BIPOLAR DISORDER COMORBID WITH ALCOHOLISM: A \(^1\)H MAGNETIC RESONANCE SPECTROSCOPY STUDY

Fabiano G. Nery\(^{1,2,3}\), Jeffrey A. Stanley\(^4\), Hua-Hsuan Chen\(^5\), John P. Hatch\(^{1,6}\), Mark A. Nicoletti\(^7\), E. Serap Monkul\(^{1,3}\), Beny Lafer\(^3\), and Jair C. Soares\(^7\)

\(^1\) Department of Psychiatry, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA
\(^2\) South Texas Veterans Health Care System, Audie L. Murphy, San Antonio, Texas, USA
\(^3\) Bipolar Disorder Research Group (PROMAN), Department of Psychiatry, University of São Paulo Medical School, São Paulo, Brazil
\(^4\) Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI
\(^5\) Department of Radiology, The University of Texas Health Science Center at San Antonio (UTHSCSA), San Antonio, TX, USA
\(^6\) Department of Orthodontics, UTHSCSA, San Antonio, Texas, USA
\(^7\) Department of Psychiatry and Behavioral Sciences, Harris County Psychiatric Center at University of Texas Medical School, Houston, TX, USA

Abstract

Alcoholism is highly prevalent among bipolar disorder (BD) patients, and its presence is associated with a worse outcome and refractoriness to treatment of the mood disorder. The neurobiological underpinnings that characterize this comorbidity are unknown. We sought to investigate the neurochemical profile of the dorsolateral prefrontal cortex (DLPFC) of BD patients with comorbid...
alcoholism. A short-TE, single-voxel $^1$H spectroscopy acquisition at 1.5 Tesla from the left DLPFC of 22 alcoholic BD patients, 26 non-alcoholic BD patients and 54 healthy comparison subjects (HC) were obtained. Absolute levels of N-acetyl aspartate, phosphocreatine plus creatine, choline-containing compounds, myo-inositol, glutamate plus glutamine (Glu+Gln) and glutamate were obtained using the water signal as an internal reference. Analysis of co-variance was used to compare metabolite levels among the three groups. In the primary comparison, non-alcoholic BD patients had higher glutamate concentrations compared to alcoholic BD patients. In secondary comparisons integrating interactions between gender and alcoholism, non-alcoholic BD patients presented significantly higher glutamate plus glutamine (Glu+Gln) than alcoholic BD patients and HC. These results appeared to be driven by differences in male subjects. Alcoholic BD patients with additional drug use disorders presented significantly lower myo-inositol than BD patients with alcoholism alone. The co-occurrence of BD and alcoholism may be characterized by neurochemical abnormalities related to the glutamatergic system and to the inositol second messenger system and/or in glial pathology. These abnormalities may be the neurochemical correlate of an increased risk to develop alcoholism in BD, or of a persistently worse clinical and functional status in BD patients in remission from alcoholism, supporting the clinical recommendation that efforts should be made to prevent or early diagnose and treat alcoholism in BD patients.

Keywords
Bipolar disorder; alcoholism; magnetic resonance spectroscopy; prefrontal cortex; glutamate

INTRODUCTION

Alcoholism is highly prevalent among bipolar disorder (BD) patients, and its presence is associated with a significant negative impact on the course of BD (Frye et al., 2006). The reasons for the increased risk of alcoholism in BD patients are unknown, and shared neurobiological substrates may potentially explain this high comorbidity. The neuroanatomic model of BD comprises brain regions that substantially overlap with the neuroanatomic model of addiction, including limbic-thalamic-cortical, limbic-striatal-pallidal-thalamic, and striatal-ventral pallidal-thalamic-cortical neurocircuits (Soares, 2003; Adinoff, 2004; Sullivan & Pfefferbaum, 2005). The dorsolateral prefrontal cortex (DLPFC) is a key brain area in both models. The DLPFC corresponds to the middle frontal gyrus (Brodmann areas 9 and 46) and is functionally responsible for high cognitive functions, such as working memory, language, attention, judgment and behavior planning (D'Esposito, 2003). Controlled $^1$H spectroscopy studies of the DLPFC in BD patients have revealed lower levels of N-acetyl aspartate (NAA), phosphocreatine plus creatine (PCr+Cr) and choline-containing compounds (GPC+PC), and higher levels of glutamate plus glutamine (Glu+Gln) (Winsberg et al., 2000; Chang et al., 2003; Michael et al., 2003; Sassi et al., 2005; Molina et al., 2007; Frey et al., 2007). Although these findings are not consistent across different $^1$H MRS studies, they support a current hypothesis that BD is associated with impairments in neuroplasticity and cellular resilience (Schloesser et al., 2008), as these metabolites are considered to be markers of neuronal integrity (NAA), energy metabolism (PCr+Cr), membrane phospholipid breakdown and synthesis (GPC+PC), and glutamatergic abnormalities (Glu+Gln) (Stanley et al., 2002). Reductions of NAA and GPC+PC in the frontal lobes of alcohol abuse/dependence patients as a consequence of the neurotoxic effects of chronic alcohol consumption have also been described (Bendszuz et al., 2001; Parks et al., 2002; Ende et al., 2005; Bartsch et al., 2007). In spite of the high prevalence of the co-occurrence between these two conditions, no $^1$H spectroscopy study has investigated the neurochemical profile of BD patients with comorbid alcohol abuse/dependence. Such a study would help to characterize the neurochemical profile of alcoholic BD patients and potentially help to plan better preventive and therapeutic options for this highly prevalent comorbidity. Therefore, we conducted this single-voxel, short-echo time (TE) $^1$H
spectroscopy study to measure neuro-metabolite levels in the left DLPFC of BD patients with and without a prior diagnosis of alcohol abuse/dependence and healthy comparison subjects (HC) and to determine whether alcoholic BD patients present differences in neurometabolite levels as a function of alcoholism diagnosis.

