Published in final edited form as:

Psychopharmacology (Berl). 2016 July ; 233(14): 2675–2686. doi:10.1007/s00213-016-4313-y.

Concomitants of alcoholism: differential effects of thiamine deficiency, liver damage, and food deprivation on the rat brain in vivo

Natalie M Zahr, Ph.D.^{*,a,b}, Edith V Sullivan, Ph.D.^a, Torsten Rohlfing, Ph.D.^b, Dirk Mayer, Ph.D.^{b,c}, Amy M. Collins, B.S.^b, Richard Luong, B.V.Sc.^d, and Adolf Pfefferbaum, M.D.^{a,b}

^aPsychiatry & Behavioral Sciences, Stanford University School of Medicine, 401 Quarry Rd., Stanford, CA 94305, USA

^bNeuroscience Program, SRI International, Menlo Park, CA, 94025, USA

^cDiagnostic Radiology and Nuclear Medicine, University of Maryland, School of Medicine, Baltimore, MD, 21201, USA

^dDepartment of Comparative Medicine, Stanford University, Stanford, CA, 94305, USA

Abstract

Rationale—Serious neurological concomitants of alcoholism include Wernicke's Encephalopathy (WE), Korsakoff's Syndrome (KS) and hepatic encephalopathy (HE).

Objectives—This study was conducted in animal models to determine neuroradiological signatures associated with liver damage caused by carbon tetrachloride (CCl₄), thiamine deficiency caused by pyriethamine treatment, and nonspecific nutritional deficiency caused by food deprivation.

Methods—Magnetic resonance imaging (MRI) and spectroscopy (MRS) were used to evaluate brains of wild-type Wistar rats at baseline and following treatment.

Results—Similar to observations in ethanol (EtOH) exposure models, thiamine deficiency caused enlargement of the lateral ventricles. Liver damage was not associated with effects on cerebrospinal fluid volumes, whereas food deprivation caused modest enlargement of the cisterns. In contrast to what has repeatedly been shown in EtOH exposure models, in which levels of choline-containing compounds (Cho) measured by MRS are elevated, Cho levels in treated animals in all 3 experiments (i.e., liver damage, thiamine deficiency, food deprivation) were lower than baseline or controls.

Conclusions—These results add to the growing body of literature suggesting that MRS-detectable Cho is labile and can depend on a number of variables that are not often considered in human experiments. These results also suggest that reductions in Cho observed in humans with alcohol use disorder (AUD) may well be due to mild manifestations of concomitants of AUD such as liver damage or nutritional deficiencies and not necessarily to alcohol consumption per se.

*corresponding author: nzahr@stanford.edu, Phone: 650-859-5243, Fax: 650-859-2743.

Conflict of interest: The authors declare no competing financial interest in relation to the work described here.

Keywords

pyrithiamine; carbon tetrachloride; hematology; magnetic resonance spectroscopy

Introduction

Chronic alcoholism is a complex disease associated with cognitive decline, brain structural degradation, and connectivity disruption (e.g., Camchong et al. 2013; Chanraud et al. 2013; Nixon et al. 2014; Oscar-Berman et al. 2014; Pfefferbaum and Sullivan 2005; Pitel et al. 2012). Whether alcohol per se or the complications that arise with chronic alcohol use contributes to brain compromise remains controversial. Serious neurological concomitants of alcoholism include hepatic encephalopathy (HE), Wernicke's Encephalopathy (WE), and Korsakoff's Syndrome (KS)(Mann et al. 2003; Prasad et al. 2007). HE is associated with confusion, altered levels of consciousness, or coma as a result of liver failure (Losowsky and Scott 1973; Vaquero et al. 2003). WE is an acute, potentially reversible, neurological disorder resulting from thiamine (vitamin B1) deficiency also characterized by acute mental confusion, as well as ataxia and ophthalmoplegia. When WE is diagnosed, treatment with thiamine can result in rapid clinical improvement (Sechi and Serra 2007). When left undiagnosed and untreated, WE patients can develop the severe, devastating neurologic disorder KS, characterized by memory loss, confabulation, and severe ataxia (Butters 1981; Harper et al. 1986)}.

Questions persist regarding the relative contributions of mild, recurrent thiamine deficiency, liver damage, or other concomitants of alcoholism on the human brain. Animal models can be used to distinguish components of the processes associated with damage to the brain in response to alcohol exposure (Pfefferbaum et al. 2008; Zahr et al. 2010b; Zahr et al. 2014b; Zahr et al. 2013; Zahr et al. 2009b; Zahr et al. 2015b). Our in vivo magnetic resonance imaging (MRI) studies of rats have consistently shown increases in cerebrospinal fluid (CSF) volumes following ethanol (EtOH) exposure. Chronic EtOH exposure via vapor inhalation with blood alcohol levels (BALS) up to 450mg% resulted in modest (30%) enlargement of the lateral ventricles (Pfefferbaum et al. 2008). Intragastric (i.g.) EtOH for 4-days with BALS approaching 300mg% was associated with reversible enlargements (upwards of 100% increase) of the lateral ventricles (Zahr et al. 2010b; Zahr et al. 2014b; Zahr et al. 2013). Repeated exposure to EtOH (5 cycles of 4 days of EtOH i.g. + 10 days of recovery) showed enlargement of CSF volumes of the lateral ventricles and cisterns with each EtOH exposure, but recovery with each abstinence period.

MR Spectroscopy (MRS) in the same models showed in the chronic study that N-acetyl-aspartate (NAA) levels were lower in the EtOH-exposed relative to the control group but did not attain a statistical significance, while levels of choline-containing compounds (Cho) demonstrated a dose-response curve (i.e., increasing levels with higher/longer EtOH exposure) (Zahr et al. 2009b). Changes in MRS metabolite levels were reversible following 1 week of recovery from 4 days of EtOH exposure (i.g.): levels of NAA decreased, while those of Cho increased (Zahr et al. 2010b; Zahr et al. 2014b; Zahr et al. 2013). With repeated EtOH (i.g.) exposure, changes in metabolite levels did not accrue: levels of NAA

decreased, while those of Cho increased with each EtOH exposure cycle, but then recovered during each abstinence period (Zahr et al. 2015b). Although these EtOH exposure studies were informative, they did not fully model the persistent ventricular enlargement seen in chronic alcoholism in humans (Pfefferbaum et al. 2012; Rosenbloom et al. 2010) nor the low levels of NAA (frontal regions: Bendszus et al. 2001; Durazzo et al. 2004; Durazzo et al. 2010; Fein et al. 1994; Jagannathan et al. 1996; Parks et al. 2002; Schweinsburg et al. 2003; Seitz et al. 1999) and Cho (Bendszus et al. 2001; Durazzo et al. 2004; frontal regions: Ende et al. 2005; Fein et al. 1994; Parks et al. 2002; cerebellar regions: Seitz et al. 1999) observed in recently sober alcoholics relative to healthy controls.

