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Neurometabolite concentration and clinical features of chronic alcohol use: A proton magnetic resonance spectroscopy study

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Abstract

Chronic, heavy alcohol consumption may affect the concentration of neurometabolites assessed with proton magnetic resonance spectroscopy (^{1}H-MRS). We investigated the largest sample reported to date (N = 213) with the primary goal of determining how specific clinical features impact neurometabolite concentrations in an anterior cingulate gray matter voxel. This community dwelling sample included both treatment seeking and non-treatment seeking individuals. A healthy control group (N = 66) was matched for age and education. In multivariate analyses predicting neurometabolite concentrations, the heavy drinking group had greater concentrations overall. An age by group interaction was noted, as group difference across neurometabolites increased with age. More years drinking, but not more drinks per drinking day (DPDD) predicted greater concentrations of choline-containing compounds (Cho), creatine-phosphocreatine (Cre), glutamate-glutamine (Glx), and n-acetyl-aspartate (NAA). The effects of other clinical variables (depression, cigarette smoking, marijuana use) were negligible. After controlling for DPDD and years drinking, treatment-seeking status had no impact on neurometabolites. In the very oldest portion of the sample (mean age = 50), however, a negative relationship was seen between NAA and years drinking. These results suggest that the nature of neurometabolite abnormalities in chronic heavy drinkers may vary as a function of duration of abuse.

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Author Contributions

All authors contributed to writing the manuscript. Ronald A. Yeo, Kent E. Hutchison, Robert J. Thoma, Timothy C. Durazzo, Nicole Harlaar, and Mollie Monig additionally contributed to the design and analyses. Kent E. Hutchison, Andrew Mayer, and Robert J. Thoma collected the data. Charles Gasparovic, Ravi Kalyanam, and Vince D. Calhoun supervised image acquisition and ^{1}H-MRS analyses.

Keywords

alcohol use disorders; magnetic resonance spectroscopy; choline; NAA

1. Introduction

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a useful tool for identifying abnormality of brain metabolites in individuals with alcohol use disorders (AUD) and histories of heavy drinking. In contrast to other common neuroimaging modalities, ^{1}H-MRS investigates the metabolic milieu of working neurons, providing insights on neural integrity, energy metabolism, and membrane turnover. A rich ^{1}H-MRS literature has emerged characterizing both psychiatric disorders (Maddock and Buonocore, 2012) and brain injury (Yeo et al., 2011), providing an informative context in which to interpret results from studies of chronic, heavy alcohol consumption.

A recent review of MRS studies of alcohol abuse (Meyerhoff, Durazzo and Ende, 2011) notes the need to elucidate the impact of comorbid conditions such as smoking or depression on neurometabolite concentrations. Variation in the severity and duration of alcohol abuse also likely contributes to the observed clinical heterogeneity in MRS findings. Moreover, individuals who are actively seeking treatment may differ from those who are not on a number of potentially salient dimensions (Smith and Fein, 2010). Treatment-seeking status overlaps with severity and chronicity of alcohol consumption, as well as with comorbid substance use disorders and general psychopathology (Cohen et al., 2007; Fein and Landman, 2005). To date, one study directly compared brain metabolite levels in treatment-seeking and non-treatment-seeking individuals with AUD, reporting lower NAA, Cho, and mI in the treatment-seeking group (Gazdzinski, Durazzo, Weiner et al.,, 2008). For the most part, these differences were not accounted for by demographic factors, drinking severity, comorbid psychopathology, or other clinical variables.

Two major methodological issues have limited ability to delineate these and other sources of within group variability. First, many ^{1}H-MRS studies have recruited rather small samples. Three of the largest studies conducted to date have examined 25 patients (Schweinsburg, Alhassoon, Taylor et al.,, 2003), 46 patients (Meyerhoff, Blumenfeld, Truran *et al.,*, 2004), and 33 patients (Ende, Welzel, Walter et al.,, 2005), while many other studies had much smaller samples. Reduced statistical power affects comparisons with control groups and has an even greater impact on ability to detect correlations with comorbid conditions or drinking history variables within the heavy drinking group. Second, many studies have focused on more severely affected individuals, facilitating analysis of group differences, but reducing variance on measures capturing comorbid conditions or alcohol consumption.

The current study investigated absolute neurometabolite concentrations in a large, demographically diverse sample of individuals with a broad range of alcohol consumption patterns, with the central goal of detecting the impact of important components of the extended phenotype of alcohol abuse. We placed our ^{1}H-MRS in the anterior cingulate gyrus, a commonly studied region because of the high signal quality of the data obtained. This region is especially relevant for studies of substance abuse, given its roles in reward processing (Makris, Oscar-Berman, Jaffin et al.,, 2008), conflict management, and decision-making (Beckmann, Johansen-Berg and Rushworth, 2009), cognitive skills with real world relevance for substance abuse. We also compared this clinical sample to healthy controls.

