In addition, clinical studies reported that a threshold for experiencing CNS effects of ethanol is a major predictor of vulnerability to alcoholism. Several laboratories have demonstrated that glutamate is a primary target for the actions of ethanol. Glud1 is an enzyme that converts glutamate to α-ketoglutarate, and vice versa, possible rate-limiting steps in the biosynthesis of glutamate (Glu). Over-expressing Glud1 leads to excessive extraceullular accumulation of Glu, leading to neuronal oxidative stress and excessive reactive oxygen species formation.

Brain changes in aging exacerbate the effects of chronic EtOH intake on neurons. In addition, Glu release to the extracellular environment of neurons increases with advancing age. Therefore, we investigate a possible relationship between EtOH- and Glu-induced neuronal injury and aging using an animal model of excess Glu in aging using *in vivo* high resolution $^1$H MRS at 9.4 T.

## METHODS

To generate hyperglutamatergic (Glud1 over-expressing) mice, the Glud1 transgene was introduced under the control of a neuron-specific promoter and expressed only in neurons of the CNS. The wild type of mouse Glud1 cDNA was introduced into the genome of C57B16/SJL hybrid mice, which was subsequently genotyped. Then mice bearing the Glud1 transgene were selected and back-crossed to C57BI/6 for 5 generations. For each of the 5 generations, offspring from previous generation was genotyped and those having transgenes was back-crossed.

The $^1$H MRS data were measured from Glud1 tg (n = 6) and wt (n = 6) mice on a Varian 9.4 T system. MRS measurements were performed at age of 9 months before initiating EtOH treatment. Then EtOH treatments were performed on all mice using the Lieber-DeCarli diet, which increases EtOH intake from 33.3% to 100% during the first week of the treatment. Two weeks after the EtOH treatment, MRS data were acquired from both groups of mice. During the experiments, the animals were anesthetized (air:oxygen = 1:1 with 1-2% isoflurane) and their core temperatures were maintained at 37°C. Field homogeneity was adjusted using FASTMAP [1]. The spin echo, full intensity acquired localized (SPECIAL) spectroscopy (TE = 3 ms, TR = 5 s, TM = 20 ms) [2] was used to acquire $^1$H MRS data from voxels (~5 µl) in the hippocampus and striatum. The voxels were localized using $T_2$-weighted MR images acquired with fast spin echo multi-slice sequence (ETL = 16, echo spacing = 11 ms, TE/TR = 11/4000 ms, matrix = 256x256, FOV = 25.6 x 25.6 mm, slice thickness = 0.5 mm, and NT = 2). The acquired spectra were corrected for phase and frequency drift based on Cr+PCr signal at 3.03 ppm prior to the LCModel [3] analysis.

## RESULTS AND DISCUSSION

In this study, the water linewidth was in the range of 12 – 16 Hz, resulting in good spectral resolution (Fig. 1). The levels of nearly all neurochemicals in both brain regions of Glud1 mice were the same as those of wt mice. Interestingly, Glu levels were not significantly different between two groups, in either the hippocampus or striatum. These results would indicate that over-expression of Glud1 may lead to increases in Glu release upon depolarization of neurons but not tissue Glu levels. The only exceptions to this near identity between neurochemical measures in Glud1 and wt mouse brain were the ratios of ascorbate/total Cr (Cr+PCr), which were significantly lower in the striatum of Tg vs. wt mice (p=0.05, n = 6 mice per group). The lower ascorbate levels in Tg mice might be related to the activation of the Glu/ascorbate heteroexchange transport that is specific for striatum.

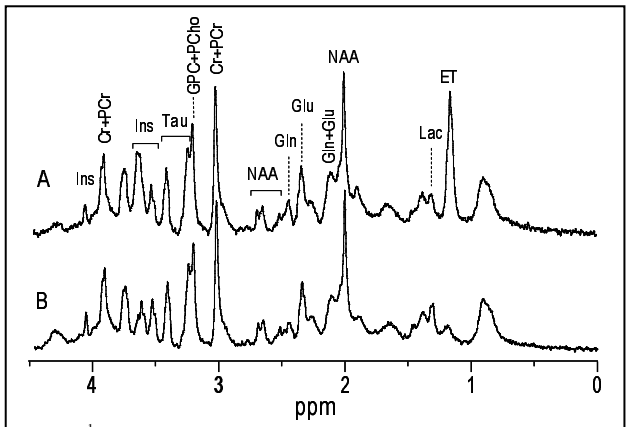


**Fig. 1.** $^1$H MR spectra acquired from hippocampus of the Glud1 tg mouse brain *in vivo* before (B) and after (A) ethanol diet. (6.3 µl, TE/TM/TR = 2/20/4000 ms)

A 2-wk period of EtOH treatment resulted in significant increases in the ratios of Glu/tCr and Gln/tCr in both hippocampus and striatum of both Glud1 and wt mice (Fig. 2). The 2-wk exposure to EtOH also caused a selectively greater increase in GABA levels in the hippocampus of Glud1 than those in wt mice (p=0.02) and exposure to EtOH for 2 wks caused a greater decrease in GSH levels in the hippocampus of Glud1 than wt mice (p = 0.02). The changes from baseline in the levels of GABA and GSH in the hippocampus of Tg mice by 2 wks of EtOH exposure were statistically significant to the same level (p = 0.02).

The decreases in GSH levels in the hippocampus of Glud1 mice may be particularly important as the hippocampus is a region of high glutamatergic activity and thus, possibly, of elevated oxidative stress. The data might indicate a further aggravation of neuronal stress in the hyper-glutamate mice following daily intake of EtOH. Such cellular stress did not lead to significant signs of neuronal injury, at least not during the 2 wk period of exposure to EtOH as shown in marginal decreases of NAA levels (p = 0.08) following 2 wks of EtOH treatment.

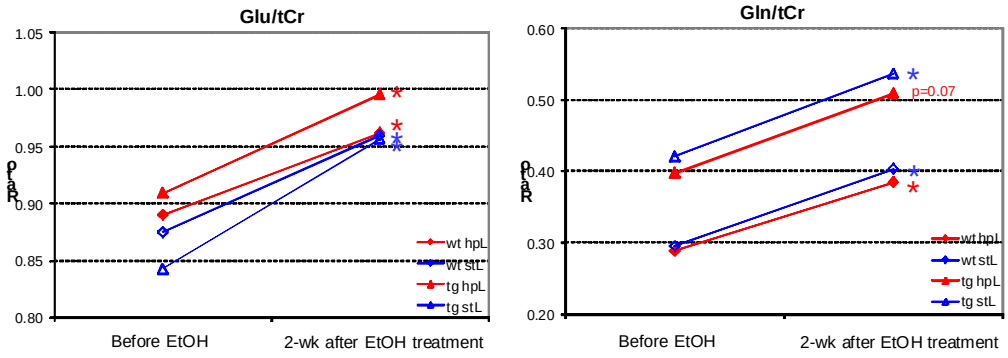


**Fig. 2.** Changes in Glu/total Cr (left) and Gln/total Cr ratios (right) in hippocampus and Str following 2-wks of EtOH treatment. * indicates p<0.05 using paired *t*-test (n = 6 mice per group). Red: H; Blue: Str; Triangles: Tg.

## REFERENCES

[1] Gruetter et al., *Magn Reson Med* 29, 804 (1993). [2] Mlynarik et al., Magn Reson Med 56:965-970 (2006) [3] Provencher, *Magn Reson Med* 30, 672 (1993).
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