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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.
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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

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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 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).

Table 1 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	$t=-15.46$, $p<0.0001$

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. $\times$ 2 weeks, 50 mg p.o. q.d. $\times$ 2 weeks, 100 mg p.o. q.d. (orally, daily) $\times$ 2 weeks, 150 mg p.o. q.d. $\times$ 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.

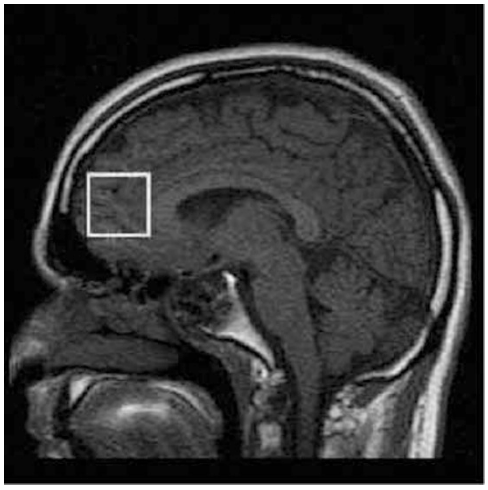

Figure 1 T1-weighted sagittal MRI location for anterior cingulate/medial prefrontal cortex 1H-MRS single-voxel acquisition ($3\,cm^3$).

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 macromolecules (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 $\times$ 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

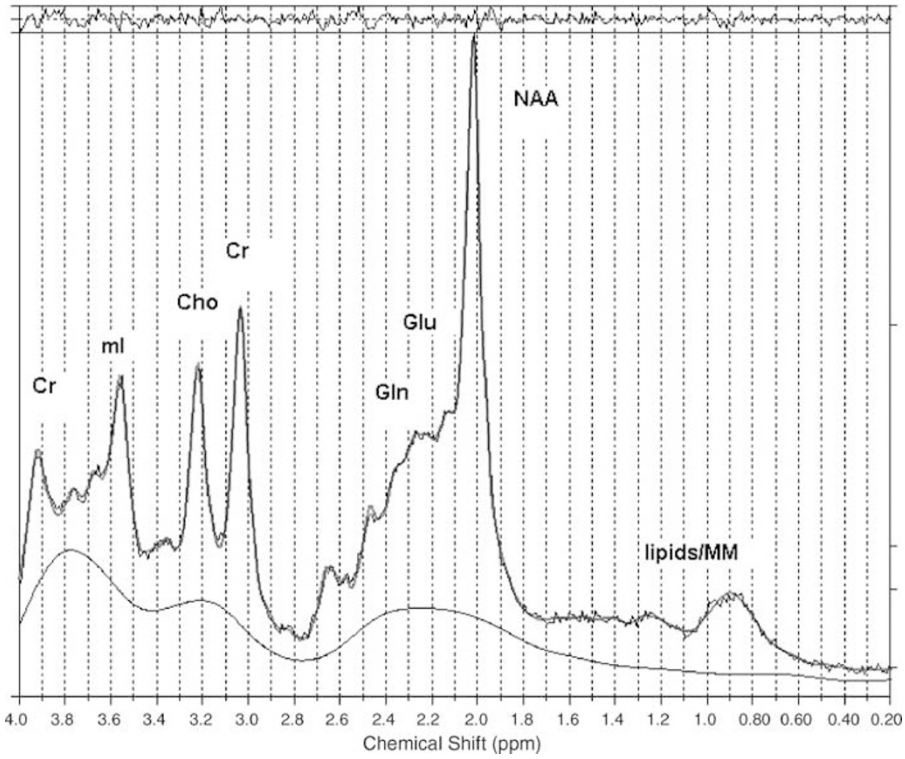

Figure 2 Raw (fine black), baseline (thick black), and LCModel fit (red) MR spectra localized in the anterior cingulate (27 cm^3) of 18-year-old male.

