Effect of ethanol treatment on axonal transport rates in hyper-glutamatergic transgenic mice

J. Kim[1], I-Y. Choi[1,2], E. K. Michaelis[3], and S-P. Lee[1,4]

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

Introduction

Alcoholism is one of the major health problems in our population. Vulnerability to the addictive properties of alcohol and the risk of developing alcoholism appear to be influenced by both environmental variables and complex genetic variations within the population. It has been suggested that that the glutamate system, the major excitatory neurotransmitter system in the brain, is a primary target of the actions of ethanol [1]. We hypothesize that hyper glutamergic status by overexpression of glutamate dehydrogenase (Glud1) transgene leads to selective hypersensitivity/vulnerability of the brain regions to the actions of ethanol, and the chronic ethanol treatment leads to morphological and physiological changes. Up to date, there has been no clear demonstration of neurochemical and physiological alterations in the living brain tissues of animals to examine the effects of high glutamate activity and age on the process of selective alcohol effects on neurons. We aim to investigate the possible changes in Glud1 transgenic mice (GLUD-tg) with ethanol treatment (ETOH) using manganese-enhanced MRI (MEMRI). Manganese (Mn^{2+}) can enter excitable cells through voltage-gated calcium channels and can be transported by microtubles in axons toward the projecting neurons and will differently accumulate in different areas of the brain. We used manganese-enhanced MRI (MEMRI) to measure fast axonal transport rate utilizing its property of shortening longitudinal relaxation rate, T_1, over time after administration of $MnCl_2$ solution.

Methods

Total seven (4 GLUD-tg, 3 age-matched wild-type) mice were studied. Animals were scanned at the age of 9 months for baseline axonal transport. After baseline scanning, all animals received a liquid diet in which initially 35% of the calories are derived from ethanol and gradually increased to 65% of calories from ethanol over a period of 1 week. Second MR scans were performed after two weeks of ethanol diet to measure the effect of ethanol intake in axonal transport. All MR studies were performed at 9.4 T Varian system equipped with a 12 cm gradient coil (40 G/cm, 250 μs) and interfaced to a Varian INOVA console (Varian Inc., CA). A 6-cm diameter Helmholtz volume transmit coil and a 7-mm diameter surface receive coil were used for MR imaging. MR data were acquired before and 1 and 6 h after unilateral and intranasal administration of $MnCl_2$ solution (160 mM, 4 µl) in three separate MRI sessions. Animals were stimulated using amyl acetate for 15 min to enhance uptake of Mn^{2+} in the olfactory neurons. Animals were anesthetized initially with 4% isoflurane mixed with 4 L/min O_2 and 1L/min air for induction and 1-1.5% isoflurane for maintenance. Body temperature was maintained at 37˚C using a circulating hot water pad and a temperature controller (Cole-Palmer, NY).. Respiration, heart rate, and blood oxygen level were also monitored via respiration pillow and mouse pulse oximeter (SA Instruments, NY; STARR Life Scicences, OH). T_1 maps were measured using a modified Look-Locker multislice sequence to acquire multiple phase encodings per inversion (TR/TE = 4/2 ms, FOV = 2 cm, matrix = 128 x 128, thk = 0.5 mm, flip angle = 20°, 22 inversion times, acquisition time = 8.5 min). B_1 maps were measured to correct the effect of flip angle variations in T1 mapping using a B1 mapping sequence [2] (TR/TE = 200/3.7 ms, matrix = 128 x 128, nt = 4, thk = 0.5 mm). High resolution T_1-weighted spin-echo data were also acquired (TR/TE = 600/10 ms, nt =2, matrix = 256 x 256, thk = 0.5 mm, scan time = 5 min). T1 and B1 maps were generated using software written in IDL (RSI, CO). Bulk axonal transport rate of olfactory neurons was calculated by linear regression of the time course of R_1 in OB.

Results and Discussion

Figure 1 shows T1 maps at olfactory bulb (OB) at 6 h post $MnCl_2$ administration before and post-2week ETOH treatment. Images clearly show unilateral $MnCl_2$ accumulation in OB over 6 h. The differential effect of the ethanol treatment on the axonal transport was visible (lowering T1 changes in GLUD-tg mice and increasing T1 changes in wt mice). Fig 2 shows quantified axonal transport rates in GLUD-tg and wt mice before and after ETOH treatment. The calculated baseline axonal transport rate for Glud1 mice (n=3) was 114% higher (P = 0.004), than that of wt mice (n=4). We do not know what might account for these intriguing differences in rates of transport from nasal epithelium to olfactory bulb.

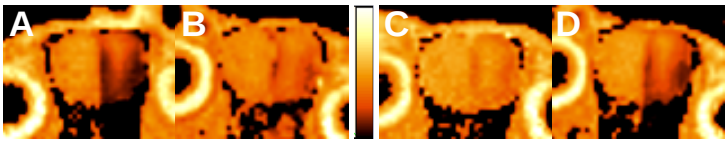

Fig. 1 T1 maps of olfactory bulbs of Glud tg (a, b) and wt (c, d) animals before (a, c) and after (b, d) 2 weeks of ethanol diet. Color scale bar indicates the range of T1 values of 0 – 2.3 s.

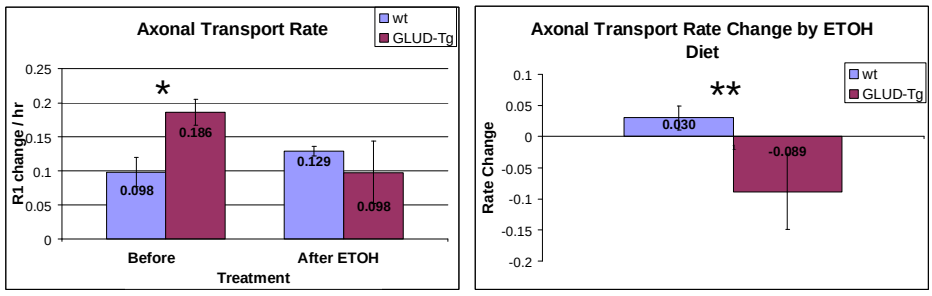

Fig. 2 Axonal transport rate before and after ethanol treatment in Glud1 tg and wt mice. *:p = 0.006, **: p = 0.03 (n=4 Glud tg, n=3 wt).

A dramatic change in transport rates in both GLUD-Tg and wt mice occurred following 2-wks of exposure to EtOH in the diet. EtOH exposure had opposite effects on Mn transport in GLUD-tg vs. wt mice. While the rates decreased by 48% in Tg mice (P = 0.04, n = 3), those of wt increased by 53% (P = 0.02, n = 4). The opposite responses to EtOH in wt and Tg mice led to a reversal from baseline transport rates being higher in Tg mice to the post-EtOH treatment rates being 27% lower in Tg than in wt mice. Currently we do not know the mechanism of the observed differential changes axonal transport following EtOH and more studies are needed to elucidate the mechanism behind the observation.

References

[1] Chen et al. J Neurochem 69: 1559-1569 (1997) [2] Pan et al, MRM 40:363-369 (1998); Supported by a grant from KCALSI.