MATERIAL AND METHODS

Participants

The sample comprised 22 BD patients with comorbid alcohol abuse or dependence (hereafter referred to as alcoholic BD patients) (mean age±S.D.: 39.1±11.4 y; males: 27.3%; 11 with other substance use disorders; who were in remission from alcoholism for at least 6 months), 26 BD patients without alcohol abuse or dependence (hereafter referred to as non-alcoholic BD patients) (mean age±S.D.: 39.8±12.5 y; males: 19.2%), and 54 HC (mean age±S.D.: 38.7 ±12.4 y; males: 25.9%). Subjects were group-matched with respect to age and gender. All of the patients and HC were residents of the San Antonio metropolitan area or surrounding cities. The subjects were outpatients or healthy volunteers recruited from the community through local media advertisements and flyers posted in the medical center. All study procedures were carried out according to the Declaration of Helsinki. Subjects gave written informed consent to participate in the study after the nature of the procedures had been fully explained. The study design was reviewed and approved by the local Institutional Review Board. Inclusion criteria for BD patients included a DSM-IV diagnosis of BD, type I or II and age over 18 y. Patients could be in any mood state, and were allowed to be on any psychotropic medication. BD patients meeting DSM-IV criteria for a lifetime diagnosis of alcohol or drug abuse or dependence (in remission for at least 6 months) formed the alcoholic BD group, and BD patients who never met DSM-IV criteria for any lifetime diagnosis of alcohol or drug abuse or dependence formed the non-alcoholic BD group. The demographic and clinical characteristics of the sample are displayed in Table 1. Exclusion criteria for all patients were the presence of any Axis I diagnosis other than BD, except a lifetime diagnosis of any anxiety disorders, presence of significant medical problems (such as hypertension, diabetes mellitus, renal or active liver disease) or neurological disorders (such as epilepsy, stroke, dyslexia or head trauma with loss of consciousness for more than one hour). The exclusion criteria for HC were the presence of any past or current Axis I psychiatric disorder, presence of significant neurological or medical problems and presence of any Axis I diagnosis in first-degree relatives.

Psychiatric assessments

The diagnostic assessments were conducted by research-trained psychiatrists, using the Structured Clinical Interview for DSM-IV (SCID), versions for patients and non-patients (First et al., 1995). The Hamilton Depression Rating Scale (HDRS) 21-item version (Hamilton, 1976) and the Young Mania Rating Scale (Young et al., 1978) were administered to all BD patients, within 2 weeks of the scanning date, to assess severity of depressive and manic symptoms. Information on course and severity of alcoholism (such as age at onset of alcoholism, length of time of heavy drinking and time in remission from alcoholism) were assessed using the substance use disorders module from the SCID.

Magnetic resonance spectroscopy (MRS) acquisition

The in vivo single-voxel, short-TE $^1$H spectroscopy study was conducted on a 1.5 T Philips Gyroscan Intera scanner. Axial, sagittal and coronal T$^1$-weighted localizer images were first obtained to verify patient positioning and determine voxel placement. Then, a 2×2×2 cm (8 cm$^3$) voxel was placed in the left DLPFC (Figure 1), using the superior frontal sulcus, the lateral fissure, and the genu of corpus callosum as anatomical landmarks (Jackson & Duncan, 1996). All $^1$H spectroscopy data were acquired using a point-resolved spectroscopy sequence (PRESS) with TE=30 ms, TR=3.0 s, bandwidth=2 kHz, 4096 complex data points and 256
averages. Water unsuppressed spectra were also acquired at the same location for absolute quantification of metabolites with units of mmol/kg wet weight (Stanley et al., 1995).

MRS post-processing

NAA, PCr+Cr, GPC+PC, myo-inositol (myo-Ins), glutamate, and the summation of glutamate plus glutamine (Glu+Gln), as well as glutamine, N-acetyl aspartylglutamate (NAAG), taurine, alanine, aspartate, gamma-aminobutyric acid (GABA), glucose, and lipid resonances and macromolecule resonances (Seeger et al., 2003) were quantified using the Linear Combination (LC) Model software (Provencher, 1993), an operator-independent fitting routine (Figure 2). The LC Model approach allows for the incorporation of a priori knowledge of the multiplet structures including signals of macromolecules and lipids, which has been demonstrated by several groups to be effective in accurately quantifying 1.5 T short-TE, $^1$H spectroscopy data (de Graaf et al., 1990; Stanley et al., 1995; Bartha et al., 2000). We included in this analysis only the $^1$H metabolites with reasonable level of fitting confidence [i.e., the metabolites with an overall mean Cramer-Rao Lower Bound (CRLB) value of less than 20%], which include NAA, GPC+PC, PCr+Cr, myo-Ins, Glu+Gln and glutamate. If the CRLB value of a metabolite of interest exceeded 20%, then the metabolite levels from that spectrum were excluded from the analysis.

