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Quantification of ethanol methyl ^1H magnetic resonance signal intensity following intravenous ethanol administration in primate brain

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Abstract

In vivo ^1H magnetic resonance spectroscopy (MRS) can be used to directly monitor brain ethanol. Previously, studies of human subjects have lead to the suggestion that the ethanol methyl ^1H MRS signal intensity relates to tolerance to ethanol's intoxicating effects. More recently, the ethanol ^1H MRS signal intensity has been recognized to vary between brain gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) due to differences in T_2 within these environments. The methods presented here extend ethanol MRS techniques to nonhuman primate subjects. Twelve monkeys were administered ethanol while sedated and positioned within a 3T MRI system. Chemical shift imaging (CSI) measurements were performed following intravenous infusion of 1g/kg ethanol. Magnetic resonance imaging (MRI) data were also recorded for each monkey to provide volume fractions of GM, WM, and CSF for each CSI spectrum. To estimate co-variance of ethanol MRS intensity with GM, WM, and CSF volume fractions, the relative contribution of each tissue subtype was determined following corrections for radiofrequency pulse profile non-uniformity, chemical shift artifacts, and differences between the point spread function in the CSI data and the imaging data. The ethanol MRS intensity per unit blood ethanol concentration was found to differ between GM, WM, and CSF. Individual differences in MRS intensity were larger in GM than WM. This methodology demonstrates the feasibility of ethanol MRS experiments and analysis in nonhuman primate subjects, and suggests GM may be a site of significant variation in ethanol MRS intensity between individuals.

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Keywords

Nonhuman primate; ethanol; brain; magnetic resonance spectroscopy

Introduction

Most drugs of abuse are self-administered in the mg/kg dose range. In this respect, ethanol is unusual, as its intoxicating effects are only experienced after many grams of it have been ingested. Consequently, during a state of intoxication, ethanol is the most concentrated small organic molecule in the brain [1], which makes it readily identifiable with present-day *in vivo* nuclear magnetic resonance spectroscopic (MRS) imaging techniques and instrumentation.

Over the course of the 20 years since the first MRS measurements of ethanol in the human brain were reported [2], there has been an interest in the extent to which the intensity of the ethanol MRS signal relates to brain ethanol concentration and/or is directly related to the pharmacological effect of brain ethanol. In MRS studies contrasting ethanol sensitive/naïve with ethanol tolerant/exposed human [3–5] or animal [6,7] subjects, it has been reported that the brain ethanol MRS intensity per unit ethanol concentration is higher in brains of tolerant individuals than in ethanol-sensitive comparison groups. Ethanol interacts with a wide variety of protein and lipid macromolecular constituents in brain parenchyma [8]. It has thus been proposed that such interactions could influence the ethanol ^1H methyl MRS intensity through reduction in the methyl ^1H T_2 value [9]. Some have suggested that the ^1H methyl T_2 value of macromolecule-bound ethanol is sufficiently short that the component of interacting molecules would be “NMR-invisible” [3,7,10]. However, others have reported nearly complete ethanol visibility, provided an appropriate value for the parenchymal ethanol methyl T_2 value is recognized [1,9,11].

Although the biophysical mechanism linking the T_2 of ethanol to the exertion of its pharmacological effects has not been established, it is known that the T_2 of ethanol is different in GM, WM, and CSF [9,12]. Therefore, quantitative study of the ethanol methyl ^1H resonance intensity within primate brain must account for the relative amounts of GM, WM, and CSF contained within the measured volume element (voxel). Studies of non-human primates can provide unique opportunities to monitor changes in ethanol MRS intensity following exposure to controlled amounts of ethanol over prolonged (months to years) time periods. The motivation for the study described here is to develop a suitable methodological approach for making comparisons between individuals with varying behavioral responses to ethanol exposure, or between longitudinal data acquired from the same individual at multiple time points. This methodology will facilitate studies of the neurobiological basis of drinking behavior.

Herein, a methodology for the acquisition of ethanol MRS data obtained from a group of 12 ethanol-naïve rhesus macaques is presented, and the abilities of five model expressions to fit the experimental data are compared. To quantify ethanol MRS intensity in the GM, WM, and CSF of individual monkeys, procedures described by Hetherington and co-workers [11] have been adapted to studies of non-human primates. This requires the acquisition of magnetic resonance imaging (MRI) data; localization of the MRS voxels within the anatomical MRI data; segmentation of the brain into GM, WM, and CSF tissue classes; and determination of relative volume fractions of each tissue class in each MRS voxel. In the future, the analytical model developed here will also be used to determine whether potential ethanol MRS intensity changes associated with exposure to ethanol can be localized specifically to GM or WM, and whether these MRS intensity changes co-vary with behavioral measures of ethanol tolerance. The model selection analysis presented here addresses two specific questions. First, it is

determined whether a parsimonious model that utilizes a single parameter representing MRS intensity in parenchymal tissue provides sufficient detail to describe the experimental data or if modeling separate parameters for ethanol MRS intensity in GM and WM provides a significantly better fit to the experimental data.. Second, to determine whether significant individual differences in ethanol MRS intensity are observed within the 12 ethanol-naïve animals, models that include individualized subject specific brain tissue parameters are compared to simpler models that estimate tissue parameters generalized across the entire group. Potential future strategies are discussed for extending the model presented here to enable comparisons between ethanol naïve subjects and those with a history of prolonged ethanol exposure, and to account for potential co-variance of ethanol MRS intensity with behavioral manifestations of ethanol tolerance.

Methods

Subjects

Twelve adult male rhesus monkeys (*Macaca Mulatta*) weighing between 7.6 – 11.8 kg served as subjects. Each monkey was individually housed in a stainless steel cage measuring $1.6 \times 0.8 \times 0.8$ m (Allentown Caging, Allentown, NJ) in a vivarium with a 12 hour light:dark cycle (with lights on at 7am) that was maintained at $21 \pm 1^\circ\text{C}$ and 30–50% humidity. The monkeys were fed a diet of fresh fruit and 1g banana-flavored pellets (consisting of 63% carbohydrate, 4% fat, and 22% protein - PJ Noyes, Lancaster, NH) in quantities sufficient to maintain a positive caloric intake. All procedures involving animals were conducted in accordance with the “Guidelines of the Committee on the Care and Use of Laboratory Animal Resources” (National Health Council, Department of Health, Education, and Welfare, ISBN 0-309-05377-3, revised 1996) and, prior to their implementation, were reviewed by the Institutional Animal Care and Use Committee of the Oregon National Primate Research Center, and found to be in compliance with all local, state, and national regulations pertaining to the humane use of animal subjects.