2. Methods

2.1 Participants

Participants were recruited from the local community through advertisements and word-of-mouth. Individuals in the heavy drinking group were recruited under two study protocols that differed as to whether the target population was seeking treatment for alcohol problems. Both studies, however, were designed to explore the biological heterogeneity among heavy drinkers. To be included, participants were between the ages of 21 and 55, reported 5 or more binge drinking episodes per month, did not report current use of illicit drugs other than marijuana, and did not have a history of traumatic brain injury with loss of consciousness. Participants were instructed to refrain from alcohol or illicit drug use for 24 hours, and blood alcohol concentration of 0 was confirmed with a Breathalyzer at the time of the scan. All participants had a Clinical Institute Withdrawal Assessment Scale (CIWA; Sullivan, Sykora, Schneiderman, et al., 1989) score less than 8, indicating no need for detoxification.

Healthy control participants were between the ages of 19 and 51 and reported neither history of substance abuse or dependence, nor other Axis I psychopathology upon interview. Exclusion criteria were: 1) history of neurological disorder or disease; 2) history of traumatic brain injury with loss of consciousness for more than 5 minutes; 3) mental retardation, dementia, or other cognitive impairment; and 4) current drug use as assessed by urine screen. These participants were originally recruited as controls for other studies, and hence, detailed information on current substance use was not available. All participants provided informed consent, and the University of New Mexico Human Research Review Committee approved this study.

2.2 Diagnostic testing

AUD participants completed the Alcohol Dependence Scale (ADS) (Skinner and Allen, 1982) and Beck Depression Inventory (BDI) (Beck et al., 1996) as well as questionnaires on demographics and drinking history. Recent use of alcohol and other substances was recorded on a timeline follow back instrument, from which drinks per drinking day (DPDD), frequency of marijuana use (number of days smoking marijuana in the past 90 days), and severity of cigarette use (number of cigarettes per smoking day) were calculated.

2.3 MRI and MRS data acquisition

^{1}H-MRS data were collected for the AUD group at a mean of 2.66 (SD = 2.85) days after the participant's self-reported last drink. MR imaging and spectroscopy were performed on a single Siemens 3-Tesla TrioTIM MR scanner using the 12-channel radiofrequency head coil. T1-weighted images were collected in the sagittal plane using a five-echo 3D MPRAGE sequence (TR/TE/TI = 2530/1.64, 3.5, 5.36, 7.22, 9.08/1200 ms, flip angle = 7°, field of view (FOV) = 256×256 mm, matrix = 25 6x 256, 1 mm thick slice, 192 slices, GRAPPA acceleration factor = 2). Using these images, a single ^{1}H-MRS voxel was positioned in the bilateral medial frontal cortex directly superior to the corpus callosum (Mullins, Chen, Xu et al., 2008), comprised mostly of the anterior cingulate gyrus (Figure 1). A PRESS sequence (TR/TE = 1.5 s/40 ms, voxel size = 20×30×20 mm, averages = 192) was collected, using a TE of 40 ms for improved detection of Glx (Mullins, Chen, Xu et al., 2008). Due to a small protocol adjustment, the raw data were acquired with either a dwell time of 1 ms or 0.625 ms. An unsuppressed water sequence was collected with 16 averages (and otherwise identical parameters) from each single voxel, for use as a concentration reference and for eddy current correction (Klose, 1990) in LCModel analysis.

2.4 MRS data analysis

Raw time-domain ^{1}H-MRS data from 4.2 – 1.8 ppm in the spectral dimension were analyzed using LCModel (Gasparovic, Yeo, Mannell et al.,, 2009; Provencher, 2001) with the unsuppressed water scan as a concentration reference (Gasparovic, Yeo, Mannell et al., 2009). Metabolite concentrations were computed for total creatine plus phosphocreatine (Cre), total choline containing compounds (Cho), total N-acetylaspartate plus N-acetyl-aspartylglutamate (NAA), myoinsitol (mI), and the combined Glu plus Gln signal (Glx). T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid using SPM5. Metabolite concentrations were then computed, correcting for partial-volume and T1 and T2 relaxation effects, using methods described previously (Gasparovic, Yeo, Mannell et al., 2009).

A variety of quality-assurance procedures were undertaken to ensure that valid estimates of neurometabolite concentration were obtained. First, cases were excluded if the CSF fraction in the MRS voxel was greater than 0.36. Second, all participants had S/N values greater than 12. Third, all participants had Cramer-Rao lower bound (CRLB) estimates of less than 20%. Fourth, all participants with any outlier MRS value (identified as “extreme outliers” with the SPSS Explore procedure) were excluded from all analyses. Figure 1 shows a representative spectrum and the voxel location.

