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ARTICLE

Altered neurochemistry in the anterior white matter of bipolar children and adolescents: a multivoxel ^{1}H MRS study

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Abstract

Abnormalities within frontal lobe gray and white matter of bipolar disorder (BD) patients have been consistently reported in adult and pediatric studies, yet little is known about the neurochemistry of the anterior white matter (AWM) in pediatric BD and how medication status may affect it. The present cross-sectional 3T ^{1}H MRS study is the first to use a multivoxel approach to study the AWM of BD youth. Absolute metabolite levels from four bilateral AWM voxels were collected from 49 subjects between the ages of 8 and 18 (25 healthy controls (HC); 24 BD) and quantified. Our study found BD subjects to have lower levels of N-acetylaspartate (NAA) and glycerophosphocholine plus phosphocholine (GPC + PC), metabolites that are markers of neuronal viability and phospholipid metabolism and have also been implicated in adult BD. Further analysis indicated that the observed patterns were mostly driven by BD subjects who were medicated at the time of scanning and had an ADHD diagnosis. Although limited by possible confounding effects of mood state, medication, and other mood comorbidities, these findings serve as evidence of altered neurochemistry in BD youth that is sensitive to medication status and ADHD comorbidity.

Introduction

Bipolar disorder (BD) is a debilitating psychiatric illness that affects 1–2% of youth and continues into adulthood [1]. Along with other mood disorders, BD is regarded as a neurodevelopmental illness but remains, however, not fully understood [2]. Structural neuroimaging studies have sought to define the neuroanatomical profile of the disorder. Magnetic resonance imaging (MRI) studies have consistently reported smaller prefrontal cortex volumes in BD children and adults compared to healthy controls (HCs) [3–5]. The anterior white matter (AWM) has also been implicated by diffusion tensor imaging studies showing lower fractional anisotropy values, which reflects reduced anisotropic behavior of water diffusion in the WM microstructure [6, 7]. This, in turn, suggests that the signal conductance of neural information of these WM tracts to the frontal cortex, which is necessary for processes such as cognitive control and emotional regulation [8, 9], may be compromised. While these neuroimaging studies help to better understand the extent of structural alterations, little is known as to whether the neurochemistry in the AWM of BD patients is also impacted. This information may serve to further characterize neuropathology, predict illness severity, and guide treatment.

Proton magnetic resonance spectroscopy (^{1}H MRS) is a well-established technique that can access the neurochemistry in vivo by quantifying levels of key metabolites [10, 11]. These include: N-acetylaspartate (NAA), a marker of functioning neuroaxonal tissue that includes aspects of the formation and/or maintenance of myelin [12, 13]; glutamate (Glu), the main excitatory neurotransmitter [14];

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myo-inositol (MI), a metabolite that is exclusively localized in astrocytes and therefore, a marker of gliosis [15, 16]; phosphocreatine plus creatine (PCr + Cr), are high-energy phosphate metabolites that reflect total energy reserves [10, 17]; and glycerophosphocholine plus phosphocholine (GPC + PC), metabolites that are part of the catabolic and anabolic pathways of membrane phospholipids, respectively [18].

Compared to HCs, BD adults have been reported to have different metabolite levels in the frontal lobe, with some studies noting lower NAA and higher GPC + PC levels in BD [19–22]. Although ^{1}H MRS studies have reported similar variations in metabolite levels in pediatric BD patients [23–25], the literature is relatively scant with no studies examining the AWM. There are, however, two ^{1}H MRS studies assessing AWM differences in adult BDs, with one reporting decreases only in the NAA/PCr + Cr ratio and the other reporting decreases in NAA, GPC + PC, MI, and PCr + Cr in AWM voxels of older adults with BD along with increases in Glu plus glutamine and decreases in NAA levels in younger BD adults [26, 27]. Therefore, it remains unclear as to whether AWM areas in pediatric BD patients also exhibit differences in neurochemistry, to what extent, and how these putative changes relate to disorder severity and symptomatology. Furthermore, BD youth are often prescribed psychoactive medications [28], emphasizing the importance of also assessing effects related to medication status in which the reporting is mixed [29–32]. In addition to the complexities raised by medication status, pediatric BD patients have high rates of mood disorder comorbidities [33], thus the accounting of the effects of comorbidity is integral for a better understanding of the neurochemistry of BD.

