Cerebral Metabolite Differences and Correlations in Short-Term Binge Ethanol-Exposed Rats: A Study of Ex Vivo Proton Nuclear Magnetic Resonance Spectroscopy at 11.7-T

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Target audience: Neurologists, psychiatrists, and clinicians interested in using magnetic resonance spectroscopy (MRS) to investigate brain disorders.

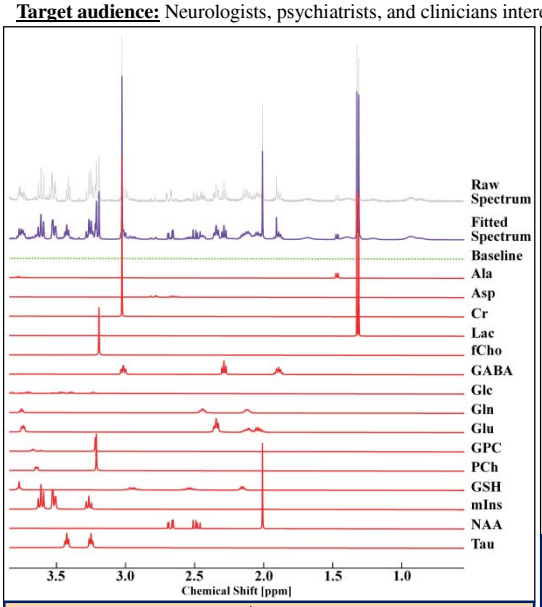

Fig. 1. Representative *ex vivo* ^{1}H NMR spectra acquired at 11.7 T from short-term binge ethanol-exposed rats in the hippocampal region. Quantified spectra are represented by several colors, as follows: Fitted spectra (purple), raw spectrum (grey), baseline (dotted green), and metabolite signals (red).

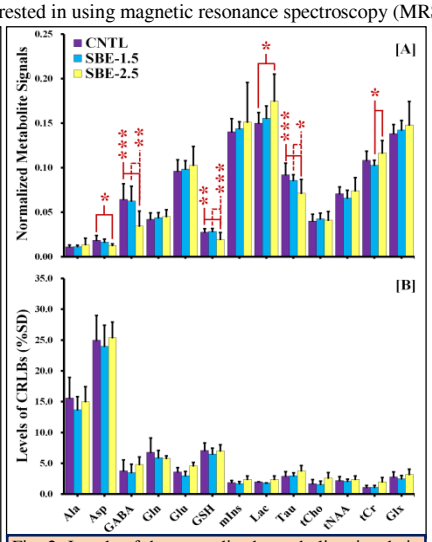

Fig. 2. Levels of the normalized metabolite signals in the hippocampal region of CNTL, SBE-1.5, and SBE-2.5, quantified using LCModel (A). One-way ANOVA tests were considered significant at $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$. Cramer–Rao lower bound (CRLB) levels are expressed as percentage standard deviation (% SD) (B). Vertical bars indicate the positive standard deviation from the mean.

Purpose: Numerous studies have shown that binge ethanol-exposed rats exhibit significant metabolic abnormalities, functional impairments, and neuronal changes. These include cerebral metabolite changes[1], cognitive deficits[2], and neuronal dysfunction and degeneration/recovery[3] in the hippocampus[1], temporal (entorhinal/ perirhinal) cortex[3], and olfactory bulb[2]. However, the short-term dose effects of binge ethanol exposure on cerebral neurochemical differences and responses in the hippocampal region have not been experimentally assessed with *ex vivo* ^{1}H NMR spectroscopy. Therefore, the first goal of this study was to determine the influence of dose-dependent short-term binge ethanol (SBE) exposure on cerebral neurochemical differences and responses among sham controls (CNTL) and low- (SBE-1.5) and high-dose (SBE-2.5) ethanol-exposed rats, using *ex vivo* ^{1}H high-resolution magic angle spinning (HR-MAS) NMR spectroscopy. The second goal was to determine the correlations between the metabolite-metabolite levels (pairs of metabolite levels) using individual metabolite data from the hippocampal region of SBE-exposed rats.