2.5 Statistical Analyses

All analyses were conducted in SPSS v.16.0. Our central analyses were based on the multivariate General Linear Model (GLM) procedure in which the five neurometabolites served as dependent variables, minimizing the multiple comparison problem. Two imaging covariates were used in most analyses, dwell time and proportion of GM in the voxel. Separate analyses in the entire sample revealed that dwell time was significantly related to all concentrations except mI, while GM proportion was related only to Glx.

3. Results

3.1 Demographics and clinical characteristics

Demographic and clinical characteristics of each group are presented in Table 1. The heavy drinking group had a slightly smaller proportion of women than the control group, but these groups did not differ in age or education. All groups were ethnically diverse.

Eight percent of the clinical sample averaged three or fewer drinks per drinking day, while 54% averaged six or more. Using conventional criteria for the BDI (Beck, 1996), 45% of our clinical sample was “minimally depressed,” 25% “mildly depressed,” 23% “moderately depressed,” and 6% “severely depressed”. In terms of cigarette smoking, 46% of our clinical sample did not smoke at all, while 16% reported smoking every day. The majority of individuals in our clinical group did not use marijuana (62%), though 12% reported using it at least one-third of days. Compared to the non-treatment seeking group, the treatment-seeking group was older, less educated, and had higher BDI scores. They also consumed less marijuana, had more DPDD, and reported more years of drinking. However, the non-treatment seeking group had an average ADS score of 9.60 (SD = 5.42), and 57% scored above 9 (vs. 77% of the treatment seekers), the cutoff suggesting likely alcohol dependence (Ross, 1990). The broad range of demographic and clinical characteristics suggests that the study was successful in capturing a reasonably representative sample of the larger population of heavy drinkers. In the combined drinking group, correlations of age with selected clinical variables were as follows: years drinking, $r = .89$, $p < 0.001$, DPDD, $r = 0.30$, $p < 0.001$, BDI, $r = 0.27$, $p < 0.001$, cigarettes per smoking day, $r = 0.17$, $p = 0.015$, and marijuana smoking days, $r = -0.09$, ns.

3.2 Neurometabolite differences across groups

Descriptive statistics for ^{1}H-MRS variables are presented in Table 2. The median intercorrelation of neurometabolite concentrations for the entire sample was r = .47. Group differences between controls and drinkers were evaluated with a multivariate GLM, with all five neurometabolites serving as dependent variables, group (control vs. AUD) and gender as fixed factors, and age, dwell time, gender, and proportion of GM in the voxel as covariates. The following interactions were also entered: group by gender, group by age, and group by both age and gender. The overall effect of group was significant ($F(5, 265) = 2.300$, $p < .042$. At the individual variable level, no specific neurometabolite was significant, though the effect of Cho showed a trend ($p = .057$, partial eta squared = .013). A significant overall effect was also noted for the group by age interaction ($F(5, 265) = 2.44$, $p = .032$), with a significant effect for Cho ($p = .039$, partial eta squared = .016). Another way to view this effect is by comparing the partial correlations (controlling for dwell time, percent GM in the voxel, and gender) of Cho with age in the combined AUD group and healthy controls. The correlation was significantly greater ($p = .04$) in the clinical sample ($r = .373$, $p < .001$) vs. controls ($r = .096$, ns). Not surprisingly, the age effect was also significant overall ($F(5, 265) = 8.72$, $p < .001$), with significant individual MRS effects for Cho ($p = .003$, partial eta squared = .032), Cre ($p < .001$, partial eta squared = .081), and Glx ($p = .016$, partial eta squared =.021). Figure 2 shows the age by group interaction, using three discrete age categories for illustrative purposes.

In an identical multivariate GLM analysis with GM, WM, and CSF from the MRS voxel as dependent variables (with the exception that percent GM in the voxel was not included as a covariate), no effects were significant except for age ($F(3, 268) = 52.76$, $p < .001$), with significant individual effects being found for GM ($p < .001$, partial eta squared = .348), and CSF ($p < .001$, partial eta squared = .274). Thus, the drinking and control groups did not differ in the tissue composition of the MRS voxel.

We next compared neurometabolite concentrations across the treatment seeking and non-treatment seeking subgroups of heavy drinkers. A multivariate GLM analysis was conducted, with the five MRS variables serving as dependent variables. The effect of group was examined along with the additional covariates of DPDD, years drinking, gender, MRS dwell time, and the proportion of GM in the voxel. The overall main effect of group was not significant ($F(5, 177) = 1.86$, $p = 0.10$), revealing that the two drinking groups did not differ in neurometabolite concentrations after controlling for group differences in drinking variables. Time since last drink was not related to concentration of any neurometabolite in the total AUD sample, as partial correlations (controlling for MRS dwell time and proportion of GM in voxel tissue) yielded r values < 0.06.