In the present multivoxel ^{1}H MRS study, the aims were to: (1) investigate differences in the neurochemistry of AWM areas of BD youth and HCs, (2) examine the relationship between neurochemistry and clinical severity scores, and (3) investigate possible effects of medication and comorbidity. Guided by the limited number of ^{1}H MRS studies of the frontal cortex in pediatric BD and of the AWM in BD adults, we hypothesized BD youth would have lower NAA levels than HCs. We predicted lower levels of NAA to correspond with increased BD severity measures and medicated youth with BDs to have higher NAA levels than unmedicated youth with BD.

Materials and methods

Subjects

Our cross-sectional study included 49 subjects between the ages of 8 and 18 (25 HC, 24 BD). Subjects were recruited using flyers, radio, and newspaper advertisements from the local community and psychiatric clinics. BD diagnoses and clinical characteristics were assessed with the K-SADS-PL [34] administered by fully trained research assistants or postdoctoral fellows who were supervised by an experienced research psychiatrist. HCs had no history of Axis-I disorders, no first-degree relatives with an Axis-I disorder, and had not used any psychoactive medication within 2 weeks of the study. Exclusion criteria for all subjects included past head trauma that resulted in 15+ min of unconsciousness and/or a prior diagnosis of a severe traumatic brain injury, neurological disorders, and/or any major medical conditions. Subjects were evaluated through a socio-demographic history form for age, gender, and years of education. The Children's Depression Rating Scale (CDRS) [35] and the Young Mania Rating Scale (YMRS) [36] were also used to assess symptom severity. All subjects and their parent or legal guardian gave written informed consent. This study was approved by the University of North Carolina at Chapel Hill IRB.

Imaging

A multivoxel approach was used to acquire the ^{1}H MRS data on a 3T Siemens system and postprocessing followed to extract and quantify the ^{1}H MRS spectra from four bilateral voxels in the AWM (Fig. 1). Regarding the protocol, scout MRI images were first acquired followed by a set of T_1-weighted MRI images using the magnetization prepared rapid acquisition gradient-echo sequence (TR = 1750 ms, TE = 4.38 ms, flip angle = 8°, field of view (FOV) = 256 × 256 mm^2, number of axial slices = 160, slice thickness = 1 mm, number of excitations = 1, matrix size = 256 × 208). The ^{1}H MRS measurement was acquired using a three-dimensional (3D) multivoxel acquisition scheme, which combined the point resolved spectroscopy (PRESS) sequence with chemical shift imaging (CSI). The PRESS volume of interest (VOI) was located in the frontal lobe (70 × 50 × 40 mm^3), which included the four voxels of interest in the AWM. The acquisition parameters included the following: TR = 1410 ms, TE = 80 ms [37], FOV = 160 × 160 × 80 mm^3, acquired CSI matrix = 14 × 14 × 8 and zero-filled to 16 × 16 × 8, nominal pixel dimension = 10 × 10 × 10 mm^3, complex spectral data points = 2048, spectral bandwidth = 2.0 kHz, water suppression using CHESS pulses [38], and number of averages = 1. Additionally, an unsuppressed water measurements for absolute quantification was acquired using the identical parameters except the acquired CSI matrix was 8 × 8 × 8 and zero-filled to 16 × 16 × 8.

Postprocessing included extracting and quantifying the ^{1}H MRS signal from four different left and right AWM voxel locations and estimating the tissue fraction within