Lower levels of both NAA and Cho compared with controls are reported in MRS case studies of human WE (Mascalchi et al. 2002; Murata et al. 2001) and models of thiamine deficiency in rats treated with pyridoxamine hydrochloride (Lee et al. 2001; Lee et al. 1995; Pfefferbaum et al. 2007; Rose et al. 1993; Zahr et al. 2014a). A reduction in Cho is also seen in patients with cirrhosis of the liver, along with reduced signal from myo-Inositol (mI) and an elevated signal from the combined resonance (Glx) of glutamate (Glu) and glutamine (Gln); typically no effect of liver cirrhosis is observed on NAA levels (Cordoba et al. 2001; Geissler et al. 1997; Kreis et al. 1992; Laubenberger et al. 1997; Lee et al. 1999; Ross et al. 1994). In animal models of liver disease, frequently reported MRS findings include reduced Cho, mI, and Glu, but elevated Gln (Bates et al. 1989; Chavarria et al. 2013; de Graaf et al. 1991; Peeling et al. 1993; Rackayova et al. 2015; Zwingmann et al. 2004).

The current study included three experiments to evaluate the patterns of MRI and MRS changes in models of concomitants of alcoholism to compare to the effects on the brain of EtOH exposure models. Acute hepatitis was induced using carbon tetrachloride (CCl₄, Chavez-Pina et al. 2009). A food deprivation experiment was performed as a non-specific nutritional deficiency and as a control for the EtOH (i.g.) exposure model (Majchrowicz 1975), which has repeatedly resulted in a 20% loss of body weight regardless of attempts to mitigate it (Zahr et al. 2009a; Zahr et al. 2014b; Zahr et al. 2013). An experiment combining thiamine deficiency and EtOH exposure was also conducted. The key hypothesis to be tested was that concomitants of alcoholism such as liver damage and thiamine deficiency, unlike EtOH exposure, cause a decrease in Cho levels. It was further expected that a combination of thiamine deficiency and EtOH exposure would result in a net neutral effect on Cho levels (i.e., decrease in Cho due to thiamine deficiency would be canceled by the increase in Cho due to EtOH exposure).

Methods

Animals

Wild-type Wistar rats from Charles River Laboratories, singly housed with free access to food and water and lights on for 12h starting at 06:00 were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). The Institutional Animal Care and Use Committees (IACUC) at SRI International and Stanford University approved all research protocols in accordance with the guidelines of the IACUC of the National Institute on Drug Abuse, National Institutes of

Health, and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

Treatment and Schedule

Liver Damage Model—This study group included 12 male rats weighing 311.6 ± 13.5 g at baseline (i.e., MR1). After acclimation to a new environment, including single housing, all animals ($n=12$) underwent baseline scanning (MR1). Two days after scanning, 6 animals were given their first dose (2.5ml/kg) of CCl_4 in olive oil (1:1) via intragastric oral gavage; a second dose (2.5ml/kg) was administered on the following day. Control animals ($n=6$) were given similar volumes of olive oil via intragastric oral gavage on each of the 2 days. Follow-up scans (MR2) were conducted the day after the second dose (Fig. 1a). Animals were euthanized the day following MR2.

Food Deprivation Model—After acclimation, all animals ($n=23$), weighing an average of 329.1 ± 22.4 g, underwent baseline scanning (MR1). For this experiment, 12 animals were fasted for 12h, then food deprived (FD, i.e., given a limited number of food pellets), for a period of 4 days to 80% of their baseline weight (i.e., 20% weight loss as observed following i.g. EtOH exposure) and also deprived of water 12h prior to the follow-up scan (MR2). Feces were removed from cages to avoid coprophagy and body weights were taken daily. Control animals ($n=11$) had *ad libitum* access to food and water during the course of the experiment. One control rat died immediately after MR2 of apparent anesthesia sensitivity. Immediately following MR2, FD animals were given *ad libitum* access to food and water, through a weeklong recovery period, after which all rats ($n=22$) underwent a recovery scan (MR3, Fig. 1b). One FD animal died immediately after the recovery scan. Of the remaining 11 FD rats, 6 were exposed to another period of food restriction (4 days) before euthanasia, which was conducted 8 days following MR3. MRS data analysis included as many animals as possible (i.e., MRS1 $n=23$, MRS2 $n=23$, MRS3 $n=22$); MRI analysis was only conducted on the total number of animals remaining at the last scan (i.e., MRI1,2,3 $n=22$).

Thiamine Deficiency Model—This study initially included 22 male rats weighing 319.5 ± 12.0 g at baseline (i.e., MR1) and lasted a total of 21 days. Three days after acclimation and baseline scanning (MR1) on day 0, all 22 rats were put on thiamine-deficient chow (Harlan TD81029) and then triaged into 3 groups. The first group (thiamine treated, EtOH treated, TE) included 5 rats given thiamine replacement for 11 days (0.4g/kg of 0.2mg/ml thiamine, by intraperitoneal (IP) injection, once per day (Q.D.)) and exposed to 2 cycles of 4 days of EtOH (i.g.) treatment starting on days 3 and 10 (with 2 days of no EtOH exposure in between the 2 EtOH exposure cycles). The second group (pyrithiamine treated, EtOH treated, PE) included 9 rats that were given pyrithiamine, an inhibitor of thiamine metabolism, for 11 days (0.5g/kg of 0.15mg/ml pyrithiamine, IP, Q.D.) and exposed to 2 cycles of 4 days of EtOH (i.g.) treatment starting on days 3 and 10 (with 2 days of no EtOH exposure in between). The third group (pyrithiamine treated, saline treated, PS) included 8 rats that were also given pyrithiamine for 11 days (0.5g/kg of 0.15mg/ml pyrithiamine, IP, Q.D.), but during exposure periods, these animals received saline instead of EtOH.