3.3 Relationships between drinking variables, comorbid factors, and neurometabolites

Our central analysis of the clinical determinants of neurometabolite concentrations was performed with a multivariate GLM analysis (Table 3). Each neurometabolite served as a dependent variable, with covariates being dwell time, gender, and percent of gray matter in the voxel. These clinical variables were entered as predictors: years drinking, DPDD, the cigarette and marijuana variables, and the BDI total score. Diagnostics revealed striking collinearity between years drinking and age, so the latter was not included in these models. For the overall model, the only significant predictor variable was years drinking. A greater number of years drinking was associated with significantly greater concentration of each neurometabolite except mI (a glial marker). A trend ($p = .09$) was noted for the overall effect of smoking, and at the level of individual neurometabolites greater smoking predicted greater concentrations of Glx ($p = .006$). Drinks per drinking day, depression, and marijuana

use were not related to the overall set of neurometabolites, and hence, were not examined further at the individual level.

We wished to compare our clinical correlations with those obtained in the ^{1}H-MRS study by Durazzo and colleagues (Durazzo et al., 2004) of recovering alcoholics (N = 24, mean age = 50). Thus, we compared the oldest portion of our drinking group, those over age 45 (N = 21, mean age = 49.57) with our younger clinical participants (N = 191, mean age = 28.67). Given the results of the previous study, which focused on NAA, and the current results suggesting the importance of Cho, we analyzed partial correlations of these two neurometabolites with three drinking variables: DPDD, years drinking, and days since last drink. Our younger and older drinking groups did not significantly differ in either DPDD or time since last drink. Significant correlations were noted between Cho and DPDD in the younger group ($r = .253$, $p = .001$), but not the older group ($r = .052$, ns). In the older group Cho was correlated with days since last drink ($r = -.465$, $p = .03$) and NAA was correlated negatively with years drinking ($r = -.462$, $p = .035$), though neither of these correlations was significant in the younger group. As these exploratory analyses were not corrected for multiple comparisons, they must be considered preliminary.

3.4 Gender

Relationships between alcohol consumption variables and neurometabolites might differ depending on gender. Independent samples t-tests revealed that in the combined group women consumed fewer DPDD ($t(211) = 2.52$, $p = 0.012$), and a trend was noted for women to have fewer years of drinking ($t(211) - 1.81$, $p = .07$). In a multivariate GLM analysis each neurometabolite was treated as a dependent variable, and covariates were DPDD, years drinking, MRS dwell time, and proportion of GM in the voxel. Group (gender) and the interaction of gender with DPDD and the interaction of gender with years drinking were included as predictors. No significant overall effect was noted for gender or either interaction.

4. Discussion

The present study conducted an MRS analysis of a predominantly gray matter voxel in the anterior cingulate region in a large sample of community dwelling heavy drinkers, with the goal of identifying clinical characteristics that may affect neurometabolite concentrations. Heavy drinkers were also compared to healthy controls. Multivariate analyses revealed (1) greater overall neurometabolite concentrations in heavy drinkers, with the effect being strongest for Cho; (2) a significant age by group interaction, such that overall neurometabolite concentrations (esp. Cho) increased more dramatically with age in the heavy drinking group; (3) more years of heavy drinking, but not more drinks per drinking day, was associated with greater concentrations overall; and (4) after taking group differences in drinking history into account, treatment seekers did not differ from nontreatment-seekers. The observed significant effect sizes were quite modest.

Before considering the implications of these observations, and how they relate to those obtained in prior studies, let us consider the strengths and weaknesses of the current study. On the positive side, our ^{1}H-MRS quantification and quality control procedures provided high quality data on gray matter neurometabolites, including Glx, which relatively few human studies have examined. Also, our large sample size and multivariate analyses afforded adequate statistical power to detect predictors of neurometabolite concentrations. A major limitation is that we have analyzed only a single GM voxel. The clinical MRS literature is replete with examples of site-specific effects, limiting our ability to generalize to other gray matter regions. Further, our quantification procedure, while producing “absolute” concentration estimates, is based on a ratio with tissue water and, as such, requires accurate

identification of the CSF voxel fraction, as well as assumptions about unchanging water densities and relaxation times. Finally, disentangling the relationships between years drinking and age was difficult, given the high correlation between these two variables.

Diverse aspects of the extended phenotype of chronic alcohol abuse had minimal impact on neurometabolite concentrations, and no group by gender interactions were found. Of course, these results are sample-specific; different results might be found, for example, in a sample with a greater amount of marijuana consumption or more severe clinical depression. A trend was noted for cigarette smoking on overall neurometabolite concentrations, and a significant univariate effect was noted for increased Glx with more smoking. Reduced NAA has been linked with smoking in some studies of heavy drinking individuals (Durazzo, Gazdzinski, Banys, et al., 2004), but not others (e.g., Gallinat and Schubert, 2007). Nicotine has been shown to increase glutamate release in the prefrontal cortex (Lambe, Picciotto and Aghajanian, 2003), an observation consistent with our results.