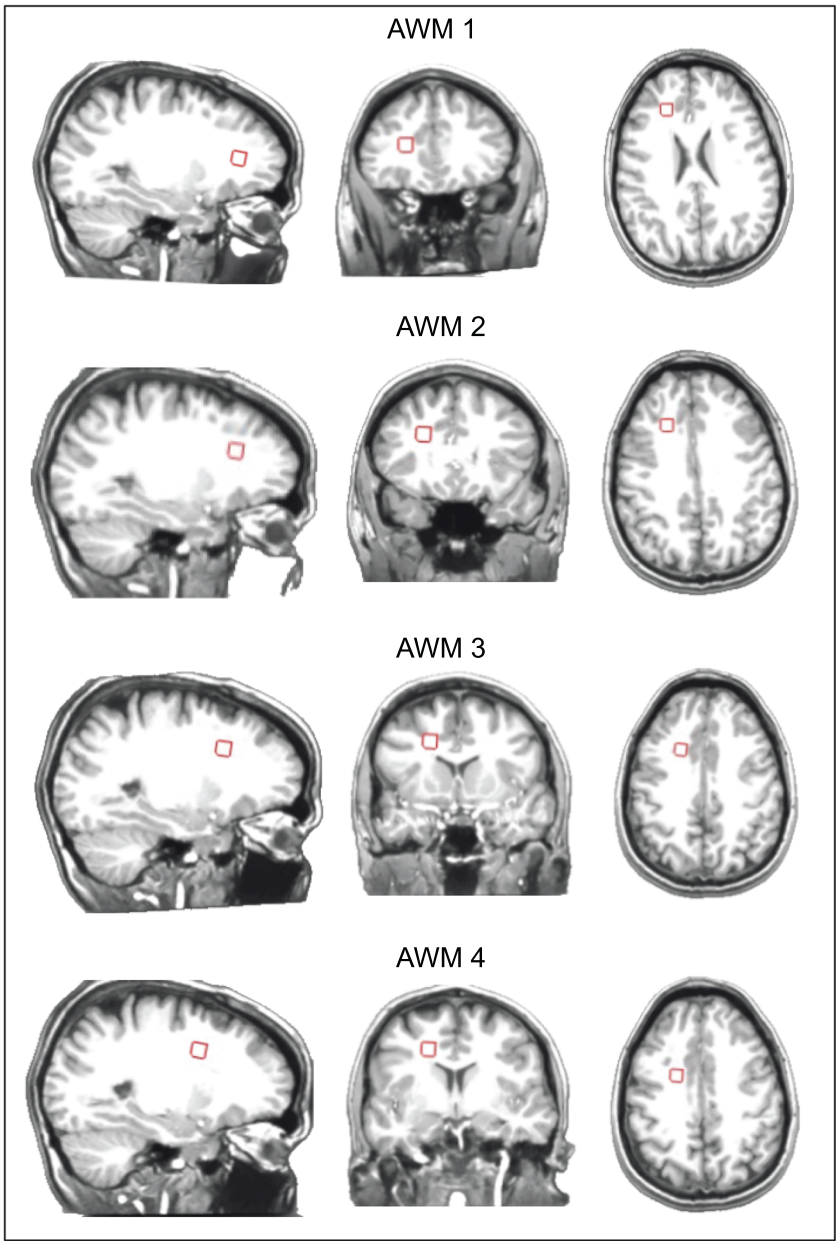

Fig. 1 Voxel locations. Metabolite levels were quantified from four voxels in the anterior white matter. Figure shows the sagittal, coronal, and axial views of each voxel. AWM anterior white matter.

those extracted ^{1}H MRS voxels [i.e., the percent gray and white matter and cerebrospinal fluid (CSF)]. The procedure was completely automated and independent of operator inputs, using Linux shell scripts, and FreeSurfer and FSL tools (e.g., FLIRT, NU_CORRECT, BET, and FAST) [39, 40]. The four AWM voxels of interest were predefined anatomically on an MNI template brain image, and the voxels were mapped to the subject space using the co-registration transformation between the T_1-weighted images and the template image. The coordinates of the voxel location in subject space were then used to shift the 3D multivoxel grid to ensure a voxel was centered at that location and then the ^{1}H MRS signal of that voxel was extracted for quantification. This innovative procedure consistently and systematically placed the voxels in the specified anatomical locations between subjects, and has been demonstrated to be accurate and reliable [41]. These voxel locations were also mapped on the tissue-segmented images, in which the tissue fraction within each voxel was estimated.

GPC + PC, NAA, PCr + Cr, MI, and Glu levels were quantified using the Linear Combination Model software with a simulated basis set for the a priori knowledge [42]. The following inclusion criteria were applied to the fitted spectrum of each voxel: the signal-to-noise ratio equal to or greater than 5, CRLB values equal to of <20%, absolute data shift ⩽0.1 p.p.m., and absolute first-order phase correction equal to or less than 30°/p.p.m.

The unsuppressed water signal along with the appropriate correction factors related to the proportions of gray matter, white matter and CSF within the voxel were applied to obtain absolute quantification values with institutional units (IU) [43].

Statistics

The General Estimated Equations (GEE) approach with repeated measures was used to investigate group effects. Specifically, GEEs with repeated measurements were chosen due to their suitability for analyzing data with multiple measurements per subject of which measurements may be correlated and without punity on missing data points. All statistical analyses were performed using RStudio Version 1.2.1335 based on R version 3.6.0.

GEEs were generated using the geeglm function in the geepack library. For each metabolite, a GEE model was created to test the main overall effect of subject group independent of voxel locations while controlling for age, gender, and hemisphere. To determine whether certain voxels differ differently between groups, a second GEE was conducted that accounted for group X voxel location interactions while also controlling for age, gender, and hemisphere. FDR corrections were used to adjust for the testing of multiple metabolites.