Data acquisition

Following a 12-hour fast, the monkeys were removed from their home cages under 10 mg/kg of ketamine anesthesia (Vedco, St. Joseph, MO) and transported to the adjacent MRI facility (< 5 min transport time). At the MRI facility, an endotracheal tube was inserted and anesthesia was maintained by the inhalation of 1.5% isoflurane gas (Butler Animal Health Supply, Dublin, OH) and oxygen (Polar Cryogenics Inc, Portland, OR). A 22G $\times$ 1" catheter (Termumo Corporation, Somerset, NJ) was inserted into the saphenous vein and kept patent with a 30 u/ml solution of heparinized saline (Baxter Healthcare Corporation, Deerfield, IL). The monkeys were then placed inside a Siemens whole-body 3T trio MRI system (Erlinger, Germany) with their heads positioned in the center of a circular polarized extremity RF coil. Once inside the MRI system, the endotracheal tube was connected to a veterinary anesthesia ventilator (Model 2002IE, Hallowell EMC, Pittsfield, MA) set to 10 breaths per minute and a tidal volume sufficient to produce an airway pressure of 15 cm H₂O to ensure a consistent level of isoflurane exposure from one subject to the next and safeguard against possible respiratory depression following the intravenous injection of ethanol. The administration of ethanol was made possible by connecting the catheter inserted in the saphenous vein to a PHD 22/2200 infusion pump (Harvard Apparatus, Holliston, MA) via 15' of PTFE Microbore Tubing (Cole Parmer, Vernon Hills, IL). Ten minutes after the ethanol infusion, a blood sample was obtained for the determination of blood ethanol concentration (BEC) by inserting a 25 gauge needle into the saphenous vein and then collecting 20 μ l of blood from the hub of the needle with a microcapillary pipet (Model #71900-20, Kimble/Kontes, Vineland, NJ). Blood samples were sealed in air-tight vials containing 500 μ l of distilled water and 20 μ l of isopropanol (10% internal standard) and stored at -20°C until assay using gas chromatography (Hewlett Packard

5890 Series II, Avondale, PA) supplied with headspace autosampler, flame ionization detector, and a Hewlett Packard 3392A integrator. Blood samples were taken in duplicate for each monkey and the average BEC value was used as an estimation of brain ethanol concentration.

Subsequent to positioning the monkey in the scanner, a series of 6 high resolution MP-RAGE (T_1 -weighted [13]) images were obtained (0.5 mm isotropic image resolution, 128 slices, $TE=4.38$ ms, $TR=2500$ ms, $TI=1100$ ms). For all 12 animals, the field of view in the frequency-encode direction was 128 mm. For seven monkeys, the field of view in the phase-encode direction was 80mm, and for the five larger monkeys, it was 96 mm. The larger field of view in the phase-encode direction increased the acquisition time from 6.7 to 8.0 minutes. These images were used to estimate GM, WM, and CSF composition as described below. The first acquired image was also used to position a single transverse 8-mm-thick CSI (chemical shift imaging) slice, centered on the inter-hemispheric axis (Figure 1). In order to perform reliable 1H CSI measurements of the brain, a suppression strategy is necessary to minimize spurious MRS intensity arising from extracranial lipid molecules. Unsuppressed lipid signals give rise to MRS intensity in the 0–3 ppm range, which can interfere with *N*-acetylaspartate (NAA) and ethanol methyl 1H quantification [14]. As described below, the quantification strategy presented here relies on accurate quantification of the NAA methyl 1H signal to provide a reference concentration. To suppress extracranial lipid signals, a point resolved spectroscopy (PRESS) [15] localization functionality has been incorporated by the vendor in the CSI pulse sequence. The CSI pulse sequence thus consisted of three rf pulses. The first of these, the “excitation” pulse, was applied in combination with a 2.47 mT/m gradient along the left/right axis to select a 32 mm-thick slab. The second rf pulse, a “refocusing” pulse, was applied in combination with a 1.13 mT/m gradient along the rostral/caudal axis to select a 48 mm-thick slab. The third rf pulse was an additional refocusing pulse, applied in combination with a 6.77 mT/m gradient along the inferior/superior axis to select an 8 mm-thick slab. All rf pulses were applied at the resonance frequency of the NAA methyl 1H . The total acquisition time (for two transients) was 3 minutes and 55 seconds. The target volume for one monkey is outlined in red in Figure 1b.

Within the selected volume, spatially-resolved spectra were obtained by Fourier-encoding an 8 by 8 square matrix, resulting in an isotropic nominal CSI resolution of $(8.0\text{ mm})^3$ (green grid, Figure 1b). Gradient pulses in the left/right and rostral/caudal directions within the interval between the excitation and first refocusing rf pulses were systematically incremented to sample an 8×8 rectilinear k-space grid, resulting in a 64 mm field of view in these directions. Additional pulse sequence parameters were $TE=150$ ms and $TR=1770$ ms. Prior to the infusion of ethanol, a baseline CSI data set was acquired (8 transients, 16 minutes). An ethanol dose of 1.0 g/kg was then delivered at a rate of 0.1 g/kg per minute. CSI data continued to be collected every 4 minutes both during the 10 minute ethanol infusion, and for an additional 70 minutes following the infusion. A TE value of 150 ms was chosen as a compromise between minimal T_2 -weighting (achieved at a short TE values) and reductions in baseline distortions in the vicinity of ethanol and NAA methyl resonance signals (achieved at long TE values). A TR value of 1770 ms was chosen to maximize the signal to noise ratio obtained per unit time under conditions of a 90° excitation pulse and a T_1 of 1.4 sec, corresponding to the published value for the NAA methyl 1H resonance at a magnetic field strength of 3T [16].

Post acquisition data processing

The overall data processing strategy is schematized in Figure 2. Except where noted, this strategy follows procedures developed by Hetherington and co-workers [9,11,17].

In Figure 3, the operation performed in processing step 2 is illustrated for a representative voxel. Time-domain data were transferred from the MRI system host computer to a PC workstation and converted to the frequency domain by Fourier transformation, with application

of an exponentially-decaying apodization filter of time constant 83 ms. Manual phase-corrections were applied with the aid of customized visualization software programmed in Matlab (MathWorks Inc., Natick, MA). Figure 3b displays a baseline (pre ethanol infusion, black) spectrum, and the sum of NMR spectra obtained 20–40 minutes following the beginning of the infusion, normalized by the number of averaged acquisitions (red). The post/pre-infusion difference spectrum is displayed in Figure 3b (lower trace, in blue). Figure 3c illustrates a spectrum in which extracranial lipids were incompletely suppressed. Although the volume selection component of the pulse sequence dramatically improved suppression of extracranial lipid signals, evidence of contamination from lipid ^1H signals was observed in several voxels. Therefore, spectra from each CSI voxel were inspected, and voxels in which NAA resonance signals were not resolved to baseline were removed from subsequent analysis. As evident in Figure 3a, lipid suppression is less effective in voxels on the periphery of the volume selected for spectroscopy. Over the 12 individuals characterized by CSI, a total of 140 voxels were removed from the analysis due to lipid contamination. Results herein are reported on the spectra from the remaining 148 CSI voxels.

In CSI voxels that exhibited sufficient suppression of extracranial lipid resonances, difference spectra were calculated by subtracting the baseline spectrum from each post-infusion spectrum, as exemplified in Figure 3b (lower trace). The ethanol methyl ^1H MRS intensity was quantified for each voxel at a given post-infusion time point by summing difference intensity values over the 1.0 to 1.2 ppm range. Ethanol methyl ^1H peak areas obtained over the time period ranging from 20 to 40 minutes following completion of the infusion were then summed to obtain the ethanol methyl ^1H MRS intensity. NAA methyl peak areas were also determined from each of the spectra used to quantify ethanol by summing spectra intensity values over the 2.0 to 2.2 ppm range. Figure 4 shows a time course of ethanol MRS intensity, expressed as the ratio of the ethanol/NAA intensity vs. time. Data shown in the shaded area were summed to provide a single ethanol MRS intensity estimate. The ethanol infusion occurred from time 0–10 minutes. Ten minutes after the ethanol infusion, a blood sample was taken for the determination of blood ethanol concentration. The average blood ethanol concentration was 120 mg of ethanol per dl of blood.