Our multivariate analyses showed that heavy drinkers, as compared to controls, showed a pattern of increased concentration of all neurometabolites, and an overall age by group interaction such that the association of age with metabolite increase was stronger in the drinkers than in controls. Within the drinking group greater years drinking had a similar effect, predicting higher overall concentrations of the neurometabolites. Perhaps it is not surprising that significant overall effects were seen for neurometabolite concentrations, given their substantial intercorrelations. We do not believe that this general effect is an artifact, as we have controlled for voxel parameters (esp. water content) that could raise or lower all concentration estimates. Nor do we believe that the overall increase merely reflects an aging effect, given the significant age by group interaction. Rather, we tentatively propose that the greater aging effect on neurometabolites in our heavy drinking sample is caused by the cumulative effect of years of heavy drinking.

The widespread increased concentrations noted in the drinking group likely reflects neural adaptation to chronic alcohol use, potentially mediated by the direct influence of alcohol on neurotransmitters and resulting receptor density changes, and up- or down-regulation of gene expression. As part of this adaptive response, a recent review of fMRI studies (Oscar-Berman and Marinkovic, 2007) revealed that in alcoholics some brain regions show decreased activation relative to controls, while others show increased activation. Interestingly, the anterior cingulate gyrus has been identified as one region showing increased activation. Alterations in gene expression by alcohol are region-specific (Flatscher-Bader, van der Brug, Landis, et al., 2006), perhaps contributing to the specific pattern of activation differences in drinkers vs. controls. Gene expression can increase or decrease with alcohol exposure; in a recent study of zebrafish, 1,914 genes demonstrated a two-fold or greater difference in expression with chronic alcohol exposure and 59% of these involved up-regulation (Pan et al., 2011). Increased concentrations of Glx, Cre, and NAA in particular suggest a metabolic up-regulation that could potentially “fuel” adaptive or compensatory neural changes to chronic exposure. There may, however, be a cost to increased Glx and NAA – increased oxidative stress, as we shall detail below.

Our most interesting specific findings concern Cho. The Cho signal, primarily from the membrane lipid metabolites phosphorylcholine and glycerol phosphorylcholine, is often interpreted as reflecting breakdown of membranes and/or increased concentrations of neurometabolites needed for repair processes, such as occur in traumatic brain injury (Garnett, Blamire, Corkill *et al.*,, 2000; Garnett, Blamire, Rajagopalan, et al., 2000; Shutter, Tong and Holshouser, 2004; Yeo, Phillips, Jung, et al., 2006). Elevated Cho in GM, reflecting greater membrane turnover, could be secondary to alcohol-induced degeneration of unmyelinated axons or dendritic processes. The significant age by group interaction

showed that Cho increased with age to a greater extent in the drinking group than in controls. In exploratory analyses we found that in the larger group of younger drinkers (under age 45), but not in the older group, higher Cho was also associated with more drinks per drinking day. In the older group alone, more recent drinking was associated with higher Cho. Taken together, these observations offer fairly strong evidence that heavy drinking may cause an increase in Cho concentration.

Cho effects have also been noted in other human and animal studies. Ende and colleagues (Ende, Walter, Welzel, et al., 2006; Ende, Welzel, Walter, et al., 2005). Zahr and colleagues (Zahr, Mayer, Vinco, et al., 2009) argue convincingly that a decrease in Cho in older patients might reflect subclinical liver dysfunction, a problem likely less common in our community dwelling sample than in older, more chronic samples. Our Cho results closely mirror those obtained in animal models of alcohol-induced neurometabolic changes. After 24 weeks of alcohol exposure, rats without thiamine deficiency or liver damage showed elevated Cho, consistent with current results (Zahr, Mayer, Vinco, et al., 2009). An older study reporting neurometabolite ratios found Cho/NAA to be elevated (in an animal model of dependence) after 16 weeks of exposure, but reduced after 44 weeks of exposure (Lee, Holburn and Price, 2003). Results from their initial time point are consistent with our Cho observations in the entire AUD sample; results from the latter time point are consistent with our analysis of the much smaller, older, more chronic subset of participants.