To address the variability in the tissue composition of the voxels of interest, the proportions of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) were estimated for each extracted $^1$H spectroscopy voxel. In a fully automated procedure, the T1-weighted images were coregistered to the axial scout images and corrected for any B1 field bias. The brain then was extracted, and the images were segmented into partial volume maps of GM, WM, and CSF/extracortical space using FSL tools (Smith et al., 2004). The tissue fractions within the voxels of interest were then extracted from the segmented images by matching the coordinates and size of the $^1$H spectroscopy voxel with the images using the FSL tools. The GM, WM, and CSF voxel content values, along with the other appropriate correction factors, were then utilized to obtain absolute quantification values, as described elsewhere (Stanley et al., 1995).

Statistical analysis

Exact $X^2$ tests for cross-tabulated qualitative data and the Mann-Whitney $U$ test or analysis of variance tests for ordinal and interval scale data were used to compare the BD groups and HC with respect to clinical and demographic variables, and in post hoc analyses to study the effects of subdiagnosis of alcoholism (abuse versus dependence, and alcoholism alone versus alcoholism plus other substance use disorders) within the alcoholic BD group. Analysis of covariance (ANCOVA) with age and gender as covariates was used to study the differences in the brain metabolites among the alcoholic BD patients, non-alcoholic BD patients, and HC. We first tested the assumption of equality of regression for the covariates. When interactions involving age or gender and diagnosis were significant in preliminary models, age (statistically controlled at the 25th, 50th, and 75th percentiles), and gender were included as a factor rather than as a covariates in the final model. Pearson correlations were used to study the associations among the brain metabolites and clinical characteristics of BD and alcoholism within the alcoholic BD group. All reported means and standard deviations (S.D.) are the unadjusted values. The level of significance was set at $p=0.05$, with Bonferroni adjustment for multiple comparisons among groups. SPSS (SPSS, Inc., Chicago, IL) version 12.0 was used to do all of the analyses.
RESULTS
The three groups were matched by age [ANOVA, F(2,99)=0.07, p=0.93] and gender (X²=0.54, df=2, p=0.76). Demographic and clinical characteristics of the two groups of BD patients are displayed in Table 1.

Regarding spectroscopy data quality, the mean signal-to-noise ratios of NAA for the alcoholic BD group, non-alcoholic BD group and HC, as estimated by LC Model, were 17.9±3.4 versus 17.8±4.4 versus 19.2±3.6, [ANOVA, F(2,99)=1.7, p=0.2], respectively. The mean full-width-half-maximum (FWHM) of the NAA peak for the same groups were 0.049±0.012 versus 0.05±0.01 versus 0.046±0.007 [ANOVA, F(2,99)=1.9, p=0.16], respectively. There were no significant differences on CRLB mean values for NAA, GPC+PC, PCr+Cr, myo-Ins, Glu +Gln and glutamate among the three groups (F values ranging from 0.09 to 2.2, p values ranging from 0.12 to 0.91). There were also no differences in the tissue composition within the voxel of interest among the three groups (Table 2).

Primary comparisons among alcoholic BD patients, non-alcoholic BD patients and HC
ANCOVA, with age and gender as covariates, showed statistical differences among the groups only regarding glutamate levels [F(2,95)=3.2, p=0.045]. Post hoc analyses showed that non-alcoholic BD patients presented significantly higher glutamate levels than alcoholic BD patients (5.86±1.26 mmol/kg versus 5.18±0.96 mmol/kg; p=0.04), and non-significantly higher glutamate levels than HC (5.55±0.98 mmol/kg; p=0.41). Alcoholic BD patients did not differ from HC on glutamate levels (p=0.43).

Effects of interaction between age, gender and alcoholism diagnosis
Analyses revealed an interaction between gender and comorbid alcoholism regarding Glu+Gln and myo-Ins levels and between age and comorbid alcoholism regarding GPC+PC levels. Therefore, these analyses were respectively conducted with gender as a factor and age as the single covariate, and with age statistically held constant at the 25th, 50th, and 75th percentiles and gender as the single covariate. We present mean±S.D. for all metabolites and final statistics of diagnosis effects in Table 3.

Regarding Glu+Gln levels, the main effects of gender and comorbid alcoholism, and the interaction between gender and comorbid alcoholism were all significant [for gender, F(1,95)=7.3, p=0.008; for comorbid alcoholism, F(2,95)=6.24, p=0.003; for the interaction between gender and comorbid alcoholism, F(2,95)=4.7, p=0.01]. Non-alcoholic BD patients presented statistically higher Glu+Gln levels than alcoholic BD patients (p=0.007) and than HC (p=0.006). The significant interaction between gender and comorbid alcoholism was deconstructed using simple effects analysis. Male non-alcoholic BD patients presented significantly higher Glu+Gln levels than male alcoholic BD patients (10.84± (s.d.=2.75) versus 7.56± (s.d.=0.98) mmol/kg, p=0.002) and than HC (8.33± (s.d.=1.19); p=0.01). Male alcoholic BD patients presented non-significantly lower Glu+Gln levels than alcoholic BD patients (p=0.7) (Figure 3). Female alcoholic BD patients did not statistically differ from female non-alcoholic BD patients (7.94±1.6 versus 8.0±1.16 mmol/kg, p=1.0) and from female HC (7.53±1.79 mmol/kg, p=0.92) on Glu+Gln levels. Female non-alcoholic BD patients did not differ from female HC on Glu+Gln levels (p=0.70).