During each EtOH exposure, EtOH-treated rats received an initial “loading” dose of 5g/kg 20% EtOH w/v via oral gavage, then a maximum of 3g/kg every 8h for 4 days. On each of the 4 days of EtOH treatment, animals were weighed; tail vein blood samples were collected 4 times per day: ~90min before each dose to determine the level of intoxication before further dosing or 90min after the 2nd dose of the day (i.e., 15:00) to determine peak BALs. Plasma was assayed for alcohol content based on direct reaction with the enzyme alcohol oxidase (Analox Instruments Ltd., UK). EtOH was administered according to body weight, BALs, and behavioral intoxication state assessed using a modified Majchrowicz scale (range 0–5: 0-normal, 1-sedation, 2-mild ataxia, 3-moderate ataxia, 4-severe ataxia, 5-loss of righting reflex) (Majchrowicz 1975). Average BALs (average of BALs measured at 07:30 on each of the 4 days) achieved during each EtOH exposure period were as follows: 1: TE = $281.8 \pm 5.7\text{mg\%}$, PE = $279.6 \pm 4.5\text{mg\%}$, PS = $15.0 \pm 0.4\text{mg\%}$; 2: TE = $237.1 \pm 5.1\text{mg\%}$, PE = $247.4 \pm 8.5\text{mg\%}$, PS = $16.4 \pm 0.7\text{mg\%}$.

Animals underwent follow-up scans immediately after each of the 2 EtOH exposure periods (MR2 on day 7 and MR3 on day 14). During the recovery period, which began immediately after the second EtOH exposure scan (i.e., MR3), all animals were put on thiamine-replete chow (Harlan 7001) and given thiamine replacement (0.4g/kg of 0.4mg/ml, IP, Q.D.) for 7 days until the recovery scan (MR4 on day 21, Fig. 1c). One PE animal died the day after MR2 for unknown reasons. MRS data from 1 PS animal were missing for MR3. The remaining animals (n=21) were euthanized the day after recovery scans (i.e., day 22). MRS data analysis included as many animals as possible (i.e., MRS1 n=22, MRS2 n=22, MRS3 n=20, MRS4 n=21); MRI analysis was only conducted on the total number of animals remaining at the last scan (i.e., MRI1,2,3,4 n=21).

Additional Methods

For additional methods, including behavioral assay, details regarding MRI and MRS scanning and analysis, liver and chemistry blood panel assays, and liver and intestinal postmortem histopathology, please refer to Supplementary Materials.

Statistical Analysis

Repeated measures-analysis of variance (ANOVA) was used to evaluate group-by-time interactions for weight, ventricular volume, and metabolite levels in each experiment. Only interactions involving group effects were of interest to this analysis. Follow-up included 3-group ANOVAs for the thiamine deficiency experiment. Otherwise, follow-up comparisons were conducted using Wilcoxon rank sum nonparametric tests. Correlations were evaluated between variables showing group effects, included all animals in each study, and used nonparametric Spearman's ρ . Table 1 presents percent change in weight and MR variables between each scan for each group in experiment.

Results

Effects of Treatment on Weight and Behavior

Liver Damage Model—A 2-group (Ctrl vs. CCl₄) by 4 time point repeated-measures ANOVA for weight showed a group-by-time interaction ($F(3, 30)=20.98$, $p<.0001$, Fig. 1a).

Follow-up indicated that CCl₄ animals weighed less than Ctrl animals on day 2 of treatment ($p=.004$) and at MR2 ($p=.013$). Animals treated with CCl₄ were hypoactive and lethargic showing piloerection and lacrimation.

Food Deprivation Model—A 2-group (Ctrl vs. FD) by 7 time point repeated-measures ANOVA for weight showed a group-by-time interaction ($F(6, 120)=71.15$, $p<.0001$, Fig. 1b), with FD animals beginning to weigh less than Ctrl animals starting on day 1 of food deprivation ($p=.003$) through MR2 ($p<.0001$). Weights between the 2 groups, however, were indistinguishable at the recovery scan ($p=.55$). Following 4 days of food deprivation, FD rats relative to Ctrl animals were hypoactive and lethargic, but showed no other physiological or behavioral impairments.

Thiamine Deficiency Model—A repeated-measures ANOVA evaluating weight among the 3 groups (TE, PS, PE) at 4 time points (baseline MRI1, MRI2, MRI3, recovery MRI4) showed a group-by-time interaction ($F(6, 54)=7.58$, $p<.0001$, Fig. 1c). Follow-up 3-group ANOVAs showed group effects at MR2 ($F(2,21)=35.68$, $p<.0001$; $TE=PE<PS$, $p<.0001$), MR3 ($F(2,20)=14.36$, $p=.0002$; $PE<TE=PS$, $p=.0004$), and MR4 ($F(2,20)=3.99$, $p=.0368$; $PE<TE=PS$, $p=.03$).

Previous studies (e.g., Zahr et al. 2014) to achieve thiamine deficiency report that approximately 12 days of pyrithiamine treatment are required to observe ataxia of gait (Langlais 1995), after which seizures can occur (Zhang et al. 1995). To avoid such potential complications in the context of combined thiamine deficiency and 2 bouts of EtOH exposure, thiamine-deficient animals (i.e., PS and PE animals) were exposed only to 11 days of thiamine deficiency.

Physiological changes in all 3 groups of animals included elevated heart rate, elevated respiration rate, and piloerection. TE animals showed no other behavioral effects. At most 2 of 8 PS animals showed behavioral changes including reduced trunk tone, gait disturbances, and tremor. PE animals most frequently showed behavioral changes with, for example, as many as 7 of 9 animals were observed in a hunched position on day 11 (see supplementary Fig. S1).

Effects of Treatment on Ventricular / CSF volume

Liver Damage Model—Exemplary MR images from treated (CCl₄) and untreated (Ctrl) animals indicating lateral ventricles and cisterns are presented in Fig. 2. Two-group (Ctrl vs. CCl₄)-by-2 time point (MRI1, MRI2) repeated measures-ANOVAs did not demonstrate group or interaction effects for volume measures (i.e., lateral ventricles, cisterns, total CSF, Fig. 3a).

Food Deprivation Model—Separate 2-group (Ctrl vs. FD)-by-3 time point (MRI1, MRI2, MRI3) repeated measures-ANOVAs for each volume showed only a modest (not statistically significant) interaction effect of food deprivation on cisterns ($F(2,40)=3.20$, $p=.05$): cisterns were larger in the FD relative to the Ctrl group following food deprivation ($p=.09$), but this nominal difference was no longer evident after 1 week of recovery ($p=.64$, Fig. 3b).

Thiamine Deficiency Model—For the TDE study, 3-group (TE, PS, PE)-by-4 time point (MRI1, MRI2, MRI3, MRI4) repeated measures-ANOVA showed significant group-by-time interactions for lateral ventricles ($F(6,54)=2.39$, $p=.04$) and total CSF ($F(6,54)=2.92$, $p=.02$) (Fig 3c). For lateral ventricles, volumes were different between groups at MRI2 ($F(2,18)=4.88$, $p=.02$: PE>PS $p=.007$), MRI3 ($F(2,18)=6.38$, $p=.008$: PE>PS $p=.02$, PE>TE $p=.004$), and MRI4 ($F(2,18)=7.09$, $p=.005$: PE>PS $p=.006$, PE>TE $p=.004$). Total CSF volume was different between groups at MRI2 ($F(2,18)=6.41$, $p=.008$: PE>PS $p=.002$) and MRI3 ($F(2,18)=5.94$, $p=.01$: PE>PS $p=.01$, PE>TE $p=.006$).

Effects of Treatment on Brain Metabolites

Liver Damage Model—The only metabolite that showed an interaction effect following liver damage was Cho ($F(1, 10)=6.92$, $p=.03$) (Fig. 4a). Follow-up tests demonstrated that Cho levels were lower (by ~20%) in liver-damaged relative to Ctrl animals at MRS2 ($p=.01$).

Food Deprivation Model—The only metabolite that showed an interaction effect following food deprivation was Cho ($F(2, 40)=6.07$, $p=.005$) (Fig. 4b). Follow-up tests demonstrated that Cho levels were nominally, but not statistically lower (by ~8%) in food-deprived relative to Ctrl animals at MRS2 ($p=.06$). At MRS3, following a week of recovery, Cho levels no longer distinguished the 2 groups ($p=.2$).

Thiamine Deficiency Model—In the TDE experiment, NAA and Cho metabolite levels were affected by treatment (Fig. 4c). A 3-group by 4 time point repeated measures-ANOVA for NAA showed a nominal (not statistically significant) interaction effect ($F(6,51)=1.95$, $p=.09$). NAA levels were different between pairs of groups at MRS3 ($F(2,17)=2.96$, $p=.08$: PE<TE $p=.05$, PS<TE $p=.04$). A 3-group by 4 time point repeated measures-ANOVA for Cho showed an interaction ($F(6,51)=4.46$, $p=.001$). Cho levels were significantly different between groups at MRS3 ($F(2,17)=8.34$, $p=.003$: PE<TE $p=.0118$, PS<TE $p=.0008$).

Additional Results

For additional results, including effects of treatment on hematological indices, liver and intestinal histopathology, and relationships between blood and brain markers, please refer to Supplementary Materials.

Discussion

The finding of note from these experiments is that in contrast to EtOH exposure models (e.g., Zahr et al. 2010a; Zahr et al. 2009b) and findings in non-abstinent chronic heavy drinkers (Meyerhoff et al. 2004) and social and moderate drinkers (Ende et al. 2006), which are associated elevations in Cho levels, liver damage caused by CCl₄ and thiamine deficiency accelerated by pyridoxamine each caused a reduction in the levels of Cho. Indeed, while unexpected, food deprivation and the accompanying weight loss were also associated with reductions, although nominal, in Cho levels.

The observed decrease in Cho in the CCl₄ model replicates findings in humans with cirrhosis (Cordoba et al. 2001; Geissler et al. 1997; Kreis et al. 1992; Laubenberger et al. 1997; Lee et al. 1999; Ross et al. 1994) and animal models of liver failure that also report reduced levels of Cho (e.g., Chavarria et al. 2013; de Graaf et al. 1991; Peeling et al. 1993; Rackayova et al. 2015). Similarly, as has previously been reported in the literature of MRS case studies of WE (Mascalchi et al. 2002; Murata et al. 2001) and models of thiamine deficiency in rats treated with pyridoxamine hydrochloride (Lee et al. 2001; Lee et al. 1995; Pfefferbaum et al. 2007; Rose et al. 1993; Zahr et al. 2014a), lower levels of both NAA and Cho were observed in pyridoxamine-treated relative to control rats. In the combined thiamine deficiency + EtOH exposure group, however, levels of Cho were not as low as in the thiamine deficiency group only.

A reduced Cho peak is observed in patients with disturbed energy metabolism (Bluml et al. 1998) and may be relevant to the primary biochemical lesion in thiamine deficiency (Lee et al. 2001; Murata et al. 1999.). On the other hand, reduced Cho may reflect disrupted osmoregulation (Hazell 2009; Hazell and Butterworth 2009) as glycerophosphocholine, one of the compounds included in the Cho signal, plays an important role in peripheral osmotic regulation (Gallazzini and Burg 2009). Changes in osmoregulation may also disrupt white matter integrity, as reported in thiamine deficiency (He et al. 2007; Langlais and Savage 1995; Langlais and Zhang 1997) and liver failure (Rovira et al. 2008; Rovira et al. 2002).

The Cho findings corroborate and extend those reported in human studies and speak to the lability of the signal arising from choline-containing compounds (i.e., Cho) (Zahr et al. 2015a; Zahr et al. 2014c). For example, in a sample of 71 human subjects with either human immunodeficiency virus (HIV) infection (n=33) or a comorbidity of HIV + alcohol use disorder (AUD, n=38), Cho levels were higher in those who had experienced an acquired immune deficiency syndrome (AIDS)-defining event or who had hepatitis C, but lower in individuals with low thiamine levels and those on highly active antiretroviral HIV treatment (HAART)(Zahr et al. 2014c). In a sample of 20 human subjects with AUD, Cho levels were higher in individuals who drank in binge-like patterns and those with longer length of dependence, but lower with more recent drinking and those AUD individuals with past stimulant use (Zahr et al. 2015a). The fact that the MRS-detectable Cho signal is composed of phosphocholine, glycerophosphocholine, and free choline, among other choline derivatives (Boulanger et al. 2000), may explain, in part, the variability in the observed signal. In severe weight loss, for example, levels of phosphocholine can decrease while levels of glycerophosphocholine can increase (Gopel et al. 2002).

No changes in CSF volumes were observed in the liver damage model. In the thiamine deficiency model, changes in ventricular volume were compounded, with PE animals showing both lateral ventricle and total CSF volume enlargement following just 4 days of pyridoxamine treatment + a single 4-day EtOH exposure. Further treatment (7 days of pyridoxamine + another EtOH exposure) caused a greater increase in volumes of lateral ventricles and CSF in the PE group. These findings in the PE group are consistent with enlargement of lateral ventricles observed with MRI in thiamine deficient rats (i.e., equivalent to the PS group in this study)(Dror et al. 2010; Dror et al. 2014; Zahr et al. 2014a). In the previous studies, however, ventricular enlargement was more profound and

appeared to persist (Dror et al. 2010; Dror et al. 2014), possibly because the length of thiamine deficiency was longer (14 days) and thiamine replacement was shorter (3 days) (Dror et al. 2010; Dror et al. 2014). Although the TE group did not show statistically significant group effects, lateral ventricle and total CSF volumes were enlarged following the first EtOH exposure period. Unlike our repeated EtOH exposure experiment (Zahr et al. 2015b), however, there was a relatively suppressed effect of the second EtOH exposure on lateral ventricular volume in the TE group. One potential explanation for the attenuated effect of the second EtOH treatment is that these EtOH-exposed animals were being given daily injections of thiamine. This raises the intriguing possibility that thiamine given concurrently with EtOH treatment may counteract EtOH effects on ventricular volume enlargement (Zahr et al. 2013).