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 ± 2.71%; control = 70.1 ± 5.8%; t = 2.03, $p = 0.77$) or % white matter (bp = 25.8 ± 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 ± 7.1%; control = 67.6 ± 8.1%; t = 2.1, $p = 0.63$) or % white matter (bp = 26.1 ± 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 ± 6.2 weeks *vs* non-remit = 8.75 ± 6.6 weeks, df = 1,14 t = 0.49, $p = 0.63$; baseline MADRS: remit = 31 ± 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	6.84±.23	7.15±0.31	F=0.65	F=4.25
			$p=0.38$			$p=0.43$	$p=0.05$
[Glx]	8.16±0.35	6.66±0.48	F=6.4	8.38±0.54	6.82±0.71	F=3.07	F=0.0
			$p=0.016$*			$p=0.09$	$p=0.95$
[Glu]	5.66±0.27	4.62±0.37	F=5.2	5.56±0.33	5.32±0.43	F=0.19	F=1.53
			$p=0.029$**			$p=0.67$	$p=0.23$
[Gln]	2.50±0.26	2.04±0.37	F=1.04	2.72±0.3	1.57±0.44	F=4.29	F=1.53
			$p=0.32$			$p=0.05$****	$p=0.23$
[Cr]	5.78±0.19	5.14±0.26	F=4.01	5.63±0.17	5.40±0.23	F=0.66	F=1.52
			$p=0.05$***			$p=0.42$	$p=0.23$
[Cho]	1.33±0.05	1.21±0.08	F=1.69	1.29±0.06	1.21±0.08	F=0.66	F=0.13
			$p=0.2$			$p=0.42$	$p=0.72$
[mI]	4.12±0.1	3.98±0.14	F=0.61	3.88±0.12	3.98±0.14	F=0.32	F=1.1
			$p=0.44$			$p=0.58$	$p=0.3$
% CSF	4.59±0.43	5.77±.61	F=2.38	4.67±.46	5.11±0.62	F=0.33	F=1.65
			$p=0.13$			$p=0.57$	$p=0.21$

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	1.21±0.04	1.35±0.05	F=5.72	F=1.0
			$p=0.21$			$p=0.023$*	$p=0.32$
Glx/Cr	1.42±0.05	1.28±0.07	F=2.36	1.49±0.09	1.23±0.13	F=2.89	F=1.09
			$p=0.13$			$p=0.1$	$p=0.30$
Glu/Cr	0.98±0.04	0.90±0.05	F=1.63	0.99±0.05	0.97±0.06	F=0.04	F=0.48
			$p=0.21$			$p=0.84$	$p=0.49$
Gln/Cr	0.44±0.05	0.39±0.07	F=0.56	0.5±0.07	0.29±0.09	F=3.86	F=3.05
			$p=0.46$			$p=0.06$	$p=0.09$
Cho/Cr	0.23±0.01	0.23±0.01	F=0.08	0.23±0.01	0.22±0.01	F=0.38	F=0.73
			$p=0.77$			$p=0.54$	$p=0.40$
mI/Cr	0.72±0.03	0.80±0.04	F=2.57	0.70±0.02	0.74±0.02	F=2.27	F=0.56
			$p=0.12$			$p=0.14$	$p=0.46$
% CSF	4.59±0.43	5.77±0.61	F=2.38	4.67±0.46	5.11±0.62	F=0.33	F=1.65
			$p=0.13$			$p=0.57$	$p=0.21$

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]

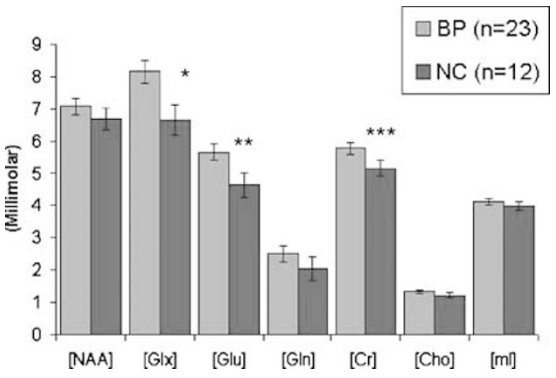

Figure 3 Mean (±SE) 1H-MRS CSF-corrected spectra: BP depressed *vs* controls. *Glx, F = 6.4, $p = 0.016$, Cohen's D = 0.9. **Glu, F = 5.2, $p = 0.029$, Cohen's D = 0.81. ***Cr, F = 4.01, $p = 0.05$, Cohen's D = 0.72.

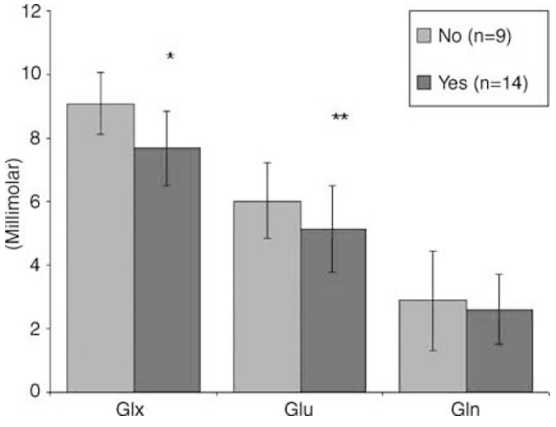

Figure 4 Mean (±SE) 1H-MRS CSF-corrected Glx, Glu, and Gln spectra in melancholic subtype *vs* Non-melancholic bipolar depression. *Glx t = −2.98, $p = 0.006$ **Glu t = −1.87 $p = 0.07$.

($F = 5.66$, 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

	LTG Remission ($n=7$)			LTG Non-Remission ($n=9$)			
[mM]	**Baseline**	**2nd scan**	**% Change**	**Baseline**	**2nd scan**	**% Change**	**ANCOVA**
[NAA]^	7.16±0.9	7.19±0.5	+0.02±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±0.2	8.35±1.9	9.14±1.9	+152±0.4	$F=2.36$ $p=0.15$
[Glu]	5.66±0.9	6.13±1.1	+0.1±0.2	5.32±1.8	5.37±1.2	+0.119±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±0.98	$F=4.84$ $p<0.05$*

CSF-corrected absolute concentration mean±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 (~10% or less). Nonetheless, 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.

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REFERENCES

Allman JM, Hakeem A, Erwin JM, Nimchinsky E, Hof P (2001). The anterior cingulate cortex: the evolution of an interface between emotion and cognition. *Ann NY Acad Sci* **935**: 107–117.

Auer DP, Putz B, Kraft E, Lipinski B, Schill J, Holsboer F (2000). Reduced glutamate in the anterior cingulate cortex in depression: an *in vivo* proton magnetic resonance spectroscopy study. *Biol Psychiatry* **47**: 305–313.

Baslow MH (2000). Functions of *N*-acetyl-L-aspartate and *N*-acetyl-L-aspartylglutamate in the vertebrate brain: role in glial cell-specific signaling. *J Neurochem* **75**: 453–459.

Baslow MH (1997). A review of phylogenetic and metabolic relationships between the acylamino acids, *N*-acetyl-L-aspartic acid and *N*-acetyl-L-histidine, in the vertebrate nervous system. *J Neurochem* **68**: 1335–1344.

Benes FM, Vincent SL, Todtenkopf M (2001). The density of pyramidal and nonpyramidal neuron in anterior cinuglate cortex of schizophrenic and bipolar subjects. *Biol Psychiatry* **50**: 395–406.

Bertolino A, Callicott JH, Mattay VS, Weidenhammer KM, Rakow R, Weinberger DR (2001). The effect of treatment with antipsychotic drugs on brain *N*-acetylaspartate measure in patients with schizophrenia. *Biol Psychiatry* **49**: 39–46.

Blumberg HP, Stern E, Martinez D, Ricketts S, de Assis J, White T *et al* (2000). Increased anterior cingulate and caudate activity in bipolar mania. *Biol Psychiatry* **48**: 1045–1052.

Bottomley PA (1987). Spatial localization in NMR spectroscopy *in vivo*. *Ann N Y Acad Sci* **508**: 333–348.

Bouras C, Kovari E, Hof PR, Riederer BM, Giannakopoulos P (2001). Anterior cingulated cortex pathology in schizophrenia and bipolar disorder. *Acta Neuropathol (Berlin)* **102**: 373–379.

Bowden CL, Calabrese JR, Sachs G, Yatham LN, Ashgar SA, Hompland M *et al* (2003). A placebo-controlled 18-month trial of lamotrigine and lithium maintanance treatment in recently manic or hypomanic patients with bipolar I disorder. *Arch Gen Psychiatry* **7**: 392–400.

Bowden CL (2005). A different depression: clinical distinction between bipolar and unipolar depression. *J Affect Disorder* **84**: 117–125.

Brambilla P, Stanley JA, Nicoletti MA, Sassi RB, Mallinger AG, Frank E (2005). 1 H magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in bipolar disorder patients. *J Affect Disord* **86**: 61–67.

Brambilla P, Stanley JA, Sassi RB, Nicoletti MA, Mallinger AG, Keshavan MS (2004). 1H-MRS study of dorsolateral prefrontal cortex in healthy individuals before and after lithium administration. *Neuropsychopharmaoclogy* **29**: 1918–1924.

Brown MS, McNitt-Gray MF, Wyckoff N, Bui A (1998). An object-oriented region of interest toolkit for workstations. In: Kim Y (ed). *Medical Imaging 1998: Image Display, Proc SPIE*, Vol. 3335, pp 627–636.

Calabrese JR, Bowden CL, Sachs GS, Ascher JA, Monaghan E, Rudd GD (1999). A double-blind placebo controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. *J Clin Psychiatry* **60**: 79–88.

Calabrese JR, Bowden CL, Sachs G, Yatham LN, Behnke K, Mehtonen OP *et al* (2003). A placebo-controlled 18-month trial of lamotrigine and lithium maintanance treatment in recently depressed patients with bipolar I disorder. *J Clin Psychiatry* **9**: 1013–1024.

Castillo M, Kwock L, Courvoisie HE, Hooper SR (2000). Proton MR spectroscopy in children with bipolar affective disorder: preliminary observations. *AJNR* **21**: 832–838.

Cecil KM, DelBello MP, Morey R, Strakowski SM (2002). Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. *Bipolar Disord* **4**: 357–365.

Chang K, Gallelli K, Howe M, Saxena K, Wagner C, Spielman D *et al* (2005). Prefrontal neurometabolite changes following Lamotrigine treatment in adolescents with bipolar depression. Presented at the 44th Annual Meeting of the American College of Neuropsychopharmacology, 11–15 December 2005.

Choudary PV, Molnare M, Evans SJ, Tomita H, Li JS, Vawter MP *et al* (2005). Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *PNAS* **102**: 15653–15658.

Cohen J (1988). *Statistical Power for the Behavioral Sciences*. Lawrence Erlbaum: Hillsdale, NJ.

Conover WJ, Iman RL (1982). Analysis of covariance using the rank transformation. *Biometrics* **38**: 715–724.

Crumrine RC, Bergstrand K, Cooper AT, Faison WL, Cooper BR (1997). Lamotrigine protects hippocampal CA1 neurons from ischemic damage after cardiac arrest. *Stroke* **28**: 2230–2236.

Cryan JF, Kaupmann K (2005). Don't worry B happy. *Trends Pharmacol Sci* **26**: 36–43.

Dager SR, Friedman SD, Parow A, Demopulos C, Stoll Al, Lyoo IK *et al* (2004). Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry* **61**: 450–458.

Davanzo P, Thomas MA, Yue K, Oshiro T, Belin T, Strober M *et al* (2001). Decreased anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. *Neuropsychopharmacology* **24**: 359–369.

Deicken RF, Eliaz Y, Feiwell R, Schuff N (2001). Increased thalamic *N*-acetylaspartate in male patients with familial bipolar I disorder. *Psychiatry Res* **106**: 35–45.

Deicken RF, Pegues MP, Anzalone S, Feiwell R, Soher B (2003). Lower concentration of hippocampal N-acetylaspartate in familial bipolar I disorder. *Am J Psychiatry* **160**: 873–882.

Devinsky O, Morrell MJ, Vogt BA (1995). Contributions of anterior cingulate cortex to behavior. *Brain* **118**: 279–306.

Drevets WC, Price JL, Simpson JR, Todd RD, Reich T, Vannier M *et al* (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* **386**: 824–827.

Eastwood SL, Harrison PJ (2001). Synaptic pathology in the anterior cingulate cortex in schizophrenia and mood disorders: A review and a western blot study of synaptophysin, GAP-43, and the complexins. *Brain Res Bull* **55**: 569–578.

First MB, Spitzer RL, Gibbon M, Williams JBW (1997). *Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, Patient Edition (SCID-I/P), in Biometrics Research*. New York State Psychiatric Institute: New York, NY.

Frey BN, Folgierini M, Nicoletti M, Machado-Vieira R, Stanley JA, Soares JC *et al* (2005). A proton magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in acute mania. *Hum Psychopharmacol* **20**: 133–139.

Friedman SD, Dager SR, Parow A, Hirashima F, Demopulos C, Stoