Increased Anterior Cingulate/Medial Prefrontal Cortical Glutamate and Creatine in Bipolar Depression

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Proton magnetic resonance spectroscopy (^{1}HMRS) is an *in vivo* brain imaging method that can be used to investigate psychotropic drug mechanism of action. This study evaluated baseline ^{1}HMRS spectra of bipolar depressed patients and whether the level of cerebral metabolites changed after an open trial of lamotrigine, an anti-glutamatergic mood stabilizer. Twenty-three bipolar depressed and 12 control subjects underwent a MRS scan of the anterior cingulate/medial prefrontal cortex. The scan was performed on a GE whole-body 1.5 T MRI scanner using single-voxel PRESS (TE/TR = 30/3000 ms, $3 \times 3 \times 3$ cm^3 and post-processed offline with LCModel. Baseline CSF-corrected absolute concentrations of glutamate + glutamine ([Glx]), glutamate ([Glu]), and creatine + phosphocreatine ([Cr]) were significantly higher in bipolar depressed subjects *vs* healthy controls. The non-melancholic subtype had significantly higher baseline [Glx] and [Glu] levels than the melancholic subtype. Remission with lamotrigine was associated with significantly lower post-treatment glutamine ([Gln]) in comparison to non-remission. These data suggest that non-melancholic bipolar depression is characterized by increased glutamate coupled with increased energy expenditure. Lamotrigine appears to reduce glutamine levels associated with treatment remission. Further study is encouraged to determine if these MR spectroscopic markers can delineate drug mechanism of action and subsequent treatment response.
Neuropsychopharmacology (2007) **32,** 2490–2499; doi:10.1038/sj.npp.1301387; published online 11 April 2007

Keywords: bipolar; depression; glutamate; lamotrigine; MR spectroscopy

INTRODUCTION

As originally proposed by Papez (1937), the cingulate cortex is a brain region linked to the cognitive and emotional aspects of affect regulation (Vogt, 2003, 2005; Devinsky *et al*, 1995; Allman *et al*, 2001) and has been implicated in the pathophysiology of bipolar disorder.

Key findings in bipolar disorder have specifically included the subgenual anterior cingulate cortex (sACC, Vogt, 2003, 2005). This is the subregion of the cingulated gyrus that lies ventral to the genu of the corpus callosum. In comparison to controls, below-normal glial cell number (Öngur *et al*, 1998), (left-sided) volume (Drevets *et al*, 1997; Hirayasu *et al*, 1999; Sassi *et al*, 2004), and regional cerebral blood flow (Drevets *et al*, 1997) have been reported in the sACC in bipolar disorder. There is additional evidence for bipolar medial prefrontal abnormalities outside the sACC including the pregenual anterior cingulate cortex (pACC,

subregion of the cingulate just anterior to the genu), anterior middle cingulate cortex (aMCC, subregion of the cingulate dorsal to the genu), and medial aspects of the superior frontal cortex. Post-mortem pathology in bipolar disorder in these regions have included reduced neuronal (Benes *et al*, 2001; Bouras *et al*, 2001) and synaptic marker density (Eastwood and Harrison, 2001).

Additional neuroimaging findings in bipolar disorder in these regions included reduced volume of superior frontal cortex (Lopez-Larson *et al*, 2002), (left-sided) reduced activation of medial frontal cortex (Strakowski *et al*, 2004), increased regional blood flow of left aMCC (Blumberg *et al*, 2000; Rubinsztein *et al*, 2001), correlations of glucose metabolic rate in medial prefrontal cortex with clinical depression severity (Osuch *et al*, 2000), post-treatment regional blood flow response to transcranial magnetic stimulation in left aMCC and medial prefrontal cortex (Speer *et al*, 2000), and increased levels of myo-inositol (mI) and choline compounds (Cho) in anterior cingulate (Davanzo *et al*, 2001; Moore *et al*, 2000c).

^{1}HMRS is an *in vivo*, non-invasive brain imaging technique that can detect alterations in brain biochemistry in the presence of apparently normal anatomy. As reviewed elsewhere (Moore and Galloway, 2002; Stork and Renshaw, 2005; Stanley, 2002; Strakowski, 2005), resonances in the [1]HMR spectrum can be reliably quantified for several

metabolites with brain concentrations in the millimolar range including N-acetyl-aspartate (NAA), a marker of neuronal viability; the excitatory amino-acid glutamate (Glu); glutamine (Gln), the glial cell reservoir storage form of glutamate; the sum of Glu and Gln (Glx); creatine + phosphocreatine (Cr), a measure of energy utilization which, given its relative stability, has historically been used as an ^{1}HMRS internal standard; choline related compounds (Cho) including glycerophosphocholine, phosphocholine, and acetylcholine some of which are involved in membrane metabolism; and myo-inositol (mI), a component of the cellular phosphoinositol-cycle second-messenger system.

In contrast to other functional imaging technologies, MR spectroscopy seems to be uniquely positioned to investigate biochemical-based psychotropic drug mechanisms of action. For example, lithium treatment has been shown to increase gray matter volume and NAA (Moore et al, 2000a, b). This work has been extended with a recent report of increased gray matter only in those bipolar depressed subjects who had a treatment response associated with lithium (Moore et al, 2005). It has been proposed that this neuroprotective/neurotrophic action of lithium, as measured by NAA, could occur via decreased glutamate-mediated neurotoxicity in pre-clinical ischemic models (Nonaka and Chuang, 1998) and increased expression of the cytoprotective protein bcl-2 (Moore et al, 2000c).