An unexpected finding was the modest increase in cistern volume following food deprivation. The pattern of hematological changes observed in the food deprivation experiment is consistent with malnutrition (e.g., low ALT, low alkaline phosphatase, low calcium, low carbon dioxide, low glucose, low cholesterol, low phosphorus) or dehydration (e.g., high albumin, high sodium, high chloride, low carbon dioxide, low creatinine). Reversible ventricular enlargement has been observed in anorexia nervosa (Enzmann and Lane 1977; Gerner et al. 1984). As the effects on ventricular volume were reversible in all the experiments presented herein, this metric does not likely index atrophy of surrounding regions, but may be related to changes in osmotic balance in the brain (c.f., Zahr et al. 2013).

The postmortem liver histopathology assays clearly distinguish effects of CCl₄ and pyrithiamine. In contrast to the necrosis and fibrosis observed in livers from the CCl₄ treated animals, the liver pathology in pyrithiamine-treated animals was mild and included minimal inflammation. The toxic metabolite of CCl₄ is produced by cytochrome P450 enzymes, which are highly concentrated in centrilobular hepatocytes. Combined with the fact that they get the least amount of oxygenated blood in the liver, relying on glycolysis for energy, CCl₄ toxicity first manifests and is overall most severe in centrilobular hepatocytes (Kumar et al. 2005; Vulimiri et al. 2011).

In summary, this study distinguishes the effects of alcohol intoxication on the brain from the effects of common concomitants of alcoholism including liver damage and thiamine deficiency. Whereas alcohol exposure models in animals (both acute and chronic) are associated with elevated Cho levels, CCl₄ and pyrithiamine treatment result in reduced Cho levels. Liver damage does not appear to independently affect CSF volume, although EtOH and pyrithiamine treatment each cause non-specific ventricular enlargement. Together, these results suggest that the pattern of changes often observed in individuals with AUD, including low levels of Cho relative to healthy controls (Bendszus et al. 2001; Durazzo et al. 2004; Ende et al. 2005; Fein et al. 1994; Parks et al. 2002; Seitz et al. 1999), may be related to common non-alcohol concomitants of chronic alcoholism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported with grant funding from the NIAAA including AA005965, AA013521, and AA017168. The authors would like to acknowledge Priya Asok, Crystal Caldwell, Cheshire Hardcastle, and Matthew Serventi for their help in data collection.

References

- Bates TE, Williams SR, Kauppinen RA, Gadian DG. Observation of cerebral metabolites in an animal model of acute liver failure in vivo: a ¹H and ³¹P nuclear magnetic resonance study. *Journal of neurochemistry*. 1989; 53:102–10. [PubMed: 2566653]
- Bendszus M, Weijers HG, Wiesbeck G, Warmuth-Metz M, Bartsch AJ, Engels S, Boning J, Solymosi L. Sequential MR imaging and proton MR spectroscopy in patients who underwent recent detoxification for chronic alcoholism: correlation with clinical and neuropsychological data. *American Journal of Neuroradiology*. 2001; 22:1926–1932. [PubMed: 11733327]
- Bluml S, Zuckerman E, Tan J, Ross BD. Proton-decoupled ³¹P magnetic resonance spectroscopy reveals osmotic and metabolic disturbances in human hepatic encephalopathy. *Journal of neurochemistry*. 1998; 71:1564–76. [PubMed: 9751190]
- Boulanger Y, Labelle M, Khat A. Role of phospholipase A(2) on the variations of the choline signal intensity observed by ¹H magnetic resonance spectroscopy in brain diseases. *Brain Res Brain Res Rev*. 2000; 33:380–9. [PubMed: 11011072]
- Butters N. The Wernicke-Korsakoff syndrome: a review of psychological, neuropathological and etiological factors. *Curr Alcohol*. 1981; 8:205–32. [PubMed: 6806017]
- Camchong J, Stenger A, Fein G. Resting-state synchrony in long-term abstinent alcoholics. *Alcoholism, clinical and experimental research*. 2013; 37:75–85.
- Chanraud S, Pitel AL, Muller-Oehring EM, Pfefferbaum A, Sullivan EV. Remapping the brain to compensate for impairment in recovering alcoholics. *Cereb Cortex*. 2013; 23:97–104. [PubMed: 22275479]
- Chavarria L, Oria M, Romero-Gimenez J, Alonso J, Lope-Piedrafita S, Cordoba J. Brain magnetic resonance in experimental acute-on-chronic liver failure. *Liver Int*. 2013; 33:294–300. [PubMed: 23295057]
- Chavez-Pina AE, Favari L, Castaneda-Hernandez G. Pharmacokinetics of acetaminophen and its active metabolite indomethacin in rats during acute hepatic damage and liver regeneration. *Annals of hepatology*. 2009; 8:141–7. [PubMed: 19502658]
- Cordoba J, Alonso J, Rovira A, Jacas C, Sanpedro F, Castells L, Vargas V, Margarit C, Kulisevsky J, Esteban R, Guardia J. The development of low-grade cerebral edema in cirrhosis is supported by the evolution of (1)H-magnetic resonance abnormalities after liver transplantation. *Journal of hepatology*. 2001; 35:598–604. [PubMed: 11690705]
- de Graaf AA, Deutz NE, Bosman DK, Chamuleau RA, de Haan JG, Bovee WM. The use of in vivo proton NMR to study the effects of hyperammonemia in the rat cerebral cortex. *NMR in biomedicine*. 1991; 4:31–7. [PubMed: 1674207]
- Dror V, Eliash S, Rehavi M, Assaf Y, Biton IE, Fattal-Valevski A. Neurodegeneration in thiamine deficient rats-A longitudinal MRI study. *Brain research*. 2010; 1308:176–84. [PubMed: 19857469]
- Dror V, Rehavi M, Biton IE, Eliash S. Rasagiline prevents neurodegeneration in thiamine deficient rats-a longitudinal MRI study. *Brain research*. 2014; 1557:43–54. [PubMed: 24525144]
- Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ. Cigarette smoking exacerbates chronic alcohol-induced brain damage: a preliminary metabolite imaging study. *Alcoholism: Clinical and Experimental Research*. 2004; 28:1849–1860.
- Durazzo TC, Pathak V, Gazdzinski S, Mon A, Meyerhoff DJ. Metabolite levels in the brain reward pathway discriminate those who remain abstinent from those who resume hazardous alcohol consumption after treatment for alcohol dependence. *J Stud Alcohol Drugs*. 2010; 71:278–89. [PubMed: 20230726]

- Ende G, Walter S, Welzel H, Demirakca T, Wokrina T, Ruf M, Ulrich M, Diehl A, Henn FA, Mann K. Alcohol consumption significantly influences the MR signal of frontal choline-containing compounds. *NeuroImage*. 2006; 32:740–6. [PubMed: 16759881]
- Ende G, Welzel H, Walter S, Weber-Fahr W, Diehl A, Hermann D, Heinz A, Mann K. Monitoring the effects of chronic alcohol consumption and abstinence on brain metabolism: a longitudinal proton magnetic resonance spectroscopy study. *Biol Psychiatry*. 2005; 58:974–980. [PubMed: 16084857]
- Enzmann DR, Lane B. Cranial computed tomography findings in anorexia nervosa. *J Comput Assist Tomogr*. 1977; 1:410–414. [PubMed: 615218]
- Fein, G.; Meyerhoff, D.; Di Sclafani, V.; Ezekiel, F.; Poole, N.; MacKay, S.; Dillon, WP.; Constans, JM.; Weiner, MW. 1H magnetic resonance spectroscopic imaging separates neuronal from glial changes in alcohol-related brain atrophy. In: Lancaster, F., editor. *Alcohol and Glial Cells*, NIAAA Research Monograph # 27. US Government Printing Office; Bethesda, MD: 1994. p. 227-241.
- Gallazzini M, Burg MB. What's new about osmotic regulation of glycerophosphocholine. *Physiology*. 2009; 24:245–9. [PubMed: 19675355]
- Geissler A, Lock G, Frund R, Held P, Hollerbach S, Andus T, Scholmerich J, Feuerbach S, Holstege A. Cerebral abnormalities in patients with cirrhosis detected by proton magnetic resonance spectroscopy and magnetic resonance imaging. *Hepatology*. 1997; 25:48–54. [PubMed: 8985263]
- Gerner RH, Cohen DJ, Fairbanks L, Anderson GM, Young JG, Scheinin M, Linnoila M, Shaywitz BA, Hare TA. CSF neurochemistry of women with anorexia nervosa and normal women. *The American journal of psychiatry*. 1984; 141:1441–4. [PubMed: 6208797]
- Gopel C, Schmidt MH, Campanini M, Klein J. Breakdown of choline-containing phospholipids in rat brain during severe weight loss. *Neuroscience letters*. 2002; 326:21–4. [PubMed: 12052529]
- Harper CG, Giles M, Finlay-Jones R. Clinical signs in the Wernicke-Korsakoff complex: a retrospective analysis of 131 cases diagnosed at necropsy. *Journal of Neurology Neurosurgery and Psychiatry*. 1986; 49:341–345.
- Hazell AS. Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. *Neurochemistry international*. 2009; 55:129–35. [PubMed: 19428817]
- Hazell AS, Butterworth RF. Update of cell damage mechanisms in thiamine deficiency: focus on oxidative stress, excitotoxicity and inflammation. *Alcohol and alcoholism (Oxford, Oxfordshire)*. 2009; 44:141–7.
- He X, Sullivan EV, Stankovic RK, Harper CG, Pfefferbaum A. Interaction of thiamine deficiency and voluntary alcohol consumption disrupts rat corpus callosum ultrastructure. *Neuropsychopharmacology*. 2007; 32:2207–16. [PubMed: 17299515]
- Jagannathan NR, Desai NG, Raghunathan P. Brain metabolite changes in alcoholism: An in vivo proton magnetic resonance spectroscopy (MRS) study. *Magn Reson Imaging*. 1996; 14:553–557. [PubMed: 8843367]
- Kreis R, Ross BD, Farrow NA, Ackerman Z. Metabolic disorders of the brain in chronic hepatic encephalopathy detected with H-1 MR spectroscopy. *Radiology*. 1992; 182:19–27. [PubMed: 1345760]
- Kumar, V.; Abbas, A.; Fausto, N.; Robbins, SL.; Cotran, RS. *Robbins and Cotran Pathological Basis of Disease*. 7th. Elsevier Saunders; Philadelphia: 2005. p. 25-26.
- Langlais PJ, Savage LM. Thiamine deficiency in rats produces cognitive and memory deficits on spatial tasks that correlate with tissue loss in diencephalon, cortex and white matter. *Behavioural brain research*. 1995; 68:75–89. [PubMed: 7619308]
- Langlais PJ, Zhang SX. Cortical and subcortical white matter damage without Wernicke's encephalopathy after recovery from thiamine deficiency in the rat. *Alcoholism, clinical and experimental research*. 1997; 21:434–43.
- Laubenberger J, Haussinger D, Bayer S, Gufler H, Hennig J, Langer M. Proton magnetic resonance spectroscopy of the brain in symptomatic and asymptomatic patients with liver cirrhosis. *Gastroenterology*. 1997; 112:1610–1616. [PubMed: 9136840]
- Lee H, Holburn GE, Price RR. In vivo and in vitro proton NMR spectroscopic studies of thiamine-deficient rat brains. *J Magn Reson Imaging*. 2001; 13:163–166. [PubMed: 11169820]

- Lee H, Tarter J, Holbourn GE, Price RR, Weinstein DD, Martin PR. In vivo localized proton NMR spectroscopy of thiamine-deficient rat brain. *Magn Reson Med*. 1995; 34:313–318. [PubMed: 7500868]
- Lee JH, Seo DW, Lee YS, Kim ST, Mun CW, Lim TH, Min YI, Suh DJ. Proton magnetic resonance spectroscopy (1H-MRS) findings for the brain in patients with liver cirrhosis reflect the hepatic functional reserve. *The American journal of gastroenterology*. 1999; 94:2206–13. [PubMed: 10445551]
- Losowsky MS, Scott BB. Hepatic encephalopathy. *Br Med J*. 1973; 3:279–81. [PubMed: 4579294]
- Majchrowicz E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia*. 1975; 43:245–54. [PubMed: 1237914]
- Mann RE, Smart RG, Govoni R. The epidemiology of alcoholic liver disease. *Alcohol Res Health*. 2003; 27:209–19. [PubMed: 15535449]
- Mascalchi M, Belli G, Guerrini L, Nistri M, Del Seppia I, Villari N. Proton MR spectroscopy of Wernicke encephalopathy. *Ajnr*. 2002; 23:1803–6. [PubMed: 12427642]
- Meyerhoff DJ, Blumenfeld R, Truran D, Lindgren J, Flenniken D, Cardenas V, Chao LL, Rothlind J, Studholme C, Weiner MW. Effects of heavy drinking, binge drinking, and family history of alcoholism on regional brain metabolites. *Alcoholism: Clinical and Experimental Research*. 2004; 28:650–661.
- Murata T, Fujito T, Kimura H, Omori M, Itoh H, Wada Y. Serial MRI and (1)H-MRS of Wernicke's encephalopathy: report of a case with remarkable cerebellar lesions on MRI. *Psychiatry research*. 2001; 108:49–55. [PubMed: 11677067]
- Murata T, Omata N, Fujibayashi Y, Waki A, Sadato N, Yoshimoto M, Omori M, Isaki K, Yonekura Y. Dynamic changes in glucose metabolism induced by thiamine deficiency and its replenishment as revealed by a positron autoradiography technique using rat living brain slices. *Journal of the neurological sciences*. 1999; 164:29–36. [PubMed: 10385044]
- Nixon SJ, Prather R, Lewis B. Sex differences in alcohol-related neurobehavioral consequences. *Handb Clin Neurol*. 2014; 125:253–72. [PubMed: 25307580]
- Oscar-Berman M, Valmas MM, Sawyer KS, Ruiz SM, Luhar RB, Gravitz ZR. Profiles of impaired, spared, and recovered neuropsychologic processes in alcoholism. *Handb Clin Neurol*. 2014; 125:183–210. [PubMed: 25307576]
- Parks MH, Dawant BM, Riddle WR, Hartmann SL, Dietrich MS, Nickel MK, Price RR, Martin PR. Longitudinal brain metabolic characterization of chronic alcoholics with proton magnetic resonance spectroscopy. *Alcoholism: Clinical and Experimental Research*. 2002; 26:1368–1380.
- Peeling J, Shoemaker L, Gauthier T, Benarroch A, Sutherland GR, Minuk GY. Cerebral metabolic and histological effects of thioacetamide-induced liver failure. *The American journal of physiology*. 1993; 265:G572–8. [PubMed: 8214078]
- Pfefferbaum A, Adalsteinsson E, Bell RL, Sullivan EV. Development and resolution of brain lesions caused by pyridoxamine- and dietary-Induced thiamine deficiency and alcohol exposure in the alcohol-preferring rat: A longitudinal magnetic resonance imaging and spectroscopy study. *Neuropsychopharmacology*. 2007; 32:1149–1177.
- Pfefferbaum A, Rosenbloom MJ, Sassoon SA, Kemper CA, Deresinski S, Rohlfing T, Sullivan EV. Regional brain structural dysmorphology in human immunodeficiency virus infection: Effects of acquired immune deficiency syndrome, alcoholism, and age. *Biol Psychiatry*. 2012; 72:361–370. [PubMed: 22458948]
- Pfefferbaum A, Sullivan EV. Disruption of brain white matter microstructure by excessive intracellular and extracellular fluid in alcoholism: evidence from diffusion tensor imaging. *Neuropsychopharmacology*. 2005; 30:423–32. [PubMed: 15562292]
- Pfefferbaum A, Zahr NM, Mayer D, Vinco S, Orduna J, Rohlfing T, Sullivan EV. Ventricular expansion in wild-type Wistar rats after alcohol exposure by vapor chamber. *Alcoholism, clinical and experimental research*. 2008; 32:1459–67.
- Pitel AL, Chetelat G, Le Berre AP, Desgranges B, Eustache F, Beaunieux H. Macrostructural abnormalities in Korsakoff syndrome compared with uncomplicated alcoholism. *Neurology*. 2012; 78:1330–3. [PubMed: 22496200]

- Prasad S, Dhiman RK, Duseja A, Chawla YK, Sharma A, Agarwal R. Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy. *Hepatology* (Baltimore, Md. 2007; 45:549–59.
- Rackayova V, Braissant O, McLin VA, Berset C, Lanz B, Cudalbu C. H and P magnetic resonance spectroscopy in a rat model of chronic hepatic encephalopathy: in vivo longitudinal measurements of brain energy metabolism. *Metabolic brain disease*. 2015
- Rose SE, Nixon PF, Zelaya FO, Wholohan BT, Zimitat C, Moxon LN, Crozier S, Brereton IM, Doddrell DM. Application of high field localised in vivo 1H MRS to study biochemical changes in the thiamin deficient rat brain under glucose load. *NMR Biomed*. 1993; 6:324–8. [PubMed: 7903547]
- Rosenbloom MJ, Sullivan EV, Pfefferbaum A. HIV infection and alcoholism: Comorbidity effects on brain structure and function. *Alcohol Research & Health*. 2010; 33:247–357. [PubMed: 23584066]
- Ross BD, Jacobson S, Villamil F, Korula J, Kreis R, Ernst T, Shonk T, Moats RA. Subclinical hepatic encephalopathy: proton MR spectroscopic abnormalities. *Radiology*. 1994; 193:457–463. [PubMed: 7972763]
- Rovira A, Alonso J, Cordoba J. MR imaging findings in hepatic encephalopathy. *Ajnr*. 2008; 29:1612–21. [PubMed: 18583413]
- Rovira A, Cordoba J, Sanpedro F, Grive E, Rovira-Gols A, Alonso J. Normalization of T2 signal abnormalities in hemispheric white matter with liver transplant. *Neurology*. 2002; 59:335–41. [PubMed: 12177365]
- Schweinsburg BC, Alhassoon OM, Taylor MJ, Gonzalez R, Videen JS, Brown GG, Patterson TL, Grant I. Effects of alcoholism and gender on brain metabolism. *Am J Psychiatry*. 2003; 160:1180–1183. [PubMed: 12777281]
- Sechi G, Serra A. Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management. *Lancet Neurol*. 2007; 6:442–55. [PubMed: 17434099]
- Seitz D, Widmann U, Seeger U, Nagele T, Klose U, Mann K, Grodd W. Localized proton magnetic resonance spectroscopy of the cerebellum in detoxifying alcoholics. *Alcoholism: Clinical and Experimental Research*. 1999; 23:158–163.
- Vaquero J, Chung C, Cahill ME, Blei AT. Pathogenesis of hepatic encephalopathy in acute liver failure. *Seminars in liver disease*. 2003; 23:259–69. [PubMed: 14523679]
- Vulimiri SV, Berger A, Sonawane B. The potential of metabolomic approaches for investigating mode(s) of action of xenobiotics: case study with carbon tetrachloride. *Mutation research*. 2011; 722:147–53. [PubMed: 20188855]
- Zahr NM, Alt C, Mayer D, Rohlfing T, Manning-Bog A, Luong R, Sullivan EV, Pfefferbaum A. Associations between in vivo neuroimaging and postmortem brain cytokine markers in a rodent model of Wernicke's encephalopathy. *Experimental neurology*. 2014a
- Zahr NM, Carr RA, Rohlfing T, Mayer D, Sullivan EV, Colrain IM, Pfefferbaum A. Brain Metabolite Levels in Recently Sober Individuals with Alcohol Use Disorder: Dependence on Use Variables. *Psychiatry Research: Neuroimaging in review*. 2015a
- Zahr NM, Mayer D, Rohlfing T, Hasak M, Hsu O, Vinco S, Orduna J, Luong R, Sullivan EV, Pfefferbaum A. Brain injury and recovery following binge ethanol: Evidence from in vivo MR spectroscopy. *Biol Psychiatry*. 2010a; 67:846–854. [PubMed: 20044076]
- Zahr NM, Mayer D, Rohlfing T, Hasak M, Hsu O, Vinco S, Orduna J, Luong R, Sullivan EV, Pfefferbaum A. Brain Injury and Recovery Following Binge Ethanol: Evidence from In Vivo Magnetic Resonance Spectroscopy. *Biological psychiatry*. 2010b; 67:846–54. [PubMed: 20044076]
- Zahr, NM.; Mayer, D.; Rohlfing, T.; Hasak, M.; Hsu, O.; Vinco, S.; Orduna, J.; Sullivan, EV.; Pfefferbaum, A. Binge Ethanol Induced Structural and Neurochemical Changes in the Rat Brain Detectable at 3T International Society on Magnetic Resonance in Medicine. Honolulu, HA: 2009a.
- Zahr NM, Mayer D, Rohlfing T, Hsu O, Vinco S, Orduna J, Luong R, Bell RL, Sullivan EV, Pfefferbaum A. Rat strain differences in brain structure and neurochemistry in response to binge alcohol. *Psychopharmacology*. 2014b; 231:429–45. [PubMed: 24030467]

- Zahr NM, Mayer D, Rohlfing T, Orduna J, Luong R, Sullivan EV, Pfefferbaum A. A mechanism of rapidly reversible cerebral ventricular enlargement independent of tissue atrophy. *Neuropsychopharmacology*. 2013; 38:1121–9. [PubMed: 23306181]
- Zahr NM, Mayer D, Rohlfing T, Sullivan EV, Pfefferbaum A. Imaging neuroinflammation? A perspective from MR spectroscopy. *Brain pathology (Zurich, Switzerland)*. 2014c; 24:654–64.
- Zahr NM, Mayer D, Vinco S, Orduna J, Luong R, Sullivan EV, Pfefferbaum A. In vivo evidence for alcohol-induced neurochemical changes in rat brain without protracted withdrawal, pronounced thiamine deficiency, or severe liver damage. *Neuropsychopharmacology*. 2009b; 34:1427–42. [PubMed: 18704091]
- Zahr NM, Rohlfing T, Mayer D, Luong R, Sullivan EV, Pfefferbaum A. Transient CNS responses to repeated binge ethanol treatment. *Addiction biology*. 2015b
- Zwingmann C, Chatauret N, Rose C, Leibfritz D, Butterworth RF. Selective alterations of brain osmolytes in acute liver failure: protective effect of mild hypothermia. *Brain research*. 2004; 999:118–23. [PubMed: 14746928]

Fig. 1.

Left: MR and treatment schedule including number of animals used at each time point and **Right:** weight changes over the course of the experiment for **a)** liver damage (carbon tetrachloride, CCl₄) model, **b)** food deprivation (FD) model, **c)** and thiamine deficiency + ethanol (EtOH) model. Ctrl=control; PE=pyrithiamine-treated+EtOH; PS=pyrithiamine-treated+saline; TE=thiamine-treated+EtOH

Fig. 2. MR images showing two time points (baseline and following treatment) in exemplary Ctrl (top) and CCL₄-treated (bottom) animals. Arrows indicate lateral ventricles (top arrow) and cisterns (bottom arrow).

Fig. 3.

Volumes of lateral ventricles (left), cisterns (middle), and total CSF (lateral ventricles + cisterns + temporal pole CSF, right) in **a)** liver damage (CCL₄) model, **b)** food deprivation (FD) model, **c)** and thiamine deficiency + ethanol (EtOH) model. Ctrl=control; PE=pyrithiamine-treated+EtOH; PS=pyrithiamine-treated+saline; TE=thiamine treated +EtOH, †p 0.1, *p 0.05.

Fig. 4. Levels of N-acetylaspartate (NAA), total creatine (tCr), choline-containing compounds (Cho), and glutamate (Glu) in **a)** liver damage (CCl₄) model, **b)** food deprivation (FD) model, **c)** and thiamine deficiency + ethanol (EtOH) model. Ctrl=control; PE=pyrithiamine-treated+EtOH; PS=pyrithiamine-treated+saline; TE=thiamine treated+EtOH, †p 0.1, *p 0.05.

Table 1

Percent Change in Weight and MR Variables between Scans

	MR1 - MR2						MR2 - MR3						MR3 - MR4					
	we igh t	lat .ve nt s	cist ern s	to ta l c sf	N A A	t C r	C h o	G lu	we igh t	lat .ve nt s	cist ern s	to ta l c sf	N A A	t C r	C h o	G lu		
Liver Damage																		
carbon tetrachloride (CCL4)	-10	+14	-3	+9	-4	-3	-19	+18	-	-	-	-	-	-	-	-		
control (Ctrl)	+4	+1	+4	+2	-1	-1	+4	-9	-	-	-	-	-	-	-	-		
Food Deprivation																		
food deprived (FD)	-21	+7	+20	+15	-1	-1	-1	+6	+36	-7	-9	-8	+1	+2	+13	+6		
control (Ctrl)	+7	+11	+11	+12	-1	-1	-8	+2	+4	+3	+5	+1	+0.1	+1	+2	+3		
Thiamine Depletion + EtOH																		
thiamine + EtOH (TE)	-13	+43	+18	+18	-5	-8	+3	+4	+8	+11	-10	-11	+6	-1	-0.5	+6		
pyrithiamine + saline (PS)	+6	+6	+14	+16	-4	-8	+0.5	-3	-14	+36	+3	+8	-17	-5	-25	+6		
pyrithiamine + EtOH (PE)	-16	+70	+27	+40	-6	-7	+11	-9	-14	+32	-11	+13	-11	+2	-18	+18		

= not applicable