The single most widely reported MRS effect is reduced NAA (Meyerhoff, Durazzo, and Ende, 2011), which we did not observe. Despite the overall increase in neurometabolite concentrations in the drinking group, the individual effect for NAA was not significant, although NAA concentration was positively correlated with years of drinking in the entire group. NAA is found almost exclusively within neurons and NAA *reductions* have typically been interpreted as reflecting loss of neurons or reduced neural integrity, as in studies of traumatic brain injury (Friedman, Brooks, Jung, et al., 1999) and Alzheimer's disease (Kantarci, 2007). However, NAA concentrations are also sensitive to variations in metabolic activity (Gasparovic, Arfai, Smid, et al., 2001), and much evidence links NAA to cell energetics, as NAA is produced from aspartate in the mitochondria (Moffett, Ross, Arun, et al., 2007). Let us offer a tentative account for why our NAA results differ from some other reports. A key observation is that in the oldest portion of our drinking sample we found a *negative* correlation between number of years drinking and NAA concentration. This suggests that actual neural damage in the anterior cingulate occurs only after many years of chronic abuse. Most other MRS studies of chronic alcohol abuse (e.g., Durazzo, Gazdzinski, Banys, et al., 2004; Ende, Welzel, Walter *et al.*, 2005) have studied groups much older than our community sample (mean age = 31), and thus are consistent with the relationship we observed only in the oldest portion of the sample.

As noted earlier, there eventually may be a cost to the cingulate's metabolic up-regulation that occurs as part of a suite of neural changes allowing adaptation to long-term alcohol exposure. One consequence of greater neural metabolic activity is greater oxidative stress, as free radicals damage cell membranes and DNA (Crews and Nixon, 2009). Indeed, an emerging literature raises the provocative question of whether increased NAA actually leads to greater oxidative stress (Pederzolli, Rockenbach, Zanin, et al., 2009; Surendran and Bhatnagar, 2011). Thus, short-term neural adaptation may carry with it the seeds of long-term neural damage. Greater chronic exposure to oxidative stress, which has been suggested to be the single most important cause of normal brain aging (Harman, 1956), may be responsible for the greater aging effects in the heavy drinking group.

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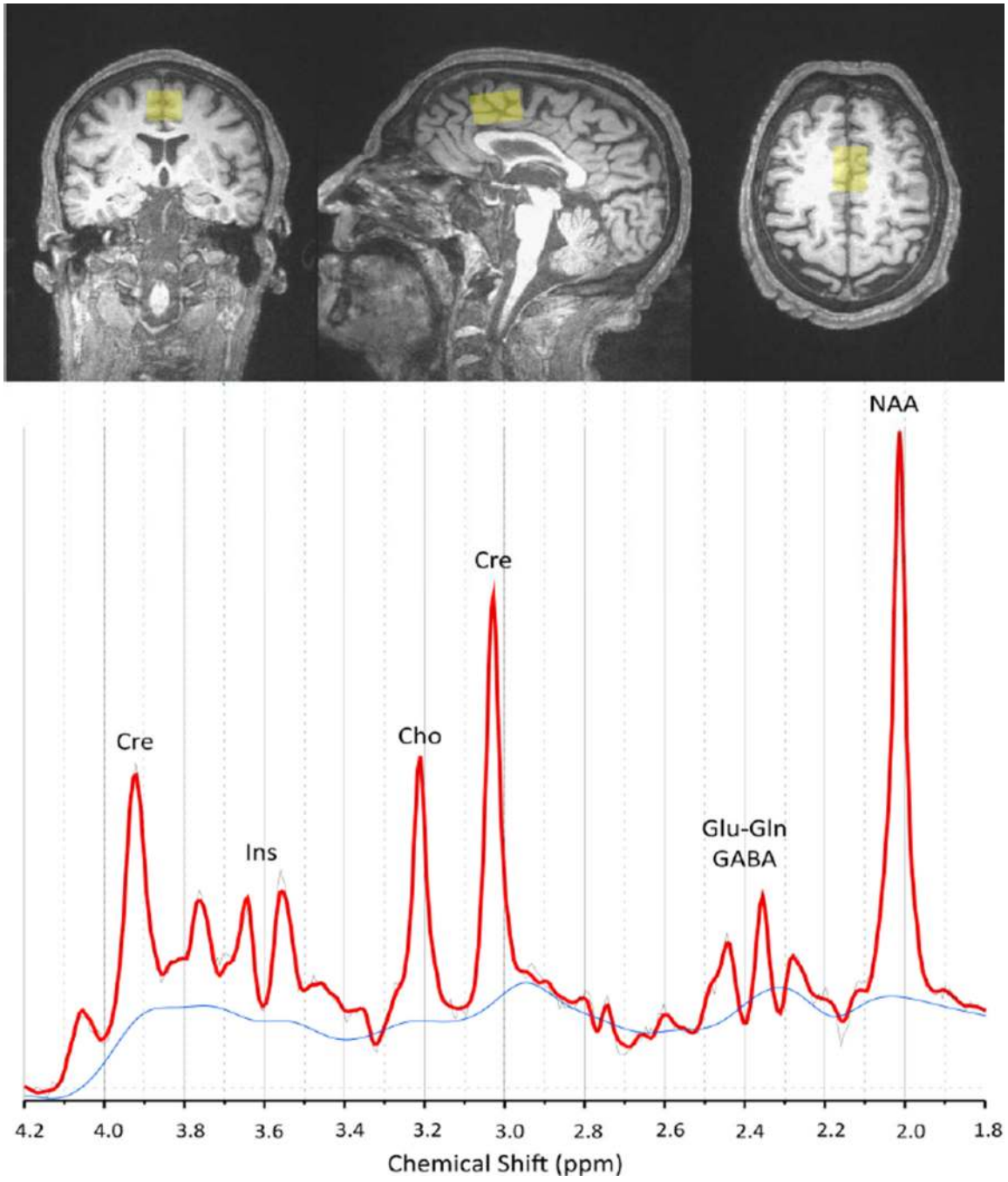

Figure 1.
Location of the ^{1}H-MRS voxel and a representative spectrum.

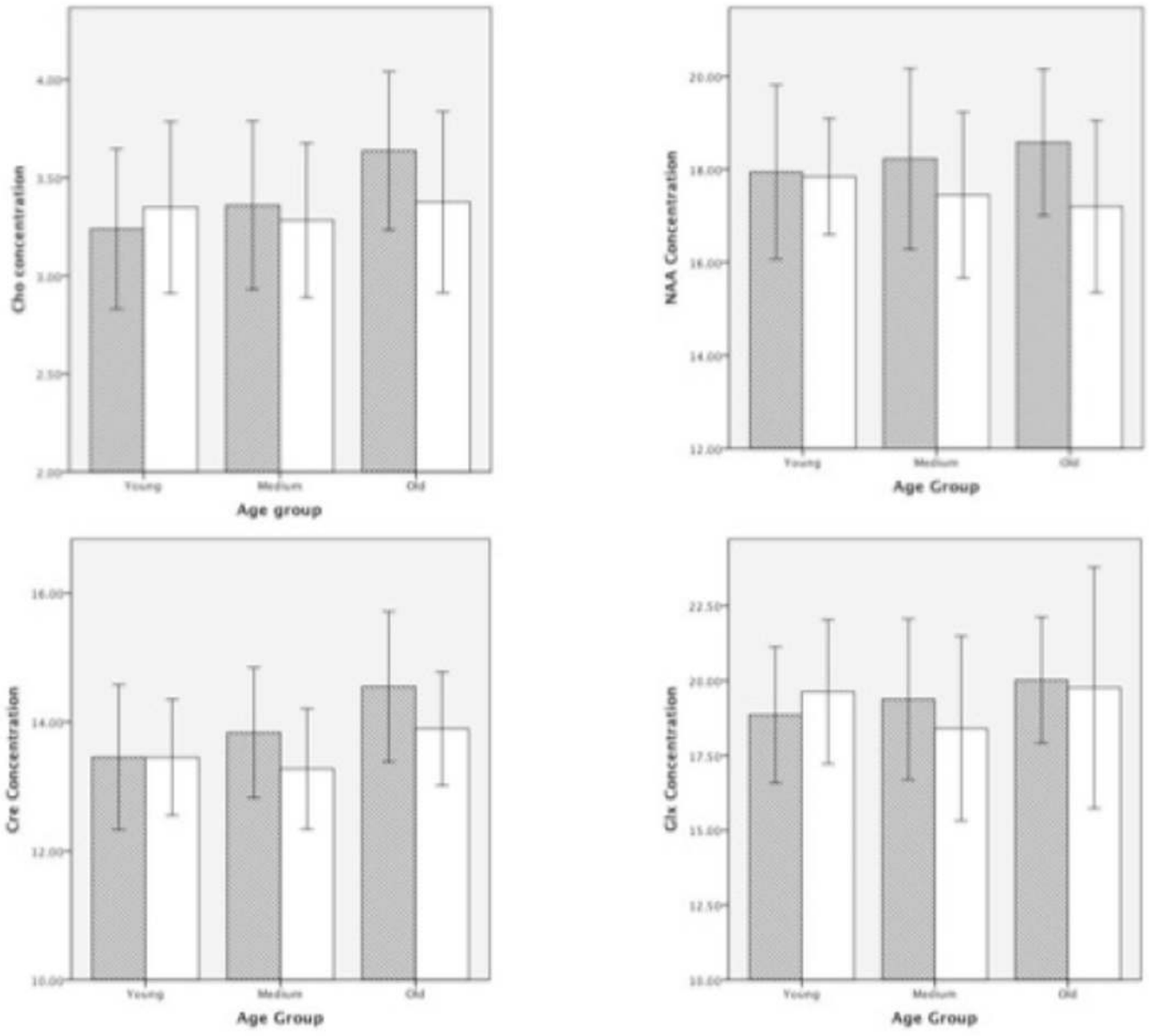

Figure 2.