For myo-Ins levels, ANCOVA examining the main effects of gender and comorbid alcoholism and their interaction showed that only the interaction was significant [main effect for gender, F(1,95)=0.06, p=0.81; main effect for comorbid alcoholism, F(2,95)=1.75, p=0.18; for the interaction between gender and comorbid alcoholism, F(2,95)=5.53, p=0.005]. The significant interaction between gender and comorbid alcoholism was deconstructed using simple effects analysis.
Female non-alcoholic BD patients presented significantly higher myo-Ins levels than female alcoholic BD patients (5.17±1.12 versus 4.53±0.58 mmol/kg, p=0.012). Female alcoholic BD patients also presented significantly lower myo-Ins levels than female HC (4.98 ±0.79, p=0.019). Male alcoholic BD patients did not statistically differ from male non-alcoholic BD patients (5.06±0.74 versus 4.42±0.46 mmol/kg, p=0.09) and from male HC (5.38±0.79 mmol/kg, p=1.0) on myo-Ins levels.

For GPC+PC levels, ANCOVA with age and gender as covariates revealed a significant interaction between age and comorbid alcoholism. Because age is a continuous variable, we compared adjusted mean GPC+PC levels with the age covariate held constant at the 25th (28.48 years), the 50th (37.58 years) and the 75th (49.54 years) percentiles of age with gender as a covariate. These analyses showed that the significant interaction between age and comorbid alcoholism on GPC+PC levels were primarily driven by differences within the oldest subjects (i.e., those subjects at the 75th percentile of the age distribution). Thus, within the oldest subjects, non-alcoholic BD patients presented statistically higher GPC+PC levels than alcoholic BD patients (1.84±0.05 versus 1.59±0.06 mmol/kg, F(1,95)=9.23, p=0.009) and than HC (1.66±0.04 mmol/kg, F(1,95)=7.70, p=0.02). Alcoholic BD patients presented non-statistically lower GPC+PC levels than HC [F(1,95)=1.43, p=0.69].

**Post hoc comparisons between alcoholic and non-alcoholic BD patients**

The alcoholic and the non-alcoholic BD groups were statistically similar with respect to most demographic and clinical characteristics of the disorder (details in Table 1). However, alcoholic BD patients presented a statistically higher frequency of prior psychotic symptoms (p=0.04) than non-alcoholic BD patients. The distribution of psychiatric medication was essentially similar between the two BD groups, with the exception of atypical antipsychotic medication, which was more frequently used among alcoholic BD patients than among non-alcoholic BD patients (p=0.05) (Table 1). We attempted to adjust for the effects of these two variables (psychosis and atypical antipsychotic medication) by adding them as covariates in an ANCOVA comparing the alcoholic and non-alcoholic BD groups. When controlling for age, gender, prior psychotic symptoms and current exposure to atypical antipsychotics, non-alcoholic BD patients presented statistically higher glutamate levels than alcoholic BD patients [F(1,42)=4.1, p=0.048]. Changing gender from covariate to factor, the effects of diagnosis, gender and interaction between diagnosis and gender were also found for Glu+Gln (diagnosis: F(1,41)=8.1, p=0.007; gender: F(1,41)=3.3, p=0.08; interaction between diagnosis and gender: F(1,41)=9.6, p=0.004), for glutamate (diagnosis: F(1,41)=6.7, p=0.01; gender: F(1,41)=6.2, p=0.02), and for myo-Ins levels (interaction between diagnosis and gender: F(1,41)=8.3, p=0.008).

**Post hoc analyses within the alcoholic BD group**

Within the alcoholic BD group, 11 (50%) subjects presented only alcoholism and 11 (50%) also met DSM-IV criteria for an additional comorbid drug use disorder (see Table 1). Comparisons between these two subgroups showed that BD patients with alcoholism and additional drug use disorders presented statistically lower myo-Ins levels (4.32±0.57 versus 5.02±0.54 mmol/kg, p=0.01, Mann Whitney U test) and marginally lower glutamate levels (4.99±1.14 versus 5.37±0.74 mmol/kg, p=0.08, Mann Whitney U test) than BD patients with alcoholism alone. There were no differences on the other brain metabolite levels between these two subgroups (p values ranging from 0.37 to 0.78).

Within the alcoholic BD group, 12 (54.5%) subjects met DSM-IV criteria for a subdiagnosis of alcohol abuse and 10 (45.5%) met criteria for alcohol dependence. There was no significant association between alcoholism subdiagnosis and brain metabolite levels (p values ranging from 0.11 to 0.74, Mann Whitney U test).
Within the alcoholic BD group, there were no significant correlations between any brain metabolites and clinical characteristics of BD or alcoholism, including current depressive or manic symptoms severity, age at onset of BD, length of illness of BD, age at onset of alcoholism, duration of heavy use of alcohol, and time in remission from alcoholism.

