Binge Ethanol Induced Structural and Neurochemical Changes in the Rat Brain Detectable at 3T

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Introduction

Human and animal studies indicate that chronic alcohol intoxication resulting in dependence damages the brain. That only a few days of intoxication can also lead to brain insult thus far has only been demonstrated in vitro in animals[1]. Binge drinking in humans is associated with high blood alcohol levels (BALs, e.g., over 500mg/dl)[2] that have been modeled in rodents with intragastric injections of 15% ethanol (EtOH) every 6h for 4d. In rodents, this intoxication protocol leads to neuronal damage in corticolimbic areas including perirhinal and entorhinal cortices and hippocampal dentate gyrus[1]. Our current study was conducted to provide in vivo structural and biochemical magnetic resonance (MR) evidence in rodents for brain damage resulting from binge EtOH exposure and potential recovery with abstinence.

Methods

The study group consisted of 19 wild-type, male, Wistar rats weighing 264.04±3.99g at the baseline scan. After the baseline scan, 11 rats were assigned to the EtOH group and received a loading dose of 5g/kg EtOH via oral gavage, then 3g/kg every 8h for 4d, for a total average cumulative EtOH dose of 45.1±1.74g/kg. Control (Con) animals received equivalent doses of 5% dextrose. The EtOH animals had average BALs of 257.84±13.18mg/dL, peak BALs of 417.16±20.68mg/dL, and lost 16% of their body weight (p=.0065) after 4d of EtOH exposure. Animals were scanned after EtOH exposure within 10h of their last dose (post-binge scan), and again after 7d of abstinence from EtOH (recovery scan). MR data were acquired on a 3T GE Signa MR scanner equipped with a high-strength insert gradient coil (500mT/m, 1800 mT/m/ms) and a custom-built RF coil (∅ = 44mm) used for both RF excitation and signal reception. Rats were scanned in sessions of ~2h each and anesthesia was provided by 2-3.5% isoflurane in oxygen (~1.5L/min). High resolution, dual echo, fast spin-echo (FSE) images were acquired in the axial plane, coronal to the magnet system bore (TE1/TE2/TR=12/60/5000ms, FOV=64x48mm^2, 256x192 matrix, echo train length=8, 50 slices, 0.3mm thick, 0mm skip, in-plane resolution=0.25mmx0.25mm). The FSE images were used to prescribe voxels (9.8x4x4mm^3) in the dorsal hippocampus centered at approximately -4.00 Bregma and extending 2mm anterior and posterior to this central point, 4.9mm to the right and left of midline, and 4mm inferior to -3.10mm Bregma, according to the atlas of Paxinos and Watson[4]. MR spectroscopy was performed with a CT-PRESS sequence (t_c=139ms, $\Delta t_1/2$=0.8ms, n_1=129, TR=2s, 6 averages)[5]. A second acquisition without water suppression ($\Delta t_1/2$=6.4ms, n_1=17) was performed to determine tissue water content used as a reference for metabolite quantification.

Results

MRI data were registered to common space on the baseline images of a reference animal[3]. Thresholding of T2 computed images was used to calculate lateral ventricular cerebral spinal fluid (CSF) volume across a defined region of interest in 5 axial slices. A 2-group, repeated-measures ANOVA yielded a group-by-time interaction (F(2,34)=10.79, p=.0002), indicating that cerebral spinal fluid (CSF) volume was modified by EtOH exposure. At the post-binge scan, CSF volume in the EtOH group had significantly increased from baseline (t(10)=3.9, p=.0029), and was higher than the Con group at the post-binge scan (t(17)=5.1, p=.0001, Fig 1). At the recovery scan, CSF volume no longer differentiated the groups.

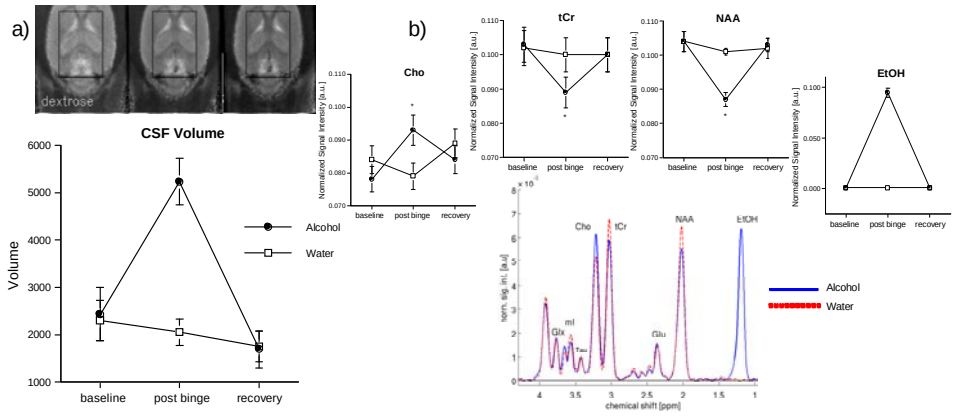

Figure 1: a) top panel, FSE images of an individual EtOH rat brain at baseline, post binge, and recovery; bottom panel, average CSF volume at the 3 time points for each group of rats. b) top panel, average signal intensity of tCr, Cho, NAA and EtOH at the 3 time points for each group of rats; bottom panel, average spectra at post binge for each group of rats.

Metabolite levels detected and expressed in arbitrary units relative to tissue water included N-acetyl-aspartate (NAA), total creatine (tCr), choline (Cho), glutamate (Glu), and Glx (Glu+glutamine). ANOVA indicated a group-by- time-by-metabolite interaction (F(14,238)=7.98, p=.0002). After binge EtOH exposure, the EtOH group had lower NAA (t(17)=5.23, p=.0001) and tCr (t(17)=3.24, p=.0048) and higher Cho (t(17)=3.48, p=.0029) than the Con group. An EtOH peak was also visible in the EtOH group at the post-binge scan (Fig 2). After 7d of recovery, there were no longer metabolite differences between groups.

Discussion and Conclusion

We have previously used MR methods to detect the effects of chronic (24wks) EtOH exposure on the wild-type Wistar rat brain and demonstrated that BALs of ~293mg/dL result in ventricular expansion[3] and increases in Cho, Glx, and Glu[6]. The current study was designed to determine whether brain changes induced by acute binge EtOH exposure could be detected using in vivo MR methods. In agreement with in vitro findings[1] and our chronic EtOH experiment, binge EtOH exposure results in brain insult detected in FSE images as an increase in ventricular CSF volume. That CSF volume returns to baseline after 7d of abstinence supports the concept of the brain's ability to recover from acute EtOH damage. In agreement with studies in recently detoxified human alcoholics, lower NAA levels in the frontal lobes generally resolve with longer abstinences[7]. The decline and recovery of NAA argues for a transient effect of EtOH on neuronal integrity. Corroborating previous evidence[6], EtOH exposure is associated with an increase in Cho that may be interpreted as inflammation, demyelination, or abnormally high glial density [8]. To our knowledge, this study is the first to provide evidence of EtOH effects on tCr. The EtOH induced decrease in tCr may be interpreted as a compromise in the brain's energy reservoir (in the form of ATP) [9]. Recovery of tCr levels in the absence of the causative agent again argues for transient effects on the brain of binge EtOH exposure.

Acknowledgements

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References

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