Concentrations of Cho, NAA, Cre, and Glx for controls and heavy drinkers, broken down into three age groups (Young, ages 21 – 24 ; Medium, ages 25 – 34; and Old, ages 35 – 56) for illustrative purposes. Heavy drinkers are represented by shaded bars and controls by white bars (error bars represent standrad deviations).

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Table 1

Descriptive statistics (means and standard deviations) for clinical and demographic variables by group. Statistical comparisons were conducted with univariate t-tests and Chi-square analyses. Controls were not assessed on every variable.

Variable	AUD (N = 213)	Treatment-seekers (N = 97)	Non-treatment seekers (N = 116)	Controls (N = 66)	AUD vs. Controls
Sex	153 M, 60F	69M, 28F	84M, 32 F	37M, 29F	.02
Age	30.99 (9.03)	37.62 (8.79)	25.45 (4.21)	29.50 (8.33)	ns
Education	14.29 (2.40)	13.46 (1.97)	14.91 (2.50)	14.78 (2.85)	ns
BMI	25.67 (4.44)	26.75 (5.07)	24.80 (3.64)	N/A	
Ethnicity	Anglo (105, 50%) Hisp. (55, 26%) Af. Am. (6, 3%) Nat. Am. (9, 4%) Mixed (31, 15%) Other (2, 1%)	Anglo (45, 46%) Hisp. (32, 33%) Af. Am. (3, 3%) Nat. Am. (6, 6%) Mixed (10, 10%) Other (1, 1%)	Anglo (60, 52%) Hisp. (23, 20%) Af. Am. (3, 3%) Nat. Am. (3, 3%) Mixed (21, 18%) Other (6, 5%)	Anglo (39, 59%) Hisp. (14, 21%) Af. Am. (2, 3%) Nat. Am. (3, 5%) Mixed (6, 9%) Other (2, 3%)	
ADS total	13.16 (8.38)	17.42 (9.27)	9.60 (5.44)		
Days since last drink	2.67 (2.87)	3.02 (3.87)	2.39 (1.62)		
Drinks/drinking day	7.45 (4.64)	9.28 (5.94)	5.93 (2.27)		
Years drinking	12.51 (9.03)	18.32 (9.24)	7.65 (5.17)		
Beck Dep. Inventory	11.22 (9.04)	15.35 (9.19)	7.77 (7.32)		
Cigarettes/smoking day	5.02 (7.13)	6.03 (7.85)	4.16 (6.37)		
Number days smoking marijuana	9.72 (22.01)	4.65 (11.96)	13.79 (26.83)	0 (.00)	NA

Table 2

Neurometabolite concentrations and voxel tissue composition (means, standard deviations) by group.

MRS variables	AUD (N = 213)	Treatment seekers (N = 97)	Non-treatment seekers (N = 116)	Controls (N = 66)
Cho	3.41 (0.44)	3.56 (.41)	3.29 (.44)	3.34 (0.43)
Cre	13.95 (1.19)	14.29 (1.11)	13.66 (1.18)	13.52 (0.93)
NAA	18.24 (1.81)	18.36 (1.66)	18.15 (1.94)	17.51 (1.63)
Glx	19.42 (2.40)	19.67 (2.33)	19.20 (2.45)	18.99 (3.74)
mI	13.41 (1.64)	13.48 (1.53)	13.36 (1.73)	12.40 (1.57)
CSF	0.21(0.05)	.23 (.05)	.18 (.04)	0.19 (0.05)
Gray Matter	0.62 (0.05)	.59 (.04)	.64 (.04)	0.63 (0.05)
White Matter	0.18 (0.04)	.17 (.04)	.18 (.03)	0.18 (0.04)

Table 3

Results of multivariate General Linear Model analyses relating clinical variables (columns) to neurometabolites assessed with Magnetic Resonance Spectoscopy (MRS). The five neurometabolites were dependent variables and the five clinical variables were predictors; dwell time and percent gray matter in the MRS voxel served as covariates. Cell entries indicate p values (with eta squared in parentheses) for the overall model in the first row, and for individual MRS effects in subsequent rows. Effects are listed only for overall models with significance of $p < .1$

	Years Drinking	Drinks per Drinking Day	Cig. per Smoking Day	Number Marijuana Days	Beck Depression Inventory
Overall model	< .001 (.20)	.39 (.03)	.09 (.05)	.74 (.01)	.59 (.02)
Cho	<.001 (.10)		.49 (.00)		
Cre	<.001 (.15)		.09 (.01)		
NAA/NAG	.03 (.02)		.17 (.01)		
Glx	.005 (.039)		.006 (.04)		
mI	.08 (.02)		.85 (.00)		