Lamotrigine is an FDA-approved agent for the maintenance phase of bipolar I disorder (Bowden et al, 2003; Calabrese et al, 2003). There are additional controlled data demonstrating acute efficacy in bipolar depression (Frye et al, 2000; Obrocea et al, 2002; Calabrese et al, 1999). As reviewed by Frye et al (2000) and Ketter et al (2003) lamotrigine has been shown to block voltage-sensitive sodium channels with the subsequent inhibition of the presynaptic excitatory amino acids aspartate and glutamate. Like lithium, lamotrigine reduces cell damage (eg, in the hippocampal CA1 region) in ischemic neuroprotective models (Crumrine et al, 1997; Lee et al, 2000). Although glutamatergic dysregulation has been less well studied in mood disorders (Frye et al, 2007; Kugaya and Sanacora, 2005), this investigation was conducted to evaluate whether lamotrigine may modulate glutamatergic tone in bipolar depression that is clinically relevant and objectively measured by MR spectroscopy.

METHODS

This study was approved both by the UCLA and the UCLA Harbor Medical Center IRB. After obtaining written informed consent, all subjects were diagnosed using the Structured Clinical Interview for DSM-IV (SCID, First et al, 1997); this diagnostic interview was administered by trained raters who had participated in an ongoing quality assurance program to prevent rater drift (Ventura et al, 1998). The inclusion criteria for this study was a DSM-IV current diagnosis of bipolar I or II depression with at least moderate symptom severity as measured by a Montgomery-Asberg Depression Rating Scale (MADRS) score $\geqslant 16$ (Montgomery and Asberg, 1979). Melancholic vs non-melancholic subtype was confirmed by DSM-IV criteria. All ratings were conducted by the principal investigator (MAF) or inter-rater reliable assistants (JF or SE). Exclusion criteria

Table I Subject Demographic Profile ($\pm$SD)

	BP Depressed ($n=23$)	Control ($n=12$)	Statistic
N/gender	17M/6F	7M/5F	$F=0.18, p=0.67$
Age (years)	35.6 ± 11.2	32.8 ± 10.9	$F=0.99, p=0.33$
Education (years)	16.3 ± 2.4	18.2 ± 3.7	$F=0.69, p=0.42$
MADRS	27.5 ± 6.2	0.8 ± 0.8	$t=-15.46, p<0.0001$

included active suicidality, current psychosis, current alcohol or substance abuse or dependence (within the last 6 months), active unstable medical condition, clinically relevant abnormal laboratory test, history of adverse reaction to lamotrigine, and antipsychotic treatment within 4 weeks as these agents have been known to increase NAA in selected regions (reviewed by Bertolino et al, 2001).

Of the 34 bipolar depressed (BP Dep) subjects who provided written informed consent, eight (five M/three F) were screen failures; therefore, 26 BP Dep (19 M/seven F) & 12 healthy controls (seven M/five F) underwent ^{1}HMRS. As described below, three bipolar subjects had non-viable spectra and thus demographics, as presented in Table 1, are on 23 depressed subjects. Five out of twenty-three (12%) bipolar depressed subjects were on lithium at the time of the scan; as lithium treatment has been shown as to increase NAA (Moore et al, 2000a), all baseline spectroscopic analyses were done with and without these five subjects. At the time of scan, depressive, and manic symptom severity was assessed utilizing the MADRS and the YMRS (Young et al, 1978) respectively.

Bipolar depressed subjects underwent a second MRS scan after a 12-week open trial of lamotrigine. The lamotrigine titration schedule was 25 mg p.o. q.d. × 2 weeks, 50 mg p.o. q.d. × 2 weeks, 100 mg p.o. q.d. (orally, daily) × 2 weeks, 150 mg p.o. q.d. × 2 weeks, and 200 mg p.o. q.d. for the remaining 4 weeks. This was a slower titration than current guidelines to simplify prescription dispensation in our research pharmacy. The dose of the study medication could be reduced for side effects or maintained at a lower dose for early clinical response. Of the original 23 subjects, five dropped out of the study before the second scan ($n=1$ hospitalization for depression, $n=2$ rash (full resolution with drug discontinuation), $n=1$ administrative discharge, $n=1$ declined second scan) and one did not have viable MRS spectrum for the second scan. Remission of bipolar depressive symptoms was defined as a MADRS <8 at the last visit/second scan.

Healthy subjects did not receive medication but underwent a second MRS scan at the same time point 12 weeks later to assess for normal variability in metabolites over time.

MRI/MRS Acquisition and Processing

Scans were conducted at the UCLA Harbor Brain Imaging Center on a 1.5 T GE MRI Scanner (GE Medical Systems, Waukesha, WI) with echo-speed gradients using a head transmit/receive coil. The scans were acquired in the following order: (1) Sagittal whole-brain scout for general orientation and positioning of subsequent scans, (2) T1-weighted and inversion-recovery (IR) weighted axial images (TR/TE = 800/8 ms, 35 slices, 4 mm thickness with no gap for MRS voxel

placement, (3) Single-voxel water-suppressed (Haase *et al*, 1985) PRESS (Bottomley, 1987) ^{1}HMRS (TR/TE = 3s/30 ms, number of averages = 256, voxel size $3 \times 3 \times 3$ cm^3) of anterior cingulated/medial prefrontal cortex. PRESS was acquired with the GE proton brain examination (PROBE) technique, whereby four unsuppressed water free-induction decays were co-acquired for eddy current compensation, phase-correction, and normalization of local absolute metabolite levels. The voxel location was guided by the T1 and IR weighted sagital images as shown in Figure 1.