In order to test the effect of medication at time of scanning, we compared metabolite levels of unmedicated BDs, medicated BDs, and HCs. For each metabolite, we created two GEE models that covaried for age, gender, and hemisphere: one model contained the grouping variable while the other was a "null" model with no grouping data. Then, an ANOVA tested whether the models were significantly different from one another, thus indicating whether there was a significant effect of medication group. FDR corrections were used to adjust for multiple comparisons. We also conducted GEE analyses that tested for medication status X voxel location interactions. Chi-square tests were used to evaluate whether the medicated and unmedicated groups had differing comorbidities.

For all significant metabolites, the average metabolite level across significant voxel locations and both hemispheres was calculated. Then, using the lm function to generate linear models, we examined the relationship between average metabolite levels in BD subjects and YMRS scores and CDRS scores. Significance levels for all analyses were set at 0.05.

Results

Demographic and clinical characteristics of our sample are detailed in Table 1. There were no significant differences between BDs and HCs in age, gender, handedness, and years of education. Regarding the quality of the ^{1}H MRS, mean SNR for the BD group was 15.3 ± 4.8 and 14.0 ± 4.4 for HCs. Mean FWHM was 6.2 ± 2.2 Hz (3.5–17.6 Hz) for BDs and 6.1 ± 2.1 Hz (3.5–14.7 Hz) for HCs. Mean, SD, and range of CRLB values for GPC + PC, PCr + Cr, NAA, MI, and Glu across all AWM voxels were 3.6 ± 1.4% (2–9%) and 3.7 ± 1.6% (2–12%), 3.4 ± 1.6% (2–11%) and 3.7 ± 1.6% (2–13%), 3.9 ± 1.5% (2–10%) and 4.2 ± 2.1% (1–16%), 9.1 ± 3.4% (5–19%) and 10.5 ± 3.4% (5–19%), and 9.8 ± 3.0% (5–19%) and 10.7 ± 3.1% (6–19%) for BDs and HCs, respectively.

Table 1 Demographic information for study participants.

	HC (25)	BD (24)	t	χ^2	p
Age (years)	12.7 ± 3.1	12.6 ± 3.0	0.15		0.88
Gender				0	1.00
Male	60.0% (15)	62.5% (15)			
Female	40.0% (10)	37.5% (9)			
Handedness				2.37	0.50
Left	4.0% (1)	12.5% (3)			
Right	92.0% (23)	79.2% (19)			
Mixed	4.0% (1)	4.2% (1)			
Unknown	0% (0)	4.2% (1)			
Education (years)	6.48 ± 3.06	5.96 ± 3.32	0.57		0.58
YMRS	0.50 ± 0.93	6.57 ± 5.16	−5.55		<0.001
CDRS	17.50 ± 1.16	29.24 ± 13.36	−3.61		0.002

HC health controls, *BD* bipolar disorder, *YMRS* Young Mania Rating Scale, *CDRS* Children's Depression Rating Scale.

Compared to HC youth, BDs had lower levels of NAA (Wald $\chi^2 = 3.78$, $p = 0.05$, $p_{corrected} = 0.13$) and GPC + PC (Wald $\chi^2 = 6.50$, $p = 0.01$, $p_{corrected} = 0.05$) (Fig. 2). NAA differences did not survive FDR correction. GEE models that included a group X voxel location term did not find any significant interactions between the two variables for any of the metabolites. As we were unable to ascertain which voxels were driving the statistical significance using the group X voxel location model, Table 2 lists the mean metabolite differences between BDs and HCs along with a 95% confidence for each voxel. Supplementary Table 1 includes mean, SD, and SE values of metabolites for HCs and BDs. Linear models examining the relationship between average metabolite levels and YMRS and CDRS scores in BD subjects were not significant.

Half of the subjects in our BD (12 out of 24) group were medicated at the time of scanning. Medicated and unmedicated BD subjects did not differ in age, gender, YMRS, and CDRS scores. ANOVAs evaluating the test and null GEE models for each metabolite indicated significant group differences in NAA (Wald $\chi^2 = 14.0$,

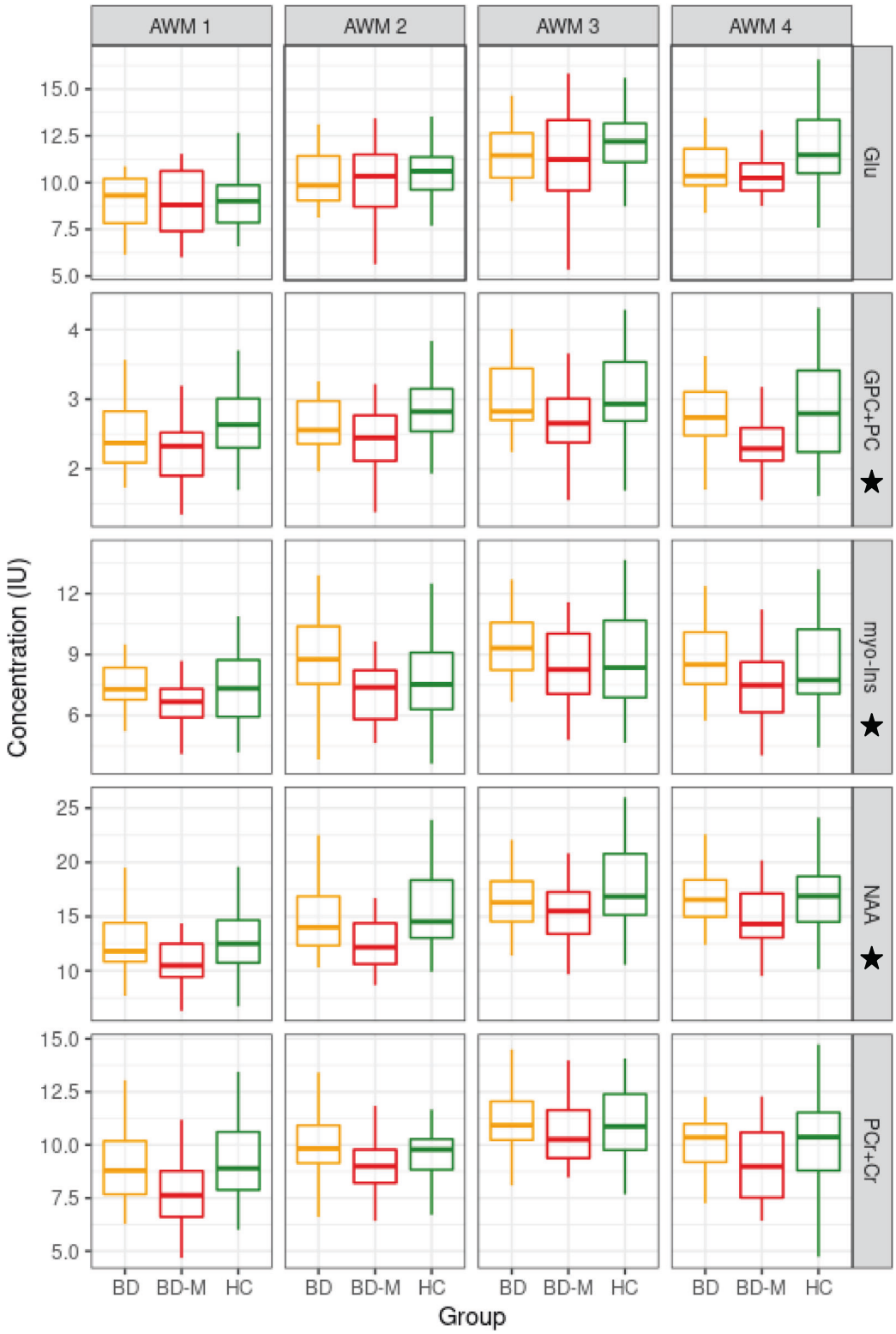

Fig. 2 Metabolite levels by subject group. Metabolites marked with ★ were significantly different across all voxels and survived FDR correction. HC healthy controls; BD unmedicated bipolar disorder; BD-M medicated bipolar disorder; Glu glutamate; GPC + PC glycerophosphocholine plus phosphocholine; myo-Ins myo-inositol; NAA N-acetylaspartate; NAAG N-acetyl-aspartyl-glutamate; PCr + Cr phosphocreatine plus creatine.

Table 2 GEE analysis results.

Metabolite (IU)	HC vs BD	3 Group GEE	UnMed BD vs Med BD	HC vs Med BD	HC vs UnMed BD
NAA	$p = 0.05$	$p = 9.0 \times 10^{-4}$	$p = 9.14 \times 10^{-3}$	$p = 3.8 \times 10^{-4}$	$p = 0.84$
AWM 1	1.49 [0.20, 2.79]		2.14 [0.60, 3.68]	2.54 [1.13, 3.95]	0.40 [−1.16, 1.96]
AWM 2	1.52 [0.07, 2.97]		2.43 [0.57, 4.29]	2.76 [1.26, 4.26]	0.33 [−1.55, 2.21]
AWM 3	1.49 [−0.12, 3.10]		1.33 [−0.64, 3.30]	2.14 [0.30, 3.98]	0.81 [−1.14, 2.75]
AWM 4	0.80[−0.93, 2.53]		2.50 [−0.44, 4.56]	2.11 [0.24, 3.98]	−0.39 [−2.48, 1.70]
GPC + PC	$p = 0.01$	$p = 7.5 \times 10^{-3}$	$p = 0.03$	$p = 1.8 \times 10^{-3}$	$p = 0.29$
AWM 1	0.32 [0.12, 0.52]		0.18[−0.09, 0.45]	0.41 [0.17, 0.65]	0.23 [−0.02, 0.48]
AWM 2	0.30 [0.08, 0.52]		0.25 [−0.03, 0.54]	0.43 [0.16, 0.70]	0.18 [−0.08, 0.43]
AWM 3	0.22 [−0.04, 0.48]		0.38 [0.06, 0.71]	0.42 [0.10, 0.73]	0.03 [−0.27, 0.33]
AWM 4	0.20 [0.01, 0.57]		0.41 [0.08, 0.75]	0.50 [0.16, 0.85]	0.09 [−0.21, 0.39]
MI	$p = 0.89$	$p = 1.6 \times 10^{-3}$	$p = 4.4 \times 10^{-4}$	$p = 0.02$	$p = 0.10$
AWM 1	0.13 [−0.72, 0.97]		1.06 [−0.06, 2.17]	0.70 [−0.21, 1.61]	−0.36 [−1.45, 0.73]
AWM 2	−0.35 [−1.23, 0.53]		1.80 [0.62, 2.98]	0.55 [−0.30, 1.41]	−1.25 [−2.44, −0.06]
AWM 3	−0.19 [−1.19, 0.81]		1.42 [0.06, 2.77]	0.56 [−0.70, 1.81]	−0.86 [−2.01, 0.29]
AWM 4	0.22 [−0.97, 1.41]		1.36 [−0.05, 2.77]	0.96 [−0.50, 2.43]	−0.40 [−1.68, 0.88]
Glu	$p = 0.13$	$p = 0.17$	–	–	–
AWM 1	0.01 [−0.97, 0.98]		0.78 [−0.79, 2.35]	0.41 [−0.70, 1.52]	−0.37 [−1.79, 1.05]
AWM 2	0.34 [−0.38, 1.06]		0.31 [−0.81, 1.42]	0.50 [−0.47, 1.46]	0.19 [−0.70, 1.08]
AWM 3	0.61 [−0.26, 1.48]		0.40 [−1.00, 1.81]	0.82 [−0.47, 2.11]	0.42 [−0.56, 1.39]
AWM 4	1.26 [−0.33, −2.20]		0.63 [−0.51, 1.78]	1.56 [0.47, 2.65]	0.93 [−0.18, 2.04]
PCr + Cr	$p = 0.08$	$p = 0.05$	$p = 0.04$	$p = 0.02$	$p = 0.86$
AWM 1	0.93 [0.20, 1.65]		1.25 [0.28, 2.21]	1.54 [0.67, 2.40]	0.29 [−0.57, 1.15]
AWM 2	0.36 [−0.34, 1.05]		1.03 [0.01, 2.05]	0.88 [0.01, 1.76]	−0.15 [−1.00, 0.70]
AWM 3	0.26 [−0.50, 1.02]		0.79 [−0.36, 1.94]	0.67 [−0.38, 1.72]	−0.12 [−0.99, 0.76]
AWM 4	0.62 [−0.31, 1.54]		1.14 [0.10, 2.19]	1.23 [0.13, 2.33]	0.08 [−0.92, 1.09]

p values reflect group differences across all voxels. Mean metabolite differences and 95% confidence intervals are included for each voxel.

HC healthy controls, *BD* bipolar disorder, *UnMed BD* unmedicated bipolar disorder, *Med BD* medicated bipolar disorder, *NAA* N-acetylaspartate, *GPC + PC* glycerophosphocholine plus phosphocholine, *MI* myo-inositol, *Glu* glutamate, *PCr + Cr* phosphocreatine plus creatine.

$p = 9.0 \times 10^{-4}$, $p_{\text{corrected}} = 3.8 \times 10^{-3}$), GPC + PC (Wald $\chi^2 = 978$, $p = 7.5 \times 10^{-3}$, $p_{\text{corrected}} = 0.01$), PCr + Cr (Wald $\chi^2 = 6.07$, $p = 0.05$, $p_{\text{corrected}} = 0.06$), and MI (Wald $\chi^2 = 12.9$, $p = 1.6 \times 10^{-3}$, $p_{\text{corrected}} = 3.8 \times 10^{-3}$) levels. Evaluation of the test GEE indicated that medicated BDs had lower levels of NAA, GPC + PC, PCr + Cr, and MI than HCs and unmedicated BDs (Table 2). HCs and unmedicated BDs were not significantly different for any of the metabolites. GEE models that included a medication group X voxel location term did not find any significant interactions between the two variables for any of the metabolites. Mean differences and 95% confidence intervals between HCs and medicated BDs, HCs and unmedicated BDs, and medicated and unmedicated BDs for AWM 1–4 are shown in Table 2.

Comorbidity data are presented in Supplementary Table 2. All our medicated BD subjects had ADHD comorbidities while 4 out of the 12 unmedicated BD subjects had an ADHD diagnosis ($\chi^2 = 8.18$, $p = 4.24 \times 10^{-3}$). No other comorbidity differed between medicated and unmedicated BD groups.

Discussion

Our study found lower levels of NAA and GPC + PC within the AWM of BD youth. Further analysis indicated that the observed patterns were mostly driven by BD subjects who were medicated at the time of scanning.

NAA is a metabolite synthesized in neural mitochondria and has been implicated in a vast array of processes, including myelin and lipid formation, neuronal protection from osmotic stress, and mitochondrial energy production [44–46]. In a postmortem study examining hippocampal tissues in BD brains, mitochondrial proteins necessary for energy production through oxidative phosphorylation and proteasomal degradation processes were underexpressed [47]. MRS studies investigating frontal white matter regions in adults have also detected lower NAA levels [26, 27]. Hence, the observed decreases in NAA may signal early disruption in mitochondrial processing in the AWM of BD children remains present in adulthood within the frontal regions, basal ganglia, and hippocampus [21, 22, 48, 49].

Along with decreased NAA levels, we also detected lower GPC + PC levels in our BD subjects. The GPC + PC signal is a measure of free choline compounds, which are the primary building blocks of the neuronal cell membranes and are integral to the generation of the neurotransmitter acetylcholine [50, 51]. However, due to the identical spectral profile of GPC and PC in ^{1}H MRS, these two compounds are classified under one choline peak. As such, it is not possible to pinpoint whether the observed decrease in GPC + PC is due to decreases in membrane turnover, which would be prompted by decreases in GPC, or decreases in de novo phosphocholine formation, which is necessary for the building of cellular membranes [52]. Furthermore, the literature regarding the role of GPC + PC in BD is mixed with certain adult studies reporting adult BD subjects exhibiting increased levels of GPC + PC than HCs in the anterior cingulate cortex and basal ganglia [41, 53, 54], while some adult and pediatric studies focusing on the prefrontal cortex have reported lower GPC + PC levels in BD subjects [49, 55, 56]. Nevertheless, there appears to be a dysregulation in the pathways responsible for cellular membrane integrity in BD. Markedly, a study investigating the effects of administering choline bitartrate to lithium treated BD adults on their metabolite levels and behavioral profiles found a decrease in the severity of manic and mood disorder symptoms accompanied the treated subjects' elevated choline levels, suggesting a therapeutic effect of choline and a possible target for future treatments [57]. Of the two ^{1}H MRS studies on the AWM in BD subjects, one reported decreases in GPC + PC in older BD adults while the other found no significant differences in GPC + PC [26, 27, 49]. Therefore, with such a scant body of the literature, future MRS studies should investigate the AWM of BD subjects to further characterize the metabolic profile of BD and to determine whether these signatures are related with white matter structural integrity.

We did not find any differences in Glu. As our PRESS sequence is optimized for Glu detection with TE = 80 ms at 3T, we do not believe this null finding to be due to a lack of signal sensitivity, and a power analysis based on effect sizes from prior publications indicated that our study had a power of 91% [58]. Although abnormal Glu activity is a hallmark of BD [59], these differences are largely reported in GM areas [60]. Studies reporting on the neurochemistry in the AWM of BD patients, however, are scant with only one study reporting significant alterations of Glu + Glutamine in AWM areas in BD young adults [27]. A meta-analysis examining the role of Glu in BD reported a lack of significant differences in both Glu and Glu + Glutamine levels in pediatric BD vs HCs in brain areas that included the anterior cingulate cortex, and gray matter in frontal cortices [60]. Therefore, more studies are needed in order to better characterize the role of Glu in pediatric BD neuropathology.

Due to the cross-sectional nature and the limited sample size of our study, we are unable to attribute the observed differences in medicated BD subjects to medication effects or other extraneous factors, including comorbidities, mood states, and illness duration. Thus, interpretations of our results and conclusions derived from them must bear in mind these study limitations. We did determine, however, that the medicated and unmedicated BD subjects did not differ in age, gender, mood status, handedness, YMRS, and CDRS score distributions. In addition, we have included the results of a GEE analysis performed on the metabolite levels of only right-handed and euthymic subjects in Supplementary Table 3. These findings were largely in accordance with the results from the full sample, thus suggesting that handedness and mood state are not main drivers of the patterns observed. We also examined whether the proportions of BD I, II, and NOS differed between medicated and unmedicated groups and found that while this trended toward significance, both groups had BD NOS subjects as the majority. We were unable to find studies that assessed metabolite level differences between all three BD subtypes, and studies that included just BD I and II subjects have reported conflicting findings [61, 62]. As the BD literature tends to focus more on BD I and BD II subtypes, the large proportion of BD NOS subjects in our sample may limit the generalizability of our results. Nevertheless, studies have shown BD NOS youth to have similar symptomologies, comorbidities, medications, functional impairments, and demographic profiles as the other BD subtypes [63–65]. In addition, 45% of BD NOS subjects have been shown to convert to either BD I or BD II within 5 years, which further highlights the overlap between the BD subtypes [1, 66]. All our medicated BD subjects had ADHD comorbidities while only 4 out of the 12 unmedicated BD subjects had an ADHD diagnosis. One must also note that of the 12 medicated subjects, 9 were taking stimulants. In solely ADHD populations, stimulants tended to increase NAA and decrease GPC + PC levels [67–69]. Given that ADHD has also been associated with decreased NAA, GPC + PC, PCr + Cr, and MI metabolite levels [70], it is possible that the counterintuitive decrease in NAA in medicated subjects may be due to concurrent ADHD and BD interacting to alter the metabolite profile of patients as well as the neurochemical effects of stimulants. In turn, while MRS studies have investigated frontal and prefrontal cortical regions, this is the first ^{1}H MRS study to target the AWM in pediatric BD. Hence, more studies investigating the AWM in BD populations are needed to better conceptualize our findings. As our sample spanned a crucial period of frontal lobe neurodevelopment [71], we assured there were no age differences between groups and that age was a covariate in all analyses. Examining the covariate terms of the GEEs, however, indicated that both NAA and PCr + Cr levels had

a significant relationship with age. When age was included as an interaction term, there were no significant group X age interactions and thus the group differences observed were not due to age effects (Supplementary Fig. 1). Future longitudinal studies would be better equipped to answer neurometabolic development and should seek to characterize the effects of psychoactive medications on the neurometabolites of BD youth and how this affects behavior. Larger studies should also further characterize the relationship between ADHD and BD and the phenotypes associated with comorbidity.

This is the first multivoxel ^{1}H MRS study to examine the neurochemistry of the AWM in BD youth, an area responsible for the neuronal communication that is necessary for the facilitation of complex cognitive processes. The neurochemical changes observed in the AWM of our BD group, specifically the decreases in NAA and GPC + PC, were consistent with the literature detailing similar patterns in BD adults [53]. GPC + PC and NAA are metabolites involved in phospholipid metabolism and neuronal viability [72]. Thus, finding decreases in both metabolites during childhood and adolescence serves as preliminary evidence of an altered neurodevelopmental trajectory in BD youth that appears to extend into adulthood. Future studies may seek to characterize BD metabolites across the lifespan using longitudinal designs, assess how deviations in metabolite levels affect white and gray matter structural development, and investigate how medication may alter the biochemical profile of BD.

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Compliance with ethical standards

Conflict of interest JCS has received grants/research support from BMS, Forrest, J&J, Merck, Stanley Medical Research Institute, NIH and has been a speaker for Pfizer and Abbott. No other authors have conflicts to declare.

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