Methods: Eight-week-old male Wistar rats (n = 28) were divided into 3 groups: a control group (CNTL, ethanol dose of 0.0 g/kg; distilled water, n = 10) and two short-term binge ethanol (SBE) groups: a low-dose (SBE-1.5, ethanol dose of 1.5 g/kg, 25% w/v ethanol solution, n = 10) and high-dose group (SBE-2.5, ethanol dose of 2.5 g/kg, 25% w/v ethanol solution, n = 8). For the initial exposure (day 1; at 17:00 h), the 18 rats in the SBE-1.5 and -2.5 groups received an initial dose of 5.0 g/kg ethanol (30% w/v solution) through oral gavage, and subsequent doses of 1.5 g/kg and 2.5 g/kg (25% w/v solution), respectively, every 8 h (at 01:00, 09:00, and 17:00 h) for 4 days. The 10 rats in the sham CNTL group received an equivalent volume (about 2.85 mL) of distilled water at comparable times (at 02:00, 10:00, and 18:00 h). Oral gavage ethanol was administered according to body weight, as described by the Majchrowicz binge alcohol protocol[4]. After 4 days of oral gavage, all animals were sacrificed and brain tissues were carefully harvested from the hippocampal region. *Ex vivo* ^{1}H HR-MAS NMR spectroscopy was performed using an Agilent VNMRS-500 (11.7 T). *Ex vivo* HR-MAS spectra were acquired from all 28 tissue samples using Carr-Purcell-Meiboom-Gill sequence (complex data number = 16384, spectral width = 8 kHz, acquisition time = 2.05 sec, relaxation delay time = 5.0 sec, presaturation time = 2.0 sec, inter-pulse delay (τ) = 0.4 msec, number of acquisitions = 128, total scan time = 15 min 24 sec). Raw data obtained for the 28 samples were analyzed using LCModel with a simulated basis-set. The LCModel basis set for 11.7 T included spectra of 17 brain metabolites: Alanine (Ala), aspartate (Asp), free-choline (fCho), creatine (Cr), phosphocreatine (PCr), gamma-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glycerophospho-sphocholine (GPC), glutathione (GSH), myo-inositol (mIns), lactate (Lac), *N*-acetylaspartate (NAA), *N*-acetyl-aspartyl-glutamate (NAAG), phospho-choline (PCh), ethanol (Eth), taurine (Tau), glutamine compl-ex (Glx: Glu + Gln), total NAA (tNAA: NAA + NAAG), and total Cr (tCr: Cr + PCr). The relative signal intensity of each metabolite was calculated by dividing the peak area by the total area of all of the metabolites of interest.

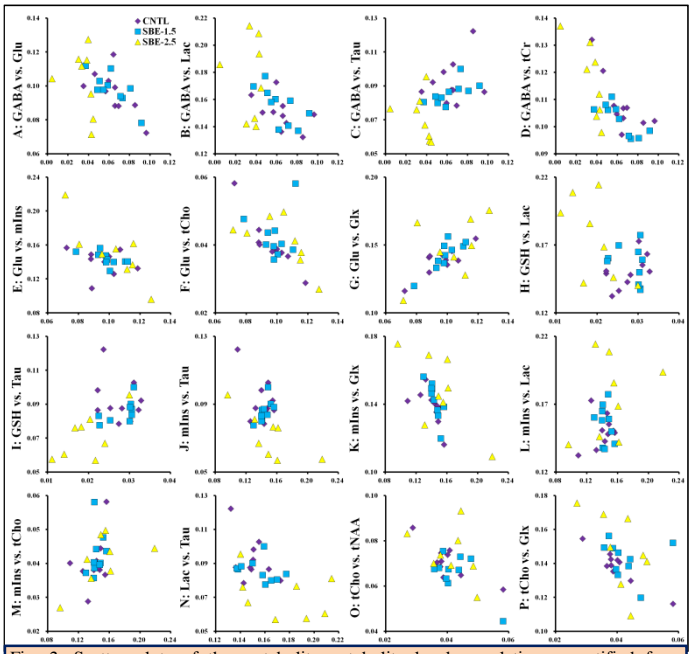

Fig. 3. Scatter plots of the metabolite-metabolite level correlations quantified from individual rats that were distinguished by symbols among the CNTL (purple rhombus), SBE-1.5 (blue square), and SBE-2.5 (yellow triangle) rats.

Results: **Figure 1** shows the representative 11.7 T NMR spectra obtained from metabolites from the hippocampal region of CNTL rats. *Ex vivo* ^{1}H NMR spectra were assigned the following cerebral metabolite signals: Ala, Asp, Cr, Lac, fCho, GABA, Glc, Gln, Glu, GPC, PCh, GSH, mIns, NAA, and Tau. **Figure 2A–B** illustrates the normalized cerebral metabolite levels quantified from the 28 acquired *ex vivo* spectra of the hippocampal region. One-way ANOVA revealed an interaction of metabolite levels among the three groups (CNTL, SBE-1.5, and SBE-2.5), which indicates a significant ethanol effect on the normalized metabolite levels. 4 days of intermittent ethanol intoxication resulted in altered normalized metabolite levels among the three groups (CNTL vs. SBE-1.5 vs. SBE-2.5) for Asp [$F(2,21) = 2.768$, $p = 0.046$], GABA [$F(2,22) = 5.913$, $p = 0.009$], GSH [$F(2,22) = 6.586$, $p = 0.006$], Lac [$F(2,22) = 3.500$, $p = 0.048$], Tau [$F(2,22) = 5.668$, $p = 0.010$], and tCr [$F(2,27) = 3.488$, $p = 0.048$]. Additionally, there were significantly lower Asp ($p = 0.029$), GABA ($p = 0.004$), GSH ($p = 0.005$), and Tau ($p = 0.003$) signals in SBE-2.5 rats compared to CNTL rats. Moreover, there were significantly higher GABA ($p = 0.006$), GSH ($p = 0.002$), and Tau ($p = 0.030$) signals in SBE-1.5 rats compared to SBE-2.5 rats. The Lac ($p = 0.016$) and tCr ($p = 0.015$) signals in the SBE-2.5 rats were significantly higher than in the CNTL and SBE-1.5 rats, respectively. To visualize the normalized metabolite levels quantified from the individual rat data and assess the relationship among them, the pairs of normalized metabolite levels that changed the most were selected for linear scatter plots (**Figure 3A–P**). The clusters of individual data from the 28 rats were significantly correlated in sixteen scatter plots. The illustrations in A–P show the relationships between the pairs of normalized metabolite levels as follows: GABA vs. Glu: $***p = 0.003$ (A), GABA vs. Lac: $***p = 0.002$ (B), GABA vs. Tau: $***p = 0.004$ (C), GABA vs. tCr: $****p < 0.001$ (D), Glu vs. mIns: $***p = 0.001$ (E), Glu vs. tCho: $***p = 0.001$ (F), Glu vs. Glx: $****p < 0.001$ (G), GSH vs. Lac: $***p = 0.003$ (H), GSH vs. Tau: $***p = 0.001$ (I), mIns vs. Tau: $***p = 0.002$ (J), mIns vs. Glx: $***p = 0.001$ (K), mIns vs. Lac: $*p = 0.047$ (L), mIns vs. tCho: $*p = 0.014$ (M), Lac vs. Tau: $***p = 0.001$ (N), tCho vs. tNAA: $***p = 0.001$ (O), and tCho vs. Glx: $***p = 0.004$ (P). The selected correlated scatter plots exhibited highly significant levels and reliable correlation coefficients.

Discussion and Conclusion: In the present study, we conducted *ex vivo* NMR spectroscopy in a rat model to quantitatively assess the dose-dependent influences of SBE exposure on cerebral neurochemical changes in the rat hippocampal region. In line with the findings of previous studies, we report altered Asp, GABA, GSH, Lac, Tau, and tCr signals in SBE-exposure rats. These results may indicate that SBE exposure leads to various biological changes, such as changes in the rate of GABA and glucose synthesis, impairment of an antioxidant defense system, abnormal ATP function in energy metabolism, and dysfunctions of anaerobic respiration[2,3,5]. Overall, our *ex vivo* ^{1}H NMR spectroscopy results suggest novel metabolic markers for assessing the dose-dependent effects of SBE exposure in the hippocampal region.

References: **1.** Zahr NM, et al., Biol. Psychiat. 2010;67:846–854., **2.** Cippitelli A, et al., Neurobiol. Learn. Mem. 2010;94:538–546., **3.** Crews FT, et al., Alcohol. Clin. Exp. Res. 2000;24:1712–1723., **4.** Majchrowicz E. Psychopharmacologia 1975;43:245–254., **5.** Lee DW, et al., Neuroscience 2014;262:107–117.
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