A systematic approach to referencing voxel position to identifiable anatomical landmarks was employed based on a human brain reference atlas (Mai *et al*, 1997), anterior cingulate anatomy (Vogt, 2003, 2005), and on our earlier report (Davanzo *et al*, 2001, see Figure 1). Laterally, the $3 \times 3 \times 3$ cm^3 voxel was centered on the interhemispheric fissure. The dorsal and ventral boundaries of the voxel were the cingulate sulcus and the inferior margin of the genu of the corpus callosum respectively. The anterior boundary of the voxel approximated the frontal pole and the posterior boundary was the gray/white-matter interface at the margin of the corpus callosum with the cingulate gyrus. This voxel contained bilateral pregenual anterior cingulate cortex (pACC), anterior midcingulate cortex (aMCC), and medial pre-frontal cortex (superior frontal gyrus). The relatively large voxel was used to allow comparison of this 1D MRS data with 2D MRS acquisitions developed in our laboratory (Thomas *et al*, 2001), which will be reported in a future work.

Images were transferred to a Sun Ultra 10 workstation (Sun Microsystems, Palo Alto, CA), and analyzed using an in-house developed image processing package which made use of software toolkits developed for medical image analysis (Brown *et al*, 1998). These toolkits enabled the display and manipulation of MR images, the representation of anatomic region of interest (ROI) and the calculation of the properties of the ROI. Multi-slice T1 MRIs were combined into a single file. The MRS voxel ($3 \times 3 \times 3$ cm^3) co-ordinates recorded during acquisition were used as the input values and the image analysis package used the multi-slice image file to calculate the percentages of tissue (gray matter + white matter) and cerebrospinal fluid (CSF) in each MRS voxel. Absolute metabolite levels were corrected for voxel CSF content.
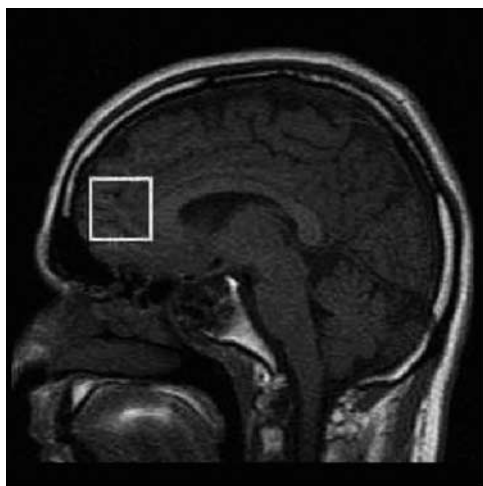

Figure 1 T1-weighted sagittal MRI location for anterior cingulate/medial prefrontal cortex 1H-MRS single-voxel acquisition (3 cm^3).

The spectroscopic raw data were transferred to an SGI^{TM} O$_2$ workstation (Silicon Graphics Inc, San Jose, CA) and processed using the LCModel package (Provencher, 1993, 2001). The basis set for TE = 30 ms was provided by the vendor for quantification. LC Model is an operator-independent commercial software package that fits *in vivo* metabolite spectra in the frequency domain using model resonances acquired under comparable scanning conditions from multiple compounds in standard phantom solutions. Using LC Model, resonances fitted with >20% variance were rejected. The entire spectrum was rejected if the signal-to-noise ratio (SNR) was less than three or the full-width at half-maximum (FWHM) was greater than 8 Hz. This occurred in three bipolar depressed subjects at baseline scan, one bipolar subject at second scan, and two controls at second scan; 91% of total scans acquired in this study were accepted. *N*-acetylaspartyl-glutamate (NAAG) and macro-molecules (MM1–MM7) were included in LC model post processing, but were not statistically analyzed.

Water-suppressed frequency-domain data were analyzed between 0.2 and 4.0 ppm without further T1 or T2 correction. An example spectrum is presented in Figure 2. Absolute concentrations of *N*-acetyl-aspartate (NAA), glutamate (Glu), glutamine (Gln), the sum of glutamate + glutamine (Glx), creatine + phosphocreatine (Cr), choline + phosphocholine (Cho), and myo-inositol (mI), corrected for voxel CSF content, are reported and denoted by brackets [ ]. Metabolite levels were also reported as ratios to creatine.

Statistical Methods

T-tests and analysis of variance were used to compare baseline demographic differences between bipolar depressed subjects *vs* controls and lamotrigine remitters *vs* non-remitters. *T*-tests were used to compare baseline spectroscopic differences between melancholic ($n = 14$) *vs* atypical ($n = 9$) depression subtypes. A 2×2 group × time mixed effects repeated measures ANOVA assuming random attrition was conducted for spectroscopic differences between bipolar depressed subjects ($n = 23$) *vs* controls ($n = 12$) at baseline and lamotrigine-treated bipolar subjects ($n = 16$) *vs* controls ($n = 10$) at second scan 12 weeks later. Effect sizes (Cohen's, 1988) were calculated for all statistically significant results. Pearson's correlation was used to evaluate associations between baseline spectra and clinical demographic variables.

The lamotrigine-associated remission ($n = 7$) and non-remission ($n = 9$) groups were compared on post-treatment spectroscopic data using analysis of covariance with baseline as the covariate and remission status as the independent variable. A preliminary analysis tested the assumption of homogeneity of the regressions of post-treatment on baseline by including the interaction of baseline and remit status. If not significant, the interaction was dropped from the model. If the interaction was significant, the covariate by treatment interaction effect was retained in the model. In that case, group means were estimated and compared using *t*-tests at the minimum, mean, and maximum values of the baseline covariate (Littell *et al*, 1996). Owing to the small sample size, only those resonances hypothesized to be associated with lamotrigine's preclinical mechanism of
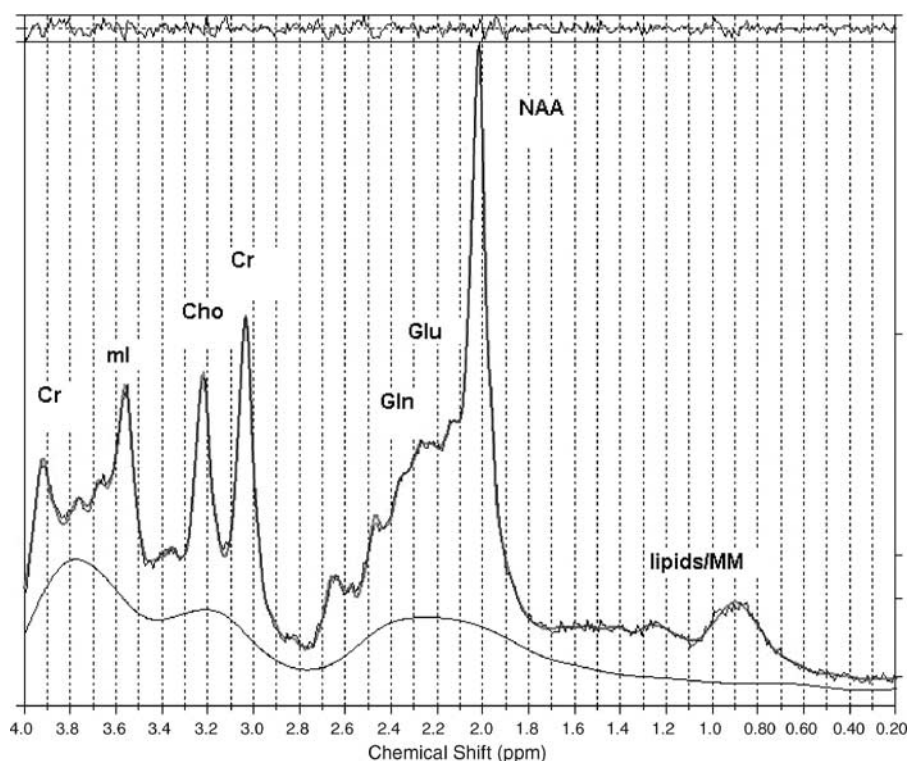
Figure 2 Raw (fine black), baseline (thick black), and LCModel fit (red) MR spectra localized in the anterior cingulate (27 cm^3) of 18-year-old male.

action (the inhibition of extracellular release of aspartate and glutamate (NAA, Glx, Glu, Gln)) were analyzed.

RESULTS

As presented in Table 1, there were no significant differences in gender, age, or years of education between BP Dep and control subjects. For bipolar depressed subjects, the mean index episode length and years ill was 10.2 ± 10.5 weeks and 17.4 ± 10.4 years respectively. The mean MADRS and YMRS were 27.5 ± 6.2 and 1.7 ± 1.9 respectively. Five out of twenty-three (4M/1F) bipolar (bp) depressed subjects were on lithium (mean dose 1170 mg, mean serum level $= 0.83$ mmol/l). At baseline scan, there was no difference in bp depressed patients ($n = 23$) vs controls ($n = 12$), in % gray matter (bp $= 70.5 \pm 2.71\%$; control $= 70.1 \pm 5.8\%$; t $= 2.03$, $p = 0.77$) or % white matter (bp $= 25.8 \pm 6.3\%$; control $n = 23.7 \pm 2.2\%$; t $= 2.04$, $p = 0.16$). As presented in Table 2a, there were no significant differences in the mean percentage voxel CSF or CSF corrected concentrations of [NAA], [Gln], [Cho], and [mI] in BP Dep subjects vs controls. However, CSF-corrected absolute concentrations of [Glx], [Glu], and [Cr] were significantly higher in BP Dep vs controls (Table 2a, Figure 3). When the five BP depressed subjects on lithium were removed, the effects for CSF-corrected absolute concentrations of [Glx] (t $= 2.60$, $p = 0.02$), [Glu] (t $= 2.45$, $p = 0.02$), and [Cr] (t $= 2.36$, $p = 0.03$, all df $= 28$) remained significant. Also, when the five BP depressed subjects on lithium were removed, there were no new CSF-corrected differences in any other spectroscopic resonances in subjects vs controls. Glx/Cr and Glu/Cr ratios in BP Dep

subjects were not significantly different from those of controls, presumably because [Cr] was also significantly elevated in BP Dep subjects. There were no other significant group differences in metabolite ratios (Table 2b).

As presented in Figure 4, the non-melancholic depressed subjects had significantly higher [Glx] and [Glu] than the melancholic depressed subjects. In all BP Dep subjects there was no significant correlation between any clinical demographic variable (MADRS, episode duration, or age of illness onset) and CSF-corrected absolute concentrations of [Glx] (MADRS $n = 23$, $r = -0.30$, $p = 0.17$; episode duration $r = 0.002$, $p = 0.99$; age mood onset $r = 0.11$, $p = 0.63$), [Glu] (MADRS $n = 23$, $r = -0.08$, $p = 0.70$; episode duration $r = 0.13$, $p = 0.63$; age mood onset $r = 0.08$, $p = 0.75$), and [Cr] (MADRS $n = 23$, $r = 0.27$, $p = 0.22$; episode duration $r = -0.26$, $p = 0.31$; age mood onset $r = -0.14$, $p = 0.54$).

At the second scan 12 weeks later, there was no difference, bp patients ($n = 16$) vs controls ($n = 10$), in % gray matter (bp $= 69.2 \pm 7.1\%$; control $= 67.6 \pm 8.1\%$; t $= 2.1$, $p = 0.63$) or % white matter (bp $= 26.1 \pm 7.0\%$; control $n = 27.35 \pm 7.9\%$; t $= 2.1$, $p = 0.69$). The only significant group differences were a significantly higher [Gln] and significantly lower NAA/Cr in BP Dep subjects vs controls (Table 2a and b).

For bipolar depressed subjects, the overall remission rate was 38% ($n = 7/18$). There was one serious adverse event for depression associated with hospitalization and two rashes that resolved with drug discontinuation. For those subjects with viable pre- and post-MRS scans, there was no significant difference in mean index episode length, baseline MADRS, and end point lamotrigine dose between remit ($n = 7$) and non-remit ($n = 9$) groups (episode length: remit $= 12 \pm 6.2$ weeks vs non-remit $= 8.75 \pm 6.6$ weeks, df $= 1,14$ t $= 0.49$, $p = 0.63$; baseline MADRS: remit $= 31 \pm 5.3$ vs non-remit $=$

Table 2a CSF Corrected Absolute Concentrations [mM] in BP Dep vs Controls at Baseline and Time 2 (12 weeks)

	Baseline			Time 2			
	BP Dep (n = 23)	Control (n = 12)	BP Dep vs Control[a]	BP LTG (n = 17)	Control (n = 10)	BP LTG vs Control[a]	BP vs Control × Time[a]
[NAA]	7.08 ± 0.25	6.7 ± 0.34	F = 0.79 p = 0.38	6.84 ± .23	7.15 ± 0.31	F = 0.65 p = 0.43	F = 4.25 p = 0.05
[Glx]	8.16 ± 0.35	6.66 ± 0.48	F = 6.4 p = 0.016*	8.38 ± 0.54	6.82 ± 0.71	F = 3.07 p = 0.09	F = 0.0 p = 0.95
[Glu]	5.66 ± 0.27	4.62 ± 0.37	F = 5.2 p = 0.029**	5.56 ± 0.33	5.32 ± 0.43	F = 0.19 p = 0.67	F = 1.53 p = 0.23
[Gln]	2.50 ± 0.26	2.04 ± 0.37	F = 1.04 p = 0.32	2.72 ± 0.3	1.57 ± 0.44	F = 4.29 p = 0.05****	F = 1.53 p = 0.23
[Cr]	5.78 ± 0.19	5.14 ± 0.26	F = 4.01 p = 0.05***	5.63 ± 0.17	5.40 ± 0.23	F = 0.66 p = 0.42	F = 1.52 p = 0.23
[Cho]	1.33 ± 0.05	1.21 ± 0.08	F = 1.69 p = 0.2	1.29 ± 0.06	1.21 ± 0.08	F = 0.66 p = 0.42	F = 0.13 p = 0.72
[ml]	4.12 ± 0.1	3.98 ± 0.14	F = 0.61 p = 0.44	3.88 ± 0.12	3.98 ± 0.14	F = 0.32 p = 0.58	F = 1.1 p = 0.3
% CSF	4.59 ± 0.43	5.77 ± .61	F = 2.38 p = 0.13	4.67 ± .46	5.11 ± 0.62	F = 0.33 p = 0.57	F = 1.65 p = 0.21

2 × 2 group × time mixed effects repeated measures analysis of variance assuming random attrition.
BP, Bipolar; DEP, Depression; LTG, Lamotrigine.
[a]All statistics BP DEP vs. Control baseline n = 35, df = 1,34, mean ± SE.
*Cohen's D = 0.9, **Cohen's D = 0.81, ***Cohen's D = .72, ****Cohen's D = 0.82.

Table 2b Metabolite Ratios/Creatine in BP Dep vs Controls at Baseline and Time 2 (12 Weeks)

	Baseline			Time 2			
	BP Dep (n = 23)	Control (n = 12)	BP Dep vs Control[a]	BP LTG (n = 17)	Control (n = 10)	BP LTG vs Control[a]	BP vs Control × Time[a]
NAA/Cr	1.24 ± 0.03	1.31 ± 0.04	F = 1.62 p = 0.21	1.21 ± 0.04	1.35 ± 0.05	F = 5.72 p = 0.023*	F = 1.0 p = 0.32
Glx/Cr	1.42 ± 0.05	1.28 ± 0.07	F = 2.36 p = 0.13	1.49 ± 0.09	1.23 ± 0.13	F = 2.89 p = 0.1	F = 1.09 p = 0.30
Glu/Cr	0.98 ± 0.04	0.90 ± 0.05	F = 1.63 p = 0.21	0.99 ± 0.05	0.97 ± 0.06	F = 0.04 p = 0.84	F = 0.48 p = 0.49
Gln/Cr	0.44 ± 0.05	0.39 ± 0.07	F = 0.56 p = 0.46	0.5 ± 0.07	0.29 ± 0.09	F = 3.86 p = 0.06	F = 3.05 p = 0.09
Cho/Cr	0.23 ± 0.01	0.23 ± 0.01	F = 0.08 p = 0.77	0.23 ± 0.01	0.22 ± 0.01	F = 0.38 p = 0.54	F = 0.73 p = 0.40
ml/Cr	0.72 ± 0.03	0.80 ± 0.04	F = 2.57 p = 0.12	0.70 ± 0.02	0.74 ± 0.02	F = 2.27 p = 0.14	F = 0.56 p = 0.46
% CSF	4.59 ± 0.43	5.77 ± 0.61	F = 2.38 p = 0.13	4.67 ± 0.46	5.11 ± 0.62	F = 0.33 p = 0.57	F = 1.65 p = 0.21

2 × 2 group × time mixed effects repeated measures analysis of variance assuming random attrition.
BP, Bipolar; DEP, Depression; LTG, Lamotrigine.
[a]All statistics BP DEP vs control baseline n = 35, df = 1,34, mean ± SE.
*Cohen's D = 0.95.

26 ± 6.4, t = 1.85, p = 0.09; end point lamotrigine dose: remit = 128.6 ± 48.8 mg vs non-remit = 162.5 ± 66.1 mg, t = 1.13, p = 0.28) groups. The end point MADRS was significantly lower in the remit (mean = 3.7 ± 1.5) vs non-remit group (mean = 16.78 ± 9.7, df = 1,14, t = 3.50, p = 0.004).

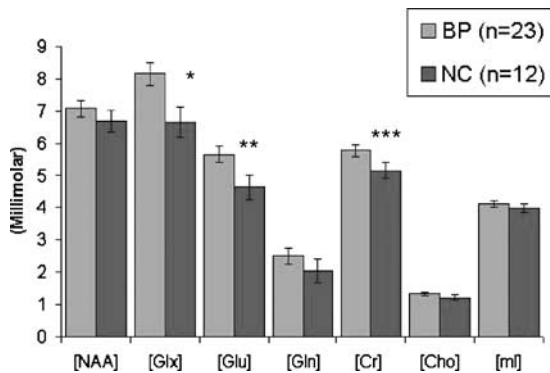The preliminary analysis of covariance revealed a significant interaction between remit status and baseline [NAA]

Figure 3 Mean (±SE) IH-MRS CSF-corrected spectra: BP depressed vs controls. *Glx, F = 6.4, p = 0.016, Cohen's D = 0.9. **Glu, F = 5.2, p = 0.029, Cohen's D = 0.81. ***Cr, F = 4.01, p = 0.05, Cohen's D = 0.72.
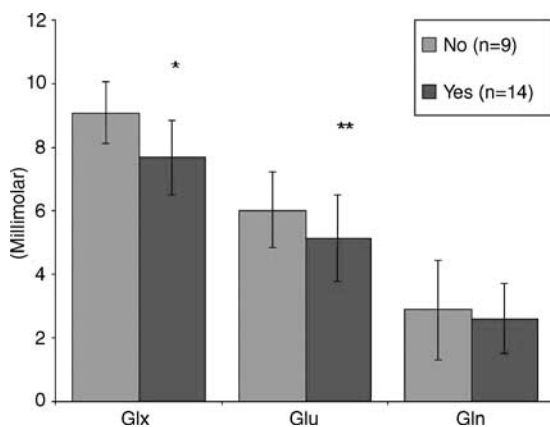

Figure 4 Mean (±SE) IH-MRS CSF-corrected Glx, Glu, and Gln spectra in melancholic subtype vs Non-melancholic bipolar depression. *Glx t = −2.98, p = 0.006 **Glu t = −1.87 p = 0.07.

(F = 5.66, df = 1,14, p = 0.03). Estimated at the baseline minimum (4.6 mM), the percent changes in [NAA] at time of second scan were 6.2% for non-remitters and 40.1% for remitters (t = 2.78, df = 1,14, p = 0.017). At the baseline mean (6.9 mM), the estimated percent changes in [NAA] were 2.9% for non-remitters and 5.8% for remitters (t = 1.92, p = 0.08). At the baseline maximum (8.6mM), the estimated percent changes in [NAA] were 9.7% for non-remitters and −19.6% for remitters (t = 1.19, p = 0.26).

The interaction between remit status and baseline level was not significant for Glx (F = 3.35, p = 0.09), Glu (F = 0.36, p = 0.56), or Gln (F = 1.25, p = 0.29, all df = 1, 14). As presented in Table 3, controlling for baseline differences, [NAA], [Glx], and [Glu] were not significantly different at study end point between remit and non-remit subjects. However, [Gln] was significantly lower in remit vs non-remit subjects (remit baseline = 2.38 ± 0.76, percent change = −0.17 ± 0.5 vs non-remit baseline = 3.04 ± 1.33, percent change = 0.46 + ±0.98, F = 4.84, p = 0.046); the large mean percent change in the non-remit group was driven by one outlier who had a 293% increase. The median percent change was 17%, more in line with the baseline and post-treatment means. Reanalysis using an analysis of covariance on ranks (Conover and Iman, 1982) yielded a trend group difference (F = 3.94, df = 1,13, p < 0.07).

DISCUSSION

These findings of increased anterior cingulate/medial prefrontal cortical glutamate (quantified as [Glx] and as [Glu] itself) and creatine [Cr] suggest that bipolar depression is characterized by an increased excitatory amino-acid signal coupled with increased energy expenditure. The effect sizes of the Glx (d = 0.9), Glu (d = 0.8), and Cr (d = 0.7) are quite large despite the small sample size. As the glutamate-glutamine cycle (Shen and Rothman, 2002) and energetic metabolism (Magistretti et al, 1993) involve neurons and glia, our two findings may reflect dysregulation of neurons and/or glia in bipolar depression. In a mixed outpatient group of bipolar patients studied by Dager et al (2004), gray matter lactate and Glx (glutamate + glutamine) were increased; the authors proposed that these increases might suggest a dynamic shift in energy redox state from oxidative phosphorylation towards glycolysis. [Glx], [Glu], and [Cr] levels in our sample may be coupled in a similar manner.

In MR spectroscopic research, the absolute level of creatine + phosphocreatine (Cr), a measure of energy utilization, has tended to be stable in most studies and conventionally has been used as an internal standard in spectroscopic research. In this study, however, [Cr] was significantly higher in bipolar depressed subjects at the first baseline scan and was not significantly different from controls at the second scan. This would suggest that Cr may not be a stable resonance to be used as an internal standard or reference ratio in bipolar depression research. This certainly warrants further investigation as the current literature in bipolar disorder, although different in methodology, mood state, and brain region, has reported increased creatine (Deicken et al, 2001; Michael et al, 2003—trend, Hamakawa et al, 1999—men only), decreased creatine (Deicken et al, 2003), or no change in creatine (Hamakawa et al, 1998; Friedman et al, 2004; Cecil et al, 2002; Brambilla et al, 2005; Frey et al, 2005).

Our findings of increased glutamate confirm previous spectroscopic studies in bipolar disorder. As mentioned above, Dager et al (2004) found above-normal Glx in left cingulate. In bipolar patients with acute mania, dorsolateral prefrontal cortical [Glx] and [Cr] (trend only) were increased in comparison to age-matched controls (Michael et al, 2003). Frontal cortex and basal ganglia Glx elevations have also been reported in pediatric bipolar disorder; the acute mood state and Cr levels were not reported (Castillo et al, 2000). In contrast, below-normal anterior cingulate [Glx] has been reported in unipolar depression with (Rosenberg et al, 2005; Mirza et al, 2004) or without (Auer et al, 2000; Pfleiderer et al, 2003) below-normal [Cr]. Despite phenomenologic similarities between bipolar and unipolar depression, the epidemiology, course of illness (age of onset, associated comorbidities), and potential liability of antidepressant treatment suggest that these are in fact, two different disease states (Bowden, 2005). Further study is encouraged to evaluate whether anterior cingulate/medial prefrontal cortex [Glx] and/or [Cr] are potential biomarkers differentiating bipolar and unipolar depression.

It will be important for future studies to not only confirm differential diagnostic criteria of bipolar vs unipolar depression, but also to delineate subtype of depression by melancholic vs atypical phenotype. Our data suggest that

Table 3 CSF-Corrected [NAA], [Glx], [Glu], and [Gln] at Baseline and 2nd Scan in Lamotrigine (LTG) Associated Remission *vs* Non-Remission

[mM]	LTG Remission (*n* = 7)			LTG Non-Remission (*n* = 9)			ANCOVA
	Baseline	**2nd scan**	**% Change**	**Baseline**	**2nd scan**	**% Change**	
[NAA]^	7.16 ± 0.9	7.19 ± 0.5	$+0.02 \pm 0.2$	6.75 ± 1.4	6.53 ± 1.1	-0.023 ± 0.1	$F = 1.77$ $p = 0.21$
[Glx]	8.04 ± 0.9	8.13 ± 2.0	$+0.01 \pm 0.2$	8.35 ± 1.9	9.14 ± 1.9	$+152 \pm 0.4$	$F = 2.36$ $p = 0.15$
[Glu]	5.66 ± 0.9	6.13 ± 1.1	$+0.1 \pm 0.2$	5.32 ± 1.8	5.37 ± 1.2	$+0.119 \pm 0.5$	$F = 0.15$ $p = 0.70$
[Gln]	2.38 ± 0.8	2.00 ± 1.2	-0.17 ± 0.5	3.04 ± 1.3	3.77 ± 1.3	$+0.46 \pm 0.98$	$F = 4.84$ $p < 0.05*$

CSF-corrected absolute concentration mean $\pm$ SD.
*The effect size (Cohen's D) of the unadjusted means = 0.79. The effect size of the adjusted means = 1.16.
^Analysis of covariance revealed a significant interaction between remit status and baseline [NAA] ($F = 5.66$, df = 1,14, $p = 0.03$). Estimated at the baseline minimum (4.6 mM), the percent changes in [NAA] at time of 2nd scan were 6.2% for non-remitters and 40.1% for remitters ($t = 2.78$, df = 1,14, $p = 0.017$). There were no significant differences in percent change at baseline mean (6.9 mM) and baseline maximum (8.6 mM).

non-melancholic depressed bipolars have a significantly higher glutamate signal than melancholic bipolar depressed subjects. It is unclear if the potential for ^{1}HMRS anterior cingulate glutamate to distinguish bipolar from unipolar depression is related to the different disease state (bipolar glutamate increase/unipolar glutamate decrease) or to the current phenomenologic presentation (atypical glutamate increase/melancholic glutamate decrease). The unipolar studies that reported reduced anterior cingulate [Glx] (Rosenberg *et al*, 2005; Mirza *et al*, 2004; Auer *et al*, 2000; Pfleiderer *et al*, 2003) did not report glutamate levels based on subtype or pattern of depression.

Our preliminary results are in contrast to the Sanacora *et al* (2004) study which found below-normal glutamate and above-normal GABA in the occipital lobes in melancholic unipolar patients. It is important to emphasize however, that the two studies differ in primary cohort (bipolar *vs* unipolar) and brain region of interest (anterior cingulated/medial prefrontal cortex *vs* occipital lobe). Nonetheless, recent preclincal data have highlighted that CRF1 receptors (activation associated with anxiogenic behavior; Liebsch *et al*, 1995) and CRF2 receptors (activation involved in stress mediated coping behavior; Liebsch *et al*, 1999) differentially regulate glutamate transmission (Liu *et al*, 2004). In the central nucleus of the amygdala, CRF decreased glutamatergic transmission through a CRF1 mediated post-synaptic action; conversely, in the lateral septum mediolateral nucleus, CRF caused a CRF1 mediated facilitation of glutamatergic transmission. It may be that CRF-mediated glutamate transmission may be differentially regulated in bipolar *vs* unipolar disorder or in atypical *vs* melancholic depressive subtypes.

These data also suggest that lamotrigine-associated remission of bipolar depression may be associated with a reduction in glutamine. Although the sample size is small, the effect size is quite large and is consistent with the *a priori* defined mechanism of drug action (ie inhibition of extracellular release of glutamate decreasing the glial cell reservoir of glutamine). Further research is encouraged to understand the clinical implications of these findings and to evaluate whether baseline glutamate levels could predict treatment response or predict the subsequent reduction of glutamine when given an antiglutamatergic drug such as lamotrigine.

Once glutamate has been released into the synapse, glial cells re-uptake glutamate by excitatory amino-acid (EAA) transporters (Shegeri *et al*, 2004; Mathews and Diamond, 2003). EAA one and two shuttle glutamate into astrocytes where it is converted to glutamine by glutamine synthetase (GS). Glutamine is then released, taken up by the neuronal terminals, and is reconverted to glutamate and GABA by glutaminase (GLS) and glutamic acid decarboxylase (GAD1) respectively. It is not known if there is differential enzymatic regulation of the glutamate–glutamine cycle in bipolar disorder patients who respond *vs* do not respond to treatment, but there are data suggesting reduced activity of GS in area 24 of anterior cingulate in depressed subjects (Choudary *et al*, 2005). There are also findings of decreased glutamate–glutamine cycling in the plasma and cerebrospinal fluid of depressed patients (Cryan and Kaupmann, 2005) and epileptic human hippocampus (Petroff *et al*, 2002). Theoretically, downregulation of GS could account for the increased Glx and Glu resonances reported here although current ^{1}HMRS technology is unable to distinguish extracellular *vs* intraneuronal glutamate.

Our data also suggest that lamotrigine treatment may be associated with an increase in NAA for those subjects with low levels at baseline; this increase was approximately 40% in remitters and was significantly greater than non-remitters (6%). The differential increase in NAA as a function of remit status was not present when estimated at the either baseline mean or baseline maximum. This means that the difference between remit and non-remit groups in NAA increase appears to exist only in those who start with a baseline deficit.

This is consistent with recent data with lamotrigine in adolescent bipolar disorder (Chang *et al*, 2005). Eleven adolescent bipolar depressed subjects underwent an 8-week open label trial of lamotrigine (mean dose 136 ± 28 mg qd). At study end point, there was a significant increase in left dorsal lateral prefrontal cortex NAA/Cr (1.59 ± 0.13 to 1.66 ± 0.1, $p = 0.04$). Like the Chang study, our data show a spectral change (ie increased NAA) consistent with the

drug mechanism of action (ie blocking the extracellular release of aspartate thereby increasing intraneuronal levels). The difference in our study *vs* the Chang study is that the increase in our cohort was only identified in those subjects with low levels at baseline. The baseline NAA by remit status interaction is as well consistent with the hypothesis originally proposed by Brambilla *et al*, (2004) whereby treatment-associated increases in NAA may only be identified in NAA deficient disease states. In their study, lithium administration to healthy controls was not associated with an increase in NAA. It may be that psychotropic drug treatment-associated increases in NAA may only be possible in subjects where NAA baseline deficits are present.

NAA metabolism is maintained via a unique tricellular pathway involving a neuron, an astrocyte, and an oligodendrocyte proximal to a glutamatergic neuronal synapse (Baslow, 2000). NAA synthesis occurs in the neuronal mitochondrion through the NAA-synthase-catalyzed union of acetyl-CoA with aspartate: Acetyl-CoA + Asp → NAA + CoAsh (Baslow, 1997; Patel and Clark, 1979). Thus, inhibition of aspartate release may increase intracellular synthesis of NAA. It will be important to replicate these NAA spectroscopic changes associated with lamotrigine in a larger, placebo-controlled investigation. Furthermore, it would be valuable to evaluate whether baseline levels of NAA predict lamotrigine-associated increases in NAA or treatment response.

There are several limitations to this study including the large voxel size and lack of gray- *vs* white-matter tissue segmentation. The large voxel size was chosen *a priori* to standardize the 1D MRS data presented here to subsequent 2D MRS data currently being analyzed (Thomas *et al*, 2001). Second, given the size of the voxel, our region of interest expands beyond a single anatomic region of interest and includes bilateral pregenual anterior cingulate (pAC), anterior midcingulate cortex (aMCC), and medial prefrontal cortex (superior frontal gyrus). A future study, with a smaller voxel size and careful regional volumetric analysis might determine which, if either, cingulate subregion or medial prefrontal cortex is driving the present results.

Uncertainties, particularly at 1.5 T, attend the quantification of overlapping resonances of Glx, Glu, and Gln. Generally, the Glx resonance is comprised of glutamate (60–70%) and glutamine (20–30%; Pouwels and Frahm, 1998). In this study, acceptably reliable peaks, as defined by LCModel quality control criteria, were recorded consistently for each of these. Thereby, the Gln resonance was smaller and relatively more variable than Glx and Glu, as has been seen by others (Auer *et al*, 2000; Rosenberg *et al*, 2005). Higher field strength may better quantify Gln and separate it from Glu as evidenced by a 4-T study of left anterior cingulate finding reductions in both Glu and Gln in schizophrenia (Theberge *et al*, 2003). Furthermore, in addition to the Glu and Gln included in the LCModel fit of the Glx signal, some authors include a GABA contribution since their curve fitting algorithms do not differentiate GABA from the overlapping Glx peaks (Sanacora *et al*, 1999); nonetheless, this contribution is considered to be quite small.

A further limitation of our study was lack of segmentation of brain tissue into gray and white matter, although CSF was distinguished from these two. Fortunately, the midline anterior cingulate placement of the MRS voxel assured a low contribution of white matter ($\sim$10% or less). None-theless, the ability to segment gray and white matter from each other might help further localize our findings vis-à-vis tissue type.

These limitations notwithstanding, this report suggests that elevated anterior cingulate [Glx] and/or [Cr] may represent an important metabolic concommittant of bipolar depression, possibly even distinguishing it from unipolar depression. Confirmatory studies are in order.

DISCLOSURE DECLARATION

Mark A Frye, MD
Consultant
Abbott Laboratories, Astra-Zeneca, Bristol-Myers Squibb, Eli Lilly and Co., GlaxoSmithKline, Janssen-Cilag, Johnson & Johnson PRD, Novartis, Ortho McNeil, Otsuka Pharmaceuticals, Pfizer Inc., Shire Pharmaeuticals, Solvay Pharmaceuticals, Wyeth.
Grants
Abbott Laboratories, American Foundation for Suicide Prevention Cephalon, GlaxoSmithKline, National Institute of Mental Health, Pfizer Inc., Solvay Pharmaceuticals, Stanley Medical Research Institute.
Speakers' Bureau
Astra Zeneca Pharmaceuticals, Bristol Myers Squibb, Eli Lilly and Co., Otsuka Pharmaceuticals, Pfizer.
Financial interest/stock ownership/royalties
None.

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