**DISCUSSION**

Alcoholic BD patients, in long-term remission from alcoholism, presented statistically lower Glu+Gln and glutamate levels in their left DLPFC than BD patients who never developed alcoholism or any other substance use disorder. These results are consistent with a defect in the glutamatergic system as a risk factor for the development of alcoholism. One of the strongest predictors for development of alcoholism is a reduced sensitivity to the dysphoric and adverse effects of ethanol (Schuckit & Smith, 1996). Reduced sensitivity to alcohol effects might be consequent to an altered N-methyl D-aspartate (NMDA) glutamate receptor function. This is suggested by the finding that a blunted response to the NMDA glutamate receptor antagonist, ketamine, is present in healthy individuals at high risk to develop alcoholism (Petakis et al., 2004). Also, a genetic variation in the NR2A, a gene that expresses the NMDA glutamate receptor, is associated with an increased risk for heavy drinking in adolescents or with alcohol dependence in adults (Schumann et al., 2008).

Non-alcoholic BD patients presented significantly higher Glu+Gln levels than HC. This is in line with several $^1$H spectroscopy studies in adult BD subjects that report statistically higher Glu+Gln levels in different brain areas (DLPFC, anterior cingulate, occipital cortex) compared to HC (Michael et al., 2003; Dager et al., 2004; Bhagwagar et al., 2007; Frye et al., 2007), and with a hypothesis that a hyperglutamatergic state is present in the pathophysiology of BD (Krystal et al., 2002).

Abnormalities in Glu+Gln levels are usually interpreted as indirect evidence for abnormalities in the glutamatergic neurotransmitter system, because this spectral region is dominated by the contribution of glutamate (Rothman et al., 1992), and the metabolism of glutamate and glutamine is highly coupled through the removal of glutamate from the synapses and conversion of glutamate to glutamine by the glia cells (Magistretti & Pellerin, 1999). It is uncertain to what extent Glu+Gln reflects a neurotransmitter pool (synaptic glutamate) or a metabolic pool (intracellular glutamate and glutamine). Eighty percent of the total brain glutamate concentration is found in glutamatergic neurons (i.e., a large “metabolic” pool of glutamate derived from glucose precursors) and only 2–20% in glial processes (small “neurotransmitter” pool involved in the glutamate-glutamine cycling) (Erecinska & Silver, 1990; Magistretti & Pellerin, 1999). Recent data show that glutamate from the “metabolic” pool can also sustain excitatory synaptic release independent of the replenishment of glutamate from the smaller glutamate-glutamine cycling compartment (Kam & Nicoll, 2007). Therefore, our findings point to a different glutamatergic neurotransmitter function in the co-occurrence of BD and alcoholism compared to BD alone.

It is noteworthy that, once the samples were divided by gender and interactions between gender and comorbid alcoholism were deconstructed, differences on Glu+Gln levels were seen only among male subjects (Figure 3). On the other hand, differences on myo-Ins levels were significant only among female subjects. Effects of gender have been previously demonstrated in BD comorbid with alcoholism (Frye et al., 2003). Although alcoholism is more prevalent among males than among females with BD, the odds ratio for alcoholism compared to the general population is much higher for females than for males with BD (Frye et al., 2003). In addition, female alcoholic BD patients present higher rates of polysubstance abuse, social phobia, family history of alcoholism, prior history of verbal abuse and more depressive episodes than female non-alcoholic BD patients. In contrast, male alcoholic BD patients present...
a higher frequency of family history of BD and of alcohol and drug abuse, prior history of physical abuse and more suicide attempts than male non-alcoholic BD patients. Thus, shared liability for BD and alcoholism may be dependent on gender-specific genetic, environmental, and – based on our findings – neurobiological factors.

An interaction between age and diagnosis was also found on GPC+PC levels. The oldest alcoholic BD patients presented significantly lower GPC+PC levels than the oldest non-alcoholic BD patients, as opposed to non-significant differences between the youngest alcoholic and non-alcoholic BD patients. GPC+PC levels are lower in prefrontal brain areas of recently detoxified alcoholic patients compared to HC (Durazzo et al., 2004; Ende et al., 2005; Lee et al., 2007). These decreased GPC+PC levels are interpreted as indicating damage to cell membranes and/or myelin caused by chronic alcohol consumption, because they increase with abstinence proportionally to the amount of gray and white matter recovery (Bartsch et al., 2007). On the other hand, gray and white matter volume losses from aging processes are accelerated in alcoholic patients (Pfefferbaum et al., 1992). Taken together, these findings suggest that aging processes interact with persistent alcohol consumption effects causing larger decrements in GPC+PC levels in older alcoholic BD patients than in younger alcoholic BD patients.

We should caution however that these abovementioned significant interactions between gender, age, and diagnosis, although intriguing, were not predicted and were based on small subsamples of patients (particularly the interactions concerning gender effects). Therefore, these findings need to be replicated by further studies and any potential explanation is speculative at this point.

All the metabolite level differences between alcoholic BD patients and HC were in opposing directions relative to the differences between non-alcoholic BD patients and HC (i.e., higher for non-alcoholic BD patients, lower for alcoholic BD patients), and alcoholic BD patients did not statistically differ from HC on most of the analyses. Whereas we cannot offer an explanation for these intriguing opposite effects, they suggest the existence of complex antagonistic neurobiological mechanisms underlying the co-occurrence of BD and alcoholism, rather than a simple synergistic or additive effect. A more parsimonious explanation is that BD comorbid with alcoholism does not have the same pathophysiology of BD alone, and that alcoholic BD patients really have normal metabolite levels in their left DLPFC. As this is the first investigation of in vivo brain metabolites in BD patients with comorbid alcoholism, further research is needed to better characterize the complexity of the neurochemical changes observed.

Within the alcoholic BD group, BD patients with alcohol and other drug use disorders presented greater left DLPFC deficits of myo-Inositol and glutamate than patients with alcoholism alone. Glutamatergic abnormalities also characterize drug use disorders (Kalivas & Volkow, 2004). Moreover, these results are partially consistent with reduced DLPFC function in patients with cannabis or cocaine use disorders (Adinoff et al., 2003; Bolla et al., 2005). Taken together, these findings suggest that a more severe glial pathology and glutamatergic system defect are present in the left DLPFC of BD patients with polysubstance use disorders compared to BD patients with alcoholism use alone.

Alcohol abuse and alcohol dependence are two distinct subcategories of alcoholism (Hasin, 2003). Some DSM-IV diagnostic criteria for alcohol dependence, e.g., including tolerance, craving, and withdrawal, correspond to symptoms that originate from well-defined biological substrates (Kalivas & Volkow, 2004) as opposed to diagnostic criteria for alcohol abuse. We did not find any differences in the left DLPFC metabolite levels between subjects with alcohol abuse versus alcohol dependence. This was an exploratory analysis, based on small numbers
in each subgroup (11 with abuse, 11 with dependence). Thus, we cannot rule out a Type II error. Future research in larger samples of BD patients should examine how alcohol abuse differs from alcohol dependence on neurobiological aspects.

This study has some limitations. First, the ability to resolve glutamate from glutamine at low magnetic field strengths such as 1.5 Tesla is technically difficult, due to the overlap between the multiple peaks of glutamate and neighboring metabolites, such as glutamine, GABA, homocarnosine, NAAG, glutathione and signals from macromolecules (Schubert et al., 2004). The reliability of quantifying glutamate is highly dependent on the signal-to-noise ratio (and hence, on the ability to shim well and minimize spectral artifacts) and on methods used to post-process and quantify the spectroscopy data (i.e., minimizing any manipulation applied to the raw signal and using the appropriate a priori knowledge) (Stanley et al., 1995; Stanley et al., 2000). In this study, we obtained reasonably good quality data, as attested by the range on CRLB values for glutamate (between 8 and 20%) and the signal-to-noise ratios and FWHM values, and we used a priori knowledge, which has been demonstrated as an effective approach to resolve overlapping signals at 1.5 T (de Graaf et al., 1990; Stanley et al., 1995; Bartha et al., 2000). Second, the current and past medication exposure is a potential confounder. Previous 1H spectroscopy studies suggest that neurometabolite levels are influenced by acute or chronic administration of selective serotonin reuptake inhibitors, lithium, mood stabilizers and atypical antipsychotics (Sanacora et al., 2002; Goff et al., 2002; Silverstone et al., 2003). Although the two patient groups presented similar exposure to psychiatric medication, we cannot completely rule out effects of current medication exposure on brain metabolites. It is important to note that current medication exposure would be expected to influence brain metabolite concentrations toward normalization of levels, and therefore, it is possible that medication-free groups would show larger differences than those we report here. The fact that most of the BD patients were symptomatic at scan time is another potential confounder. Both patients groups presented similar distribution of mood state and correlation tests did not show any significant associations between metabolite levels and depressive and manic symptoms severity. We should caution however that only the study of BD patients in remission from any mood episode would exclude any possible confounding effect of mood state on neurometabolites. A fourth limitation is the absence of an alcoholic group without BD. This control group would help to characterize whether the findings are exclusive to alcoholism or to an interaction between the two diagnoses. Finally, we focused our study in the left DLPFC, and further research will be needed to investigate the anatomical extent of these abnormalities, particularly within the specific neurocircuitries involved in mood regulation and substance addiction.

This study also has considerable strengths. It is the first report of in vivo brain metabolite abnormalities in BD patients with comorbid alcoholism, a highly prevalent and clinically severe subgroup of BD. Our sample was clinically well-characterized and well-balanced regarding other clinical characteristics of BD that could confound the study. The only statistical differences were the presence of more patients among the alcoholic BD group that had prior psychotic symptoms or were in use of atypical antipsychotics. However, the differences on Glu+Gln and on glutamate levels after statistically adjusting for these two variables were still significant. We reported absolute levels of 1H metabolites rather than expressing the results as ratios of the metabolites relative to PCr+Cr. Absolute levels of metabolites have been considered to be more reliable and accurate than metabolite levels relative to PCr+Cr (Schirmer et al., 2000). Our findings also highlight the need for a careful assessment of possible confounding effects of prior alcoholism in 1H spectroscopy studies of BD patients. Traditionally, previous studies have carefully excluded patients with substance use disorder in the previous 6 months of the scan time (Winsberg et al., 2000; Sassi et al., 2005; Molina et al., 2007; Frey et al., 2007). However, our findings demonstrate that effects of alcoholism can be detected even after 6 months of abstinence.
In conclusion, we found that BD patients in remission from alcoholism present lower Glu+Gln levels and lower glutamate levels in their left DLPFC than BD patients who were never alcoholic. In addition, male alcoholic BD patients present lower Glu+Gln levels than male non-alcoholic BD patients, female alcoholic BD patients present lower myo-Ins levels than female non-alcoholic BD patients, and oldest alcoholic BD patients present lower GPC+PC levels than oldest non-alcoholic BD patients. The combination of alcohol and other drug use disorders is associated with lower myo-Ins and glutamate levels than alcoholism alone in BD patients. These findings suggest the existence of differences in the glutamatergic neurotransmission system, inositol second messenger system and cell membrane turnover abnormalities in the pathophysiology of BD comorbid with alcoholism compared to the pathophysiology of BD alone. These abnormalities may be the neurochemical correlate of an increased risk to develop alcoholism in BD, or of a persistently worse clinical and functional status in BD patients in remission from substance use disorders compared to BD patients who never developed substance use disorders (Weiss et al., 2005). Thus, results support the clinical recommendation that, in the management of BD, efforts should be made to prevent or precociously diagnose and treat abuse and dependence of alcohol in BD patients.

Acknowledgments

ROLE OF FUNDING SOURCE

This research was partly supported by grants MH 068662, MH 068766, RR 020571, UTHSCSA GCRC (M01-RR-01346), the Krus Endowed Chair in Psychiatry (UTHSCSA) and the Veterans Administration (VA Merit Review). Dr. Nery’s scholarship was supported in part by a private donation from the Thompson Motta family. The funding sources had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

This research was partly supported by grants MH 068662, MH 068766, RR 020571, UTHSCSA GCRC (M01-RR-01346), the Krus Endowed Chair in Psychiatry (UTHSCSA) and the Veterans Administration (VA Merit Review). Dr. Nery’s scholarship was supported in part by a private donation from the Thompson Motta family.

References


de Graaf AA, Bovee WM. Improved quantification of in vivo 1H NMR spectra by optimization of signal acquisition and processing and by incorporation of prior knowledge into the spectral fitting. Magnetic Resonance in Medicine 1990;15:305–319. [PubMed: 1975420]


Figure 1.
Axial, coronal and sagittal $^1$H MRS images showing the location of the 8 cm$^3$ voxel in the left dorsolateral prefrontal cortex.
Figure 2. An example of quantifying a $^1$H spectrum acquired from the dorsolateral prefrontal cortex of an alcoholic bipolar disorder patient

Figure 2 footnote: The modeled spectrum (thick line) is superimposed on the acquired spectrum and the residuals and the individual modeled curves of the main metabolites are shown below. The abbreviations include N-acetyl-aspartate (NAA), glutamate and glutamine (Glu+Gln), phosphocreatine plus creatine (PCr+Cr), choline-containing compounds (GPC+PC), and myo-inositol (myo-Ins).
Figure 3. Glu+Glu levels in the left dorsolateral prefrontal cortex of alcoholic BD patients (n=22, 6 male and 16 female), non-alcoholic BD patients (n=26, 5 male and 18 female) and HC (n=54, 14 male and 40 female), grouped by gender.

Figure 3 footnote: Difference between means, considering effects of gender: $F_{(1,95)}=7.3$, $p=0.008$; of comorbid alcoholism, $F_{(2,95)}=6.24$, $p=0.003$; for the interaction between gender and comorbid alcoholism, $F_{(2,95)}=4.7$, $p=0.01$. Bonferroni adjusted pairwise comparisons: alcoholic BD patients versus non-alcoholic BD patients: $p=0.002$; non-alcoholic BD patients versus HC: $p=0.01$. Abbreviations: BD (bipolar disorder), HC (healthy controls), Glu+Glu (glutamate plus glutamine).
Table 1: Demographic and clinical characteristics of the bipolar disorder groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Alcoholic BD patients (n=23)</th>
<th>Non-alcoholic BD patients (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±s.d.</td>
<td>39.1±11.4</td>
<td>39.8±12.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Gender: males (%)</td>
<td>6 (27.3)</td>
<td>5 (19.2)</td>
<td>0.51</td>
</tr>
<tr>
<td>Bipolar type I (%)</td>
<td>18 (81.8)</td>
<td>18 (69.2)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age at onset of BD, y, mean±s.d.</td>
<td>15.9±6.4</td>
<td>20.4±10.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Length of BD illness, m, mean±s.d.</td>
<td>277.6±153.7</td>
<td>226.0±152.0</td>
<td>0.25</td>
</tr>
<tr>
<td>HDRS score, mean±s.d.</td>
<td>14.3±8.9</td>
<td>12.3±7.3</td>
<td>0.40</td>
</tr>
<tr>
<td>YMRS score, mean±s.d.</td>
<td>4.5±5.3</td>
<td>8.9±9.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Current mood state, (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthymic</td>
<td>9 (40.9)</td>
<td>10 (38.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Major depressive episode</td>
<td>11 (50)</td>
<td>10 (38.5)</td>
<td></td>
</tr>
<tr>
<td>Manic/mixed or hypomanic</td>
<td>2 (9.1)</td>
<td>6 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Comorbid anxiety disorders, (%)</td>
<td>13 (59.1)</td>
<td>16 (61.5)</td>
<td>0.86</td>
</tr>
<tr>
<td>Prior occurrence of psychotic symptoms, (%)</td>
<td>8 (38.1)</td>
<td>3 (11.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Family history of mood disorders in first degree relatives</td>
<td>15 (68.2)</td>
<td>15 (57.7)</td>
<td>0.55</td>
</tr>
<tr>
<td>Comorbid substance use disorder (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marijuana, (%)</td>
<td>11 (50)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cocaine, (%)</td>
<td>5 (22.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stimulants, (%)</td>
<td>6 (27.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Opioids, (%)</td>
<td>2 (9.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Number of patients in use of, (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium carbonate, (%)</td>
<td>4 (18.2)</td>
<td>6 (23.1)</td>
<td>0.73</td>
</tr>
<tr>
<td>Anticonvulsants, (%)</td>
<td>12 (54.5)</td>
<td>13 (50)</td>
<td>0.75</td>
</tr>
<tr>
<td>SSRIs, (%)</td>
<td>9 (40.9)</td>
<td>8 (30.8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Atypical antipsychotics, (%)</td>
<td>12 (54.5)</td>
<td>7 (26.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Stimulants, (%)</td>
<td>1 (4.5)</td>
<td>1 (3.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Other antidepressants, (%)</td>
<td>4 (18.2)</td>
<td>9 (34.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Benzodiazepines, (%)</td>
<td>11 (50)</td>
<td>10 (38.5)</td>
<td>0.42</td>
</tr>
<tr>
<td>Age at onset of alcoholism, y, mean±s.d. (range)</td>
<td>24.4±9.2, (13–48)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Duration of heavy use of alcohol, y, mean±s.d., (range)</td>
<td>5.7±3.6, (1–13.5)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Time in abstinence from alcohol, y, mean±s.d., (range)</td>
<td>6.8±6.5, (0.5–22)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BD (bipolar disorder), HDRS (Hamilton Depression Rating Scale), YMRS (Young Mania Rating Scale).
Table 2

Mean s.d. tissue fractions within the voxel of interest (left dorsolateral prefrontal cortex) for each group of bipolar disorder patients with and without past alcoholism and healthy comparison subjects

<table>
<thead>
<tr>
<th>Tissue fraction (%)</th>
<th>Alcoholic BD patients (n=22)</th>
<th>Non-alcoholic BD patients (n=26)</th>
<th>HC (n=54)</th>
<th>F(df)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td>0.33</td>
<td>0.33</td>
<td>0.07</td>
<td>0.33</td>
<td>0.05(2,99)</td>
</tr>
<tr>
<td>White matter</td>
<td>0.67</td>
<td>0.67</td>
<td>0.08</td>
<td>0.67</td>
<td>0.05(2,99)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>2.6(2,99)</td>
</tr>
</tbody>
</table>
Comparison of brain metabolite mean±s.d. levels in left dorsolateral prefrontal cortex of bipolar disorder patients with and without past alcoholism and healthy comparison subjects

<table>
<thead>
<tr>
<th>Brain metabolites (mmol/kg)</th>
<th>Alcoholic BD patients (n=22)</th>
<th>Non-alcoholic BD patients (n=26)</th>
<th>HC (n=54)</th>
<th>F_{1.40}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl aspartate (NAA)</td>
<td>7.11±0.60</td>
<td>7.21±0.49</td>
<td>7.38±0.62</td>
<td>2.0_{2.97}</td>
<td>0.14</td>
</tr>
<tr>
<td>Phosphocreatine plus creatine (PCr+Cr)</td>
<td>5.50±0.55</td>
<td>5.07±0.56</td>
<td>5.44±0.49</td>
<td>1.5_{2.97}</td>
<td>0.22</td>
</tr>
<tr>
<td>Choline containing-compounds (GPC+PC)</td>
<td>1.61±0.22</td>
<td>1.71±0.29</td>
<td>1.60±0.20</td>
<td>6.4_{3.95}</td>
<td>0.001</td>
</tr>
<tr>
<td>Myo-inositol (myo-Ins)</td>
<td>4.68±0.65</td>
<td>5.02±1.06</td>
<td>5.08±0.77</td>
<td>5.53_{2.95}</td>
<td>0.18</td>
</tr>
<tr>
<td>Glutamate plus glutamine (Glu+Gln)</td>
<td>7.84±1.45</td>
<td>8.55±1.89</td>
<td>7.73±1.68</td>
<td>6.24_{2.95}</td>
<td>0.003</td>
</tr>
<tr>
<td>Glutamate (Glu)</td>
<td>5.18±0.96</td>
<td>5.86±1.26</td>
<td>5.55±1.07</td>
<td>3.2_{2.97}</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Abbreviations: BD: bipolar disorder; HC: healthy comparison subjects

ANCOVA results: for NAA, PCr+Cr, and glutamate: age and gender as covariates; for GPC+PC: age as factor, gender as covariate; for Glu+Gln and myo-Ins: gender as factor, age as covariate.