Given the fact that the T_2 of ethanol differs in GM, WM, and CSF [9], the relative contributions of GM, WM, and CSF were determined for each individual voxel. To that end, steps 3 and 4 of Figure 2 utilize software included in the FMRIB Software Library (FSL, <http://www.fmrib.ox.ac.uk/>) to register the 6 MP-RAGE brain images to one another, sum them together, and then register the summed volume to a population-average MRI-based atlas of the rhesus macaque brain [18]. Specifically, for each subject, rigid-body rotation/translation transformations were applied to register the second through sixth MP-RAGE data set acquired to the first using FSL's Linear Image Registration Tool (FLIRT). The six co-registered images were then added together into a single image using the "fslmaths" functionality of FSL, and non-brain portions of the summed images were then excluded using the Brain Extraction Tool (BET). Subsequent editing of the extracted brain was necessary for all images to provide an accurate representation of the macaque frontal lobe. A 6-parameter rigid-body transformation was used to register the extracted brains to the atlas reference frame. The images were then segmented into GM, WM, and CSF components with the Statistical Parametric Mapping software package, SPM8 (www.fil.ion.ucl.ac.uk/spm/), using prior probability atlases of GM, WM, and CSF regions of the adult rhesus macaque brain (www.brainmap.wisc.edu/pages/2). Additional changes to the default segmentation settings of SPM8 were to set Cerebro-Spinal Fluid to 'Native Space,' Affine Regularisation to 'Average Sized Template,' and Sampling Distance to '2' following McLaren and co-workers (this issue). The three segmentation files output by SPM8 (GM, WM, and CSF) were then rotated and translated to a frame that is non-oblique to the CSI data using the FLIRT function of FSL. Specifically, the brain atlas that the T_1 -weighted dataset was registered to before segmentation (which is of the same orientation

as the GM, WM, and CSF prior probability maps) was aligned to the CSI volume using the rigid-body 6 parameter model of FLIRT and applying this transformation to the individual GM, WM, and CSF segmentation files as ‘secondary images.’ As exemplified in Figure 5a for GM, this segmentation procedure produces binary labelmaps for GM, WM, and CSF that are assigned a value of 1 at voxel positions overlapping the specified structure, and 0 elsewhere.

In order to quantify GM, WM, and CSF volume fractions contributing to each of the CSI spectra, the PRESS slice profiles, chemical shift artifacts, and the effects of finite CSI sampling must be considered. Figure 5a–c illustrates how non-uniformity of the PRESS slice profile is incorporated into the analysis (Figure 2, step 6). For each of the three RF/gradient pulse pairs used to provide PRESS localization, the amount of transverse magnetization was calculated as a function of offset frequency by simulating evolution of the magnetization vector according to the Bloch equations. The amplitude-modulated RF pulses were of 2.6 ms duration, and digitized by the MRI system in 400 uniform-length time intervals $\Delta t = 6.5 \mu\text{s}$. For a given offset frequency, the magnetization vector $\mathbf{M}(t_i)$ was determined at the end of each of the 400 time intervals, using the magnetization vector at the end of the preceding time interval $\mathbf{M}(t_{i-1})$ as an initial condition and the relation derived in [19]

$$\mathbf{M}(t_i) = \mathbf{R}_z(\varphi) \mathbf{R}_y(\theta) \mathbf{R}_z(\alpha) \mathbf{R}_y(-\theta) \mathbf{R}_z(-\varphi) \mathbf{M}(t_{i-1}). \quad [1]$$

In Eq. [1], $\mathbf{R}_z(\pm\varphi)$ and $\mathbf{R}_y(\pm\theta)$ are 3×3 rotation matrices around the z axis of angle $\pm\varphi$ or y axis of angle $\pm\theta$, respectively. The rotation angle φ specifies the phase of the applied RF pulse (herein defined to be 0, corresponding to a pulse of “x-phase”); θ is determined by the frequency offset from the applied RF frequency, $\Omega = (\omega - \omega_{\text{RF}})$, through the relationship

$$\theta = \tan^{-1}(\omega_{\text{RF}}/\Omega); \quad [2]$$

and $\alpha = -\omega_{\text{RF}} t \sqrt{1 + (\tan(\theta))^{-2}}$ is the effective rotation angle. Magnetization prior to the excitation pulse was assumed to be purely longitudinal ($\mathbf{M}(0) = [0 \ 0 \ 1]^T$), whereas magnetization prior to the refocusing pulses was assumed to be purely transverse ($\mathbf{M}(0) = [1 \ 0 \ 0]^T$). The transverse magnetization, $\mathbf{M}_t$, at the end of an RF pulse is defined as

$$\mathbf{M}_t = \sqrt{([1 \ 0 \ 0] \cdot \mathbf{M}(t_{400}))^2 + ([0 \ 1 \ 0] \cdot \mathbf{M}(t_{400}))^2}. \quad [3]$$

For each RF pulse, Eq. [1] was used to calculate the transverse magnetization, $\mathbf{M}_t$ at the end of the pulse assuming 101 equally-spaced values of Ω , ranging from -5 to 5 kHz. In the presence of a magnetic field gradient of strength G , the slice profile along a given coordinate r is

$$\mathbf{M}_t(r) = \mathbf{M}_t(\Omega/G). \quad [4]$$

Due to the separability of the three RF pulse/gradient pairs, the 3D magnetization profile is derived from the product of the three magnetization profiles

$$\mathbf{M}_t(x, y, z) = \mathbf{M}_t(x) \mathbf{M}_t(y) \mathbf{M}_t(z). \quad [5]$$

Figure 5b shows a projection of $\mathbf{M}_t$ on the x-y plane for the pulse sequence used in this study. As illustrated for GM in Figure 5a–c, the products of the segmentation results and the 3D slice profile are obtained to quantify the relative contributions from GM, WM, and CSF to a given CSI voxel.

A second factor that influences the volume element selected by the pulse sequence is the chemical shift artifact. Due to the 0.82 ppm chemical shift difference between NAA and ethanol methyl ^1H signals, a chemical shift artifact [14] results in shifts of the selected ethanol methyl ^1H volume relative to that of NAA. These shifts were determined to be 0.95 mm, 2.08 mm, and 0.35 mm in the left/right, rostral/caudal and inferior/superior directions, respectively, for the pulse sequence used in this study. These are illustrated in Figure 1, the selected ethanol volume outlined in blue is contrasted with the selected volume for NAA in red.

Yablonskiy and co-workers [20] have described a secondary effect of the chemical shift artifact. Ethanol ^1H methyl nuclei from different regions of a PRESS-selected volume element will experience the scalar coupling interaction with neighboring methylene ^1H nuclei to varying extent. This is because the refocusing RF pulses, applied in the presence of a magnetic field gradient, will selectively affect methyl but not methylene ^1H nuclei for a fraction of the volume element, and hence decouple the two sets of spins [20]. To account for this effect, a voxel-specific scalar coupling scaling factor, X_j^{EtOH} , is introduced below.

To account for the effects of finite CSI sampling on the relative weighting of GM, WM, and CSF in each CSI voxel (step 7 of Figure 2), the point spread function (PSF) [14,21] of the CSI experiment can be estimated, as performed in previous applications of ^1H CSI in studies of brain ethanol and other compounds [9,11,17]. The PSF relates a recorded intensity value at a 2D position, $\hat{I}(x,y)$, to the true image, $I(x,y)$, through the convolution [21]

$$\hat{I}(x, y) = PSF * I(x, y) \quad [6]$$

in which a 2-dimensional convolution operation is represented by the symbol “*”. The PSF is defined as the Fourier transform of the CSI sampling function, and is derived for the 1-dimensional case in [21] to be

$$PSF = \frac{\sin(\pi N x / FOV)}{FOV \sin(\pi x / FOV)} e^{-i\pi x / FOV} \quad [7]$$

in which N is the number of data points acquired along the direction x , and FOV is the product of N and the nominal voxel size. In two dimensions, the expression for the PSF is

$$PSF = \frac{\sin(\pi N_x x / FOV_x)}{FOV_x \sin(\pi x / FOV_x)} \frac{\sin(\pi N_y y / FOV_y)}{FOV_y \sin(\pi y / FOV_y)} e^{-i\pi(x/FOV_x + y/FOV_y)}. \quad [8]$$

Figure 5d is a magnitude image of the PSF obtained from the experimental settings used herein. Two noteworthy attributes of the function $\hat{I}(x,y)$ that result from Eq. [6] are that it is complex

and its real component can be negative [22]. As described above, only the real component of the CSI spectra were quantified, and therefore the analysis of $\hat{f}(x,y)$ performed here is restricted to its real component. Further, due to effects termed Gibbs ringing, the real component of $\hat{f}(x,y)$ can be negative (Figure 5e). This leads to the potentially counterintuitive but well-documented [21, 22] result that the ethanol and/or NAA MRS amplitudes arising from GM, WM, and/or CSF can be negative for a given voxel. Figure 5c–e shows the result of convolving the GM segmentation result in Figure 5c with the real component of the PSF using $N_x = N_y = 8$, and $FOV_x = FOV_y = 64$ mm.

Data analysis

The potential effects of tissue sub-classification and individual differences on the ethanol methyl ^1H MRS intensity are investigated by fitting the MRS data using five mathematical expressions. The dependent variable for each of the five models is the ethanol methyl ^1H signal intensity, expressed as a ratio of the NAA methyl ^1H signal intensity, $S_i^{\text{EtOH}}/S_i^{\text{NAA}}$, per unit BEC. For each voxel i , $y_i = [S_i^{\text{EtOH}}/S_i^{\text{NAA}}]/\text{BEC}$ in which the MRS intensities are in arbitrary units, and BEC is the blood ethanol concentration in units of mg of ethanol per 100 ml of blood (mg%).

$$\begin{aligned} \text{M1. } y_i &= c_{\text{Brain}} x_i + \varepsilon_i \\ \text{M2. } y_i &= c_{\text{Par}} x_{i,\text{Par}} + c_{\text{CSF}} x_{i,\text{CSF}} + \varepsilon_i \\ \text{M3. } y_i &= c_{\text{GM}} x_{i,\text{GM}} + c_{\text{WM}} x_{i,\text{WM}} + c_{\text{CSF}} x_{i,\text{CSF}} + \varepsilon_i \\ \text{M4. } y_{i,j} &= c_{j,\text{Par}} x_{i,j,\text{Par}} + c_{\text{CSF}} x_{i,\text{CSF}} + \varepsilon_i \\ \text{M5. } y_{i,j} &= c_{j,\text{GM}} x_{i,j,\text{GM}} + c_{j,\text{WM}} x_{i,j,\text{WM}} + c_{\text{CSF}} x_{i,\text{CSF}} + \varepsilon_i \end{aligned} \quad [9]$$

Model M1 is the “null” model, in which the ethanol MRS intensity is assumed to be independent of GM, WM, CSF classification and subject. The single adjustable parameter in model M1 is a coefficient reflecting the mean ethanol intensity in brain, c_{Brain} , and the independent variable $x_i = (GM_i + WM_i + CSF_i)/V_i^{\text{NAA}}$, in which GM_i , WM_i and CSF_i are relative contributions of GM, WM, and CSF to ethanol ^1H methyl MRS intensity in voxel i , respectively, and the volume fraction of parenchymal tissue contributing to the observed NAA signal for voxel i , V_i^{NAA} , is the quantity $(GM_i^{\text{NAA}} + WM_i^{\text{NAA}})$. In M2, MRS intensity is assumed to differ between brain parenchymal tissue (the sum of GM and WM tissue components) and CSF. The two adjustable parameters for model M2 are the intensity of the parenchymal tissue component, represented by the coefficient c_{Par} , and the intensity of the CSF component, c_{CSF} . The independent variables for M2 are $x_{i,\text{Par}} = (GM_i + WM_i)/V_i^{\text{NAA}}$ and $x_{i,\text{CSF}} = CSF_i/V_i^{\text{NAA}}$. In M3, the three adjustable parameters c_{GM} , c_{WM} , and c_{CSF} represent MRS intensities within GM, WM, and CSF, respectively, and the independent variables are $x_{i,\text{GM}} = GM_i/V_i^{\text{NAA}}$, $x_{i,\text{WM}} = WM_i/V_i^{\text{NAA}}$, and $x_{i,\text{CSF}}$ is as defined for model M2. Model M4 has 13 adjustable parameters. Individual differences in MRS intensity among the j animals are assumed within parenchymal tissue, as represented by the twelve coefficients $c_{j,\text{Par}}$. The thirteenth adjustable parameter in model M4 is c_{CSF} . Independent variables in model M4 include for each of the j monkeys $x_{i,j} = (GM_{i,j} + WM_{i,j})/V_i^{\text{NAA}}$, and $x_{i,\text{CSF}}$ is as defined for model M2. Model M5 has 25 adjustable parameters. Individual differences in MRS intensity among the j animals are assumed within GM and WM tissue, represented by the 12 coefficients $c_{j,\text{GM}}$ and 12 coefficients $c_{j,\text{WM}}$, respectively. The 25th adjustable parameter is c_{CSF} . Model M5 independent variables are defined as $x_{i,j,\text{GM}} = GM_{i,j}/V_i^{\text{NAA}}$, $x_{i,j,\text{WM}} = WM_{i,j}/V_i^{\text{NAA}}$, and $x_{i,\text{CSF}}$ is as defined for model M2. To obtain estimates of the coefficients for each model, the Matlab optimization toolbox function “lsqcurvefit” was used.

Within the framework of models M1–M5, variations in fitted intensity coefficients are interpreted herein to arise from differences in ethanol methyl ^1H T_2 values between tissue sub-classifications or between individuals. To convert the Eq. [9] MRS intensity coefficients to ethanol methyl ^1H T_2 values, it is necessary to account for dependencies of the measured intensity values, S_i^{EtOH} and S_i^{NAA} on several biophysical parameters.

The ethanol ^1H methyl MRS intensity is proportional to the product of the BEC and two scaling factors, $X_{T_1}^{EtOH}$ and X_J^{EtOH} , which reflect the effects of incomplete polarization of ^1H nuclei under conditions in which $TR < \sim 3 T_1$, and intensity variation due to incomplete refocusing of the scalar coupling interaction between the ethanol ^1H methyl and ^1H methylene nuclei, respectively. In addition, for the most general case considered herein (models M3 and M5), the distribution and T_2 values of ethanol differ between GM, WM, and CSF. The ethanol ^1H methyl MRS intensity can therefore be expressed as

$$S_i^{EtOH} = X_J^{EtOH} \left(\rho^{GM} X_{T_1}^{EtOH,GM} GM_i e^{-TE/T_2^{EtOH,GM}} + \rho^{WM} X_{T_1}^{EtOH,WM} WM_i e^{-TE/T_2^{EtOH,WM}} + \rho^{CSF} X_{T_1}^{EtOH,CSF} CSF_i e^{-TE/T_2^{EtOH,CSF}} \right) \quad [10]$$

in which ρ^{GM} , ρ^{WM} , and ρ^{CSF} represent equilibrium ethanol concentrations in GM, WM, and CSF, respectively, and the same notation is used to designate tissue-class-specific ethanol T_2 values. The factors $X_{T_1}^{EtOH,\gamma}$ are the steady-state magnetization for ethanol methyl ^1H nuclei within tissue components γ ($\gamma \in \{GM, WM, CSF\}$), given tissue subclass specific T_1 values and the TR [17,21]

$$X_{T_1}^{EtOH,\gamma} = 1 - \exp(-TR/T_1^{EtOH,\gamma}), \quad [11]$$

and it approaches a value of 0 if $TR \ll T_1$ and a value of unity if $TR \gg T_1$. Herein we adopt longitudinal relaxation time constants of $T_1^{EtOH,GM} = T_1^{EtOH,WM} = 2000$ ms, and $T_1^{EtOH,CSF} = 4400$ ms, according to Chiu and co-workers [3]. When combined with the TR value of 1770 ms, the resulting scaling factors are $X_{T_1}^{EtOH,GM} = X_{T_1}^{EtOH,WM} = 0.59$, and $X_{T_1}^{EtOH,CSF} = 0.33$.

The 3-bond scalar coupling interaction between the ethanol methyl and methylene ^1H nuclei, characterized by the coupling constant $^3J = 7.35$ Hz, results in a TE-dependent modulation of X_J^{EtOH} between 0 and 1, with a value of 1 being observed for integer multiples of $\frac{2}{^3J}$ [9,23,24]. The factor X_J^{EtOH} corresponding to the TE value used in this study (150 ms) was measured by applying the quantification procedures described above to a 10 mM solution of ethanol (data not shown). Following analysis of data acquired at TE = 272, 544, 816 ms, the ethanol methyl ^1H T_2 in dilute solution was found to be 725 ms. The average methyl ^1H MRS intensity obtained from the 24 PRESS-selected voxels in the CSI experiment used for these studies was found to be 0.4 that of the T_2 -corrected value expected in the absence of scalar coupling to methylene ^1H nuclei. Thus, scaling parameter X_J^{EtOH} was assigned a value of 0.4. The factors ρ^{GM} , ρ^{WM} , and ρ^{CSF} are proportional to water composition for the tissue sub-classes [25]. The water composition of CSF is 1.22 that of blood [25], and the water content of GM and WM is 0.83 and 0.71 that of CSF, respectively [26]. Thus values of 1.01, 0.87, and 1.22 were used in the analyses presented here for ρ^{GM} , ρ^{WM} , and ρ^{CSF} , respectively. The tissue-specific transverse

relaxation time constants, $T_2^{EtOH,GM}$, $T_2^{EtOH,WM}$, and $T_2^{EtOH,CSF}$ are treated as adjustable parameters, determined by the model M3 and M5 parameters c_{GM} , c_{WM} , and c_{CSF} , as described below.

The NAA methyl 1H MRS intensity, S_i^{NAA} , is determined by the NAA concentration in brain, as well as the NAA methyl 1H T_1 and T_2 values

$$S_i^{NAA} = [NAA] V_i^{NAA} X_{T_1}^{NAA} e^{-TE/T_2^{NAA}}. \quad [12]$$

The NAA concentration, [NAA], has been estimated to range from 8 mM to 12 mM, as discussed in [14,27]. Herein, a parenchymal NAA concentration of 8 mM is assumed. The NAA concentration in CSF is too small to be detectable by *in vivo* MRS [14]. The scaling factor $X_{T_1}^{NAA}$ is defined by replacing T_1^{NAA} for T_1^{EtOH} in Eq. [11]. As described above, such substitution yields $X_{T_1}^{NAA} = 0.72$. Potential differences in the NAA methyl 1H T_2 between GM and WM have not been observed [17]. Therefore an average T_2^{NAA} of 271 ms obtained from one voxel overlapping primarily GM structures and one voxel overlapping primarily WM structures [16] is used herein to reflect the T_2 of NAA within the brain parenchyma.

By combining Eqs. [9], [10], and [12], it is possible to obtain relationships between the fitted coefficients of Eq. [9] and the ethanol methyl 1H T_2 value in brain, parenchymal tissue, GM, WM, and CSF. The specific expressions used to obtain the transverse relaxation time constants are summarized in Table 1 for each of the Eq. [9] models.

Results

After visually inspecting the 288 CSI spectra from the 12 subjects, a total of 148 voxels were observed to satisfy the criteria of possessing 1H methyl NAA signals resolved to baseline. In all 12 subjects, the set of spectra satisfying this criterion corresponded to a connected set of voxels in the center of the selected volume. Among the quantified spectra, the mean S_i^{EtOH}/S_i^{NAA} ratio is 0.655, and the mean and standard deviation in the BEC 10 minutes following ethanol infusion for the 12 subjects is 117 ± 11 mg %.

Table 2 summarizes the results of fitting models M1–M5 to the 148 data values. As expected, the variance in the residuals, σ_e^2 , decreases with increasing number of model parameters, because the additional degrees of freedom in the model are used to account for variance in the data. The Akaike Information Criterion (AIC) provides a quantitative method for determining whether an observed reduction in variance could be observed due to chance. In Table 2, the AIC corrected for small sample size (AICc) [28] are given for each model. Both σ_e^2 and the AICc decrease with increasing model complexity, indicating a greater likelihood that the additional adjustable parameters account for more variance in the data than could be explained by random chance. In Figures 6a and 6c, predicted and observed data values for the 148 spectra are compared for models M4 and M5.

The fact that model M1 is characterized by the largest (least negative) AICc suggests that differences in MRS intensity between parenchymal tissue and CSF contribute a significant amount of variance in y_i values. For models M2 and M4, it is observed that the ethanol 1H methyl MRS intensity is lower per unit ethanol concentration in parenchymal tissue than within CSF. Additionally, for models M3 and M5, ethanol MRS intensity is lower within WM than it is in GM. These differences are consistent with the ethanol methyl 1H T_2 value being lower

within brain parenchymal space than in CSF, and within the parenchyma, the ethanol methyl ^1H T_2 is lower in WM than in GM. For models M2 and M5, ^1H methyl MRS intensity per unit ethanol concentration is lower within CSF than in GM. This finding is consistent with the set of $T_1^{EtOH,CSF}$, $T_1^{EtOH,GM}$, ρ^{CSF} , and ρ^{GM} values obtained from the literature, because the ratios c_{CSF}/c_{GM} obtained from models M3 and M5 in Table 2 are approximately equal to the ratio of steady-state magnetization ratio $(\rho^{CSF} X_{T_1}^{EtOH,CSF})/(\rho^{GM} X_{T_1}^{EtOH,GM})$ of 0.68. With reference to literature estimates for [NAA], T_2^{NAA} , ρ^{GM} , ρ^{WM} , ρ^{CSF} , and longitudinal relaxation time constants for ethanol and NAA methyl ^1H nuclei, it is possible to use the expressions in Table 1 to estimate the ethanol methyl ^1H T_2 within the various tissue compartments based on the parameter estimates reported in Table 2. Values resulting from models M4 and M5 for the 12 monkeys are given in Table 3, and plotted in Figures 6b and 6d.

Discussion

The results of the methodology described here demonstrate that brain ^1H MRS following intravenous infusion of ethanol can be used to perform quantitative measurements of ethanol MRS signal intensity in GM and WM within nonhuman primate subjects. Extension of this methodology to nonhuman primates enables experiments to be performed to investigate the relationships between ethanol MRS intensity and tolerance to the intoxicating effects of ethanol, and following prolonged ethanol exposure (either through self- or experimenter-administration), in a more controlled environment than is achievable in human studies. Within a group of ethanol-naïve nonhuman primates (a population that is not readily accessible in human studies), it is found that model expressions for the ethanol methyl ^1H MRS intensity that account for differences between GM, WM, and CSF (Eq. [9] models M3 and M5) explain a significantly greater amount of variance in experimental data than models that do not (Eq. [9] models M1, M2, and M4). Among Eq. [9] expressions M3 and M5, it is found that inter-animal variability in MRS intensities contributes a significant amount of variance in the observed data, and thus model M5 is considered superior for the analysis of data presented here. This result suggests that application of the methods described here are of potential utility for assigning variation in ethanol MRS intensity to specific brain tissue subclasses.

The considerably larger extent of inter-individual variation in GM MRS intensity, relative to WM intensity variation, is noteworthy. As shown in Table 2, the standard error in measurement for c_{GM} is 43% of the mean value, compared to 28% of the mean value for c_{WM} . This finding is potentially important because individual differences in ethanol signal intensity may be mechanistically related to individual differences that have been found to exist among both human and animal subjects in their sensitivity to the intoxicating effects of ethanol [e.g., 29, 30]. Localization of the source of variation in ethanol MRS intensity to GM, as supported by the data presented here, is interesting because the neuronal protein sites through which ethanol is widely believed to exert its pharmacological effects [31] reside within GM. This view that ethanol exerts its pharmacological effects through interactions with neuronal protein sites is at odds with earlier proposals that ethanol acts through non-specific effects on lipid membranes. For several reasons, the lipid theory of ethanol's actions has largely been abandoned [8]. However, this theory has recently been invoked to explain changes in ethanol MRS intensity that have been observed during the development of tolerance to ethanol [3]. Specifically, it has been suggested that with the development of tolerance to ethanol, changes in MRS intensity reflect changes in biophysical properties of membranes, such as membrane fluidity [32]. Given the finding of the current study that variability of ethanol MRS intensity is localized to GM, an alternate interpretation could be that MRS intensity is affected by interactions of ethanol with protein, as well as lipid, macromolecular constituents. It will therefore be of interest in future studies to determine whether changes in ethanol MRS intensity occur with the development of tolerance, and whether such changes are found within both GM and WM (as

would be predicted by the lipid theory), or if they are primarily associated with changes in GM (as would be predicted by the protein theory).

Comparison to previous ethanol ^1H methyl T_2 measurements

By assuming previously-published values of $[\text{NAA}]$, T_2^{NAA} , ρ^{GM} , ρ^{WM} , ρ^{CSF} , and longitudinal spin relaxation time constants for NAA and ethanol methyl ^1H nuclei, it is possible to interpret c_{GM} , c_{WM} , and c_{CSF} parameter estimates in terms of the ethanol methyl ^1H T_2 within GM, WM, and CSF, respectively, by combining Eqs. [10–12] (Table 1). Previously, Sammi et al. [9] reported directly measured ethanol ^1H T_2 values in GM and WM obtained using a pulse sequence with methyl ^1H selective refocusing RF pulses to avoid confounds in T_2 measurements associated with scalar coupling between methyl and methylene ^1H nuclei. Despite the values reported in Table 3 being derived using a less direct method, a consistency is observed in which the ethanol ^1H methyl T_2 in GM is approximately two-fold higher than in WM. The range of T_2 values reported in Table 3 (237 ± 103 ms in GM and 131 ± 36 ms in WM) possesses a larger mean value than corresponding values reported by Sammi et al. [9] (118 ± 12 and 50 ± 9 for GM and WM, respectively). One potential explanation for the longer T_2 values observed in this study is that it was performed at a 3T magnetic field strength, compared to the previous study performed at 4T [9]. At larger magnetic fields, a smaller T_2 will be observed for molecular species that undergo chemical shift modulation on the μs to ms time scale, potentially as a consequence of transient interactions with macromolecules [33–35]. A second potential explanation for the longer T_2 values observed in this study relative to Sammi et al. is that T_2 estimates reported here depend on several additional parameters derived from additional experiments on aqueous solution (such as X_j^{EtOH}) and from literature reports ($[\text{NAA}]$, T_2^{NAA} , ρ^{GM} , ρ^{WM} , ρ^{CSF} , etc.). As an example, if a value of 0.5 is used for X_j^{EtOH} rather than the experimentally-derived value of 0.4, the distributions of T_2 values for GM and WM would change to 169 ± 57 and 108 ± 25 , respectively, which is in closer agreement with results reported by Sammi et al. [9] than the distributions obtained using the experimentally-derived X_j^{EtOH} value. The degree of agreement between T_2 values inferred from the measurements presented here and those directly measured by Sammi et al. [9], as well as the expected $T_2^{\text{GM}} > T_2^{\text{WM}}$ relationship found here serve to validate that intensity differences between GM, WM, and CSF, and among individuals, are within an acceptable range.

Not included in Eqs. [10]–[12] are potential effects of an invisible sub-population of ethanol molecules, or of heterogeneous pools of ethanol within GM, WM, or CSF that could give rise to non-monoexponential transverse relaxation. Models M1 – M5 could be adjusted to incorporate a fraction $0 < \alpha < 1$ of ethanol molecules that is unobservable due to extremely rapid transverse relaxation, or is characterized by a methyl ^1H T_2 value other than the remaining molecules. Such factors were not included in this work because evidence for such heterogeneous pools have not been observed in the TE-dependent measurements of Sammi et al. [9].

Future directions

In vivo MRS studies of human subjects have indicated the ethanol ^1H methyl MRS intensity relates to acute [36], and chronic [4] tolerance to ethanol's intoxicating effects. One difficulty in the above experiments is in defining the "tolerant" and "non-tolerant" groups within the heterogeneous human population being studied. It has been repeatedly demonstrated that those with a family history of alcoholism have a less intense subjective response to ethanol than those without such a family history [for review, see 37] however, the same research group that reported differences between tolerant and non-tolerant groups found no differences between those with a family history of alcoholism and control subjects [3]. With the methodology

developed here, it will be possible to augment the data formerly limited to human studies to characterize the ethanol ^1H methyl MRS signal in nonhuman primate subjects following experimentally-controlled ethanol exposure procedures. In conjunction with experiments in which monkeys self-administer ethanol over long periods of time [38,39], it will be possible to determine whether ethanol MRS signals respond to the development of chronic tolerance over periods of time of relevance to human behavior. To address this issue, the series of models M1–M5 will be straightforwardly extended to determine whether differences in MRS intensity exist between subjects in the ethanol-naïve state, and following chronic exposure to ethanol.

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References

1. Petroff OA, Novotny EJ, Ogino T, Avison M, Prichard JW. *In vivo measurements of ethanol concentration in rabbit brain by ^1H magnetic resonance spectroscopy*. J Neurochem 1990;54(4):1188–95. [PubMed: 2313285]
2. Hanstock CC, Rothman DL, Shulman RG, Novotny EJ Jr, Petroff OA, Prichard JW. Measurement of ethanol in the human brain using NMR spectroscopy. J Stud Alcohol 1990;51(2):104–7. [PubMed: 2308346]
3. Chiu TM, Mendelson JH, Sholar MB, Mutschler NH, Wines JD, Hesselbrock VM, Mello NK. Brain alcohol detectability in human subjects with and without a paternal history of alcoholism. J Stud Alcohol 2004;65(1):16–21. [PubMed: 15000499]
4. Chiu TM, Mendelson JH, Woods BT, Teoh SK, Levisohn L, Mello NK. *In vivo* proton magnetic resonance spectroscopy detection of human alcohol tolerance. Magn Reson Med 1994;32:511–516. [PubMed: 7997118]
5. Kaufman MJ, Chiu TM, Mendelson JH, Woods BT, Teoh SK, Eros-Sarnyai M, Mercer G, Mello NK. Brain alcohol detectability increase with repeated administration in humans: a proton spectroscopy study. Magn Reson Med 1996;35:435–440. [PubMed: 8699957]
6. Govindaraju V, Meyerhoff DJ, Maudsley AA, Vermathen M, Weiner MW. *Effects of brain membranes on ^1H nuclear magnetic resonance signal intensity of ethanol in vitro*. Alcohol Alcohol 1997;32(6):671–81. [PubMed: 9463721]
7. Moxon LN, Rose SE, Haseler LJ, Galloway GJ, Brereton IM, Bore P, Doddrell DM. The visibility of the ^1H NMR signal of ethanol in the dog brain. Magn Reson Med 1991;19(2):340–8. [PubMed: 1881324]
8. Peoples RW, Li C, Weight FF. Lipid vs protein theories of alcohol action in the nervous system. Annu Rev Pharmacol Toxicol 1996;36:185–201. [PubMed: 8725387]
9. Sammi MK, Pan JW, Telang FW, Schuhlein D, Molina PE, Volkow ND, Springer CS, Hetherington HP. *Measurements of human brain ethanol T_2 by spectroscopic imaging at 4T*. Magn Reson Med 2000;44:35–40. [PubMed: 10893519]
10. Meyerhoff DJ, Rooney WD, Tokumitsu T, Weiner MW. Evidence of multiple ethanol pools in the brain: an *in vivo* proton magnetization transfer study. Alcohol Clin Exp Res 1996;20(7):1283–8. [PubMed: 8904983]
11. Hetherington HP, Telang FW, Pan JW, Sammi MK, Schuhlein D, Molina PE, Volkow ND. Spectroscopic imaging of the uptake kinetics of human brain ethanol. Magn Reson Med 1999;42:1019–1026. [PubMed: 10571922]
12. Mason G, Bendszus M, Meyerhoff D, Hetherington H, Schweinsburg B, Ross B, Taylor M, Krystal J. Magnetic resonance spectroscopic studies of alcoholism: from heavy drinking to alcohol dependence and back again. Alcohol Clin Exp Res 2005;29(1):150–158. [PubMed: 15895489]
13. Mugler JP 3rd, Brookeman JR. *Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE)*. Magn Reson Med 1990;15(1):152–7. [PubMed: 2374495]

14. De Graaf, RA. In vivo NMR spectroscopy: principles and techniques. 2. Vol. xxi. Chichester, West Sussex, England; Hoboken, NJ: John Wiley & Sons; 2007. p. 570
15. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci* 1987;508:333–48. [PubMed: 3326459]
16. Mlynarik V, Gruber S, Moser E. Proton T (1) and T (2) relaxation times of human brain metabolites at 3 Tesla. *NMR Biomed* 2001;14(5):325–31. [PubMed: 11477653]
17. Hetherington HP, Mason GF, Pan JW, Ponder SL, Vaughan JT, Twieg DB, Pohost GM. Evaluation of cerebral gray and white matter metabolite differences by spectroscopic imaging at 4.1T. *Magn Reson Med* 1994;32(5):565–71. [PubMed: 7808257]
18. McLaren DG, Kosmatka KJ, Oakes TR, Kroenke CD, Kohama SG, Matochik JA, Ingram DK, Johnson SC. A population-average MRI-based atlas collection of the rhesus macaque. *Neuroimage* 2009;45(1):52–9. [PubMed: 19059346]
19. Cavanagh, J.; Fairbrother, WJ.; Palmer, AGI.; Skelton, NJ. *Protein NMR Spectroscopy*. San Diego: Academic Press, Inc; 1996. p. 587
20. Yablonskiy DA, Neil JJ, Raichle ME, Ackerman JJ. Homonuclear J coupling effects in volume localized NMR spectroscopy: pitfalls and solutions. *Magn Reson Med* 1998;39(2):169–78. [PubMed: 9469698]
21. Haacke, M.; Brown, R.; Thompson, J.; Venkatesan, R. *Magnetic resonance imaging: physical principles and sequence design*. New York: Wiley-Liss; 1999. p. 914
22. Mareci TH, Brooker HR. Essential considerations for spectral localization using indirect gradient encoding of spatial information. *J Magn Reson* 1991;92:229–246.
23. Spielman DM, Glover GH, Macovski A, Pfefferbaum A. Magnetic resonance spectroscopic imaging of ethanol in the human brain: a feasibility study. *Alcohol Clin Exp Res* 1993;17(5):1072–7. [PubMed: 8279668]
24. Rose SE, Crozier S, Brereton IM, Moxon LN, Galloway GJ, Bore P, Doddrell DM. *Measurement of the T₂ relaxation time of ethanol and cerebral metabolites, in vivo*. *Magn Reson Med* 1992;23(2):333–45. [PubMed: 1549047]
25. Kalant, H. Absorption, diffusion, distribution, and elimination of ethanol: effects on biological membranes. In: Kissin, H.; Begleiter, H., editors. *The Biology of Alcoholism*, Vol. 1, Biochemistry. Plenum; New York: 1971. p. 1-62.
26. Whittall KP, MacKay AL, Graeb DA, Nugent RA, Li DK, Paty DW. *In vivo measurement of T₂ distributions and water contents in normal human brain*. *Magn Reson Med* 1997;37(1):34–43. [PubMed: 8978630]
27. Sager TN, Fink-Jensen A, Hansen AJ. Transient elevation of interstitial N-acetylaspartate in reversible global brain ischemia. *J Neurochem* 1997;68(2):675–82. [PubMed: 9003055]
28. McQuarrie, ADR.; Tsai, CL. *Regression and time series model selection*. Singapore: World Scientific; 1998.
29. Barr CS, Newman TK, Becker ML, Champoux M, Lesch KP, Suomi SJ, Goldman D, Higley JD. Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. *Alcohol Clin Exp Res* 2003;27(5):812–7. [PubMed: 12766626]
30. Schuckit MA. Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry* 1994;151:184–189. [PubMed: 8296886]
31. Harris RA, Trudell JR, Mihic SJ. Ethanol's molecular targets. *Sci Signal* 2008;1(28):re7. [PubMed: 18632551]
32. Rubin E, Rottenberg H. Ethanol and biological membranes: injury and adaptation. *Pharmacol Biochem Behav* 1983;18(Suppl 1):7–13. [PubMed: 6227017]
33. Bartha R, Michaeli S, Merkle H, Adriany G, Andersen P, Chen W, Ugurbil K, Garwood M. *In vivo ¹H₂O T₂^{*} measurement in the human occipital lobe at 4T and 7T by Carr-Purcell MRI: detection of microscopic susceptibility contrast*. *Magn Reson Med* 2002;47(4):742–50. [PubMed: 11948736]
34. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, Merkle H, Ugurbil K, Chen W. *Proton T₂ relaxation study of water, N-acetylaspartate, and creatine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T*. *Magn Reson Med* 2002;47(4):629–33. [PubMed: 11948722]

35. Oros-Peusquens AM, Laurila M, Shah NJ. Magnetic field dependence of the distribution of NMR relaxation times in the living human brain. *Magma* 2008;21(1–2):131–47. [PubMed: 18338191]
36. Kaufman MJ, Chiu TM, Mendelson JH, Woods BT, Mello NK, Lukas SE, Fivel PA, Wightton LG. In vivo proton magnetic resonance spectroscopy of alcohol in rhesus monkey brain. *Magn Reson Imaging* 1994;12(8):1245–53. [PubMed: 7854030]
37. Schuckit MA. An overview of genetic influences in alcoholism. *J Subst Abuse Treat* 2009;36(1):S5–14. [PubMed: 19062348]
38. Grant KA, Leng X, Green HL, Szeliga KT, Rogers LS, Gonzales SW. Drinking typography established by scheduled induction predicts chronic heavy drinking in a monkey model of ethanol self-administration. *Alcohol Clin Exp Res* 2008;32(10):1824–38. [PubMed: 18702645]
39. Vivian JA, Green HL, Young JE, Majerksy LS, Thomas BW, Shively CA, Tobin JR, Nader MA, Grant KA. Induction and maintenance of ethanol self-administration in cynomolgus monkeys (*Macaca fascicularis*): Long-term characterization of sex and individual differences. *Alcohol Clin Exp Res* 2001;25:1087–1097. [PubMed: 11505038]

Figure 1.

Figure 1a shows axial and sagittal views of the T_1 -weighted image used to position a CSI slice. In Figure 1b, axial and sagittal views of the target volume that was selected by the pulse sequence used in this study are outlined in red. All rf pulses were applied at the resonance frequency of the NAA methyl ^1H . Thus, due to the chemical shift difference between NAA and ethanol methyl ^1H signals, the selected ethanol methyl ^1H volume (shown in blue) is shifted within the CSI slab (shown in yellow). By Fourier-encoding an 8 by 8 square matrix, a nominal CSI resolution of 8.0 mm isotropic was obtained (shown in green).

Figure 2. gives a schematic overview of the overall data processing strategy employed in this study.

Figure 3.

Figure 3a shows a typical distribution of high (✓) and low (✗) quality spectra, with the majority of low quality spectra being located on the periphery of the brain, where contamination from extracranial lipid signals is the greatest. Figure 3b displays a baseline (pre ethanol infusion) spectrum (black trace), and the sum of NMR spectra obtained 20–40 minutes following the beginning of the infusion, divided by the number of averaged acquisitions (red trace). The ethanol difference spectrum (red trace minus black trace) is shown in the bottom panel of figure 3b (blue). Figure 3c is an example of a spectrum in which extracranial lipid signals were incompletely suppressed.

Figure 4.

shows the timecourse of the integrated intensity of the ethanol difference spectrum (the blue trace in the bottom panel of Figure 3b) between 1 and 1.5 ppm, expressed as a ratio of the integrated intensity of the NAA peak between 2.0 and 2.2 ppm. The ethanol was infused between 0–10 minutes. A rapid decay in signal intensity is seen immediately after the infusion as arterial and venous ethanol concentrations equilibrate. After this equilibration, the data in the shaded area were summed to provide a single ethanol MRS intensity estimate.

Figure 5. illustrates the process by which non-uniformity of the PRESS slice profile, chemical shift artifacts, and the effects of finite CSI sampling are incorporated into the estimation of the GM, WM, and CSF volume fraction of a given voxel. In this example, a typical GM segmentation volume (Figure 5a) is multiplied ($\times$) by the 3D slice profile (Figure 5b). The product (Figure 5c) is then convolved ($*$) with the point spread function (Figure 5d) to obtain the corrected GM segmentation volume.

Figure 6.

Figure 6a shows the correlation between the intensity of the ethanol signal predicted by model M4 (Eq. [11]) and the observed ethanol signal intensity for each of the 148 voxels quantified in these experiments. In Figure 6b, the T₂ value of ethanol in parenchymal tissue is given for each individual subject, assuming the following: [NAA]=10 mM; the NAA ¹H T₂= 271 ms [16]; $\rho^{GM} = \rho^{WM} = 0.74$; and $\rho^{CSF} = 1.22$ [25]. Figure 6c shows the correlation between the intensity of the ethanol signal predicted by model M5 (Eq. [11]) and the observed ethanol signal intensity for each of the 148 voxels quantified in these experiments. In Figure 6d, the T₂ value of ethanol in both grey matter and white matter are given for each individual subject. For Figures 6a and 6c, BEC is expressed in units of mg ethanol/dl blood.

Table 1

Relationships between Eq. [9] fitted parameters and ethanol transverse relaxation time constants.^a

M1	$c_{Brain} = X_J^{EtOH} \cdot \rho_{GM} \cdot X_{T_1}^{EtOH,GM} + \rho_{WM} \cdot X_{T_1}^{EtOH,WM} + \rho_{CSF} \cdot X_{T_1}^{EtOH,CSF} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$		
M2, M4	$c_{Par} = X_J^{EtOH} \cdot \rho_{GM} \cdot X_{T_1}^{EtOH,GM} + \rho_{WM} \cdot X_{T_1}^{EtOH,WM} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$	$c_{CSF} = X_J^{EtOH} \cdot \rho_{CSF} \cdot X_{T_1}^{EtOH,CSF} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$	
M3, M5	$c_{GM} = X_J^{EtOH} \cdot \rho_{CSF} \cdot X_{T_1}^{EtOH,CSF} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$	$c_{WM} = X_J^{EtOH} \cdot \rho_{CSF} \cdot X_{T_1}^{EtOH,WM} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$	$c_{CSF} = X_J^{EtOH} \cdot \rho_{CSF} \cdot X_{T_1}^{EtOH,GM} \cdot \exp\left(-TE/T_2^{EtOH}\right) \cdot S^{NAA}$

^aFor purposes of interconverting fitted Eq. [9] coefficients and ethanol methyl ¹H T₂ values, the un-subscripted NAA signal intensity, $S^{NAA} \equiv [NAA] \cdot X_{T_1}^{NAA} \cdot e^{-TE/T_2^{NAA}}$, is defined as the intensity value obtained from Eq. [12] under the condition that $V_i^{NAA} = 1$.

Table 2

Model statistics.

Model	$\sigma_{\varepsilon}^2 (\times 10^{-6})$	AICc ^a	Model Parameter Estimates
M1	2.076	-12.06	$c_{Brain} = 0.025$,
M2	1.998	-12.09	$c_{CSF} = 0.029$, $c_{j,Par} = 0.025$
M3	1.871	-12.14	$c_{CSF} = 0.023$, $c_{GM} = 0.034$, $c_{WM} = 0.020$
M4	1.589	-12.15	$c_{CSF} = 0.030$, $\langle c_{j,Par} \rangle^b = 0.024 \pm 0.003$
M5	1.210	-12.20	$c_{CSF} = 0.021$, $\langle c_{j,GM} \rangle^b = 0.035 \pm 0.012$, $\langle c_{j,WM} \rangle^b = 0.019 \pm 0.006$

^aThe AICc is defined as $\ln(\sum_i (\sigma_i^2)/n) + (n+1)/(n-k-2)$, in which n is the number of data points (here, $n=148$) and k is the number of adjustable parameters for each model ($k=1$ for M1, $k=2$ for M2, $k=3$ for M3, $k=13$ for M4, and $k=25$ for M5).

^bQuantities in angled brackets are averages of the 12 values obtained from the 12 monkeys ± 1 standard error.

Table 3

Ethanol methyl ^1H T_2 values estimated for parenchyma, GM, and WM.

	Model M4	Model M5	
Monkey	T_2 (ms) parenchyma	T_2 (ms) GM	T_2 (ms) WM
1	143	245	107
2	147	317	104
3	162	164	174
4	151	401	78.5
5	110	110	118
6	189	389	136
7	130	121	146
8	157	134	209
9	136	131	156
10	148	282	97.1
11	172	282	127
12	158	264	117
Mean (SD)	150 (20.3)	237 (103)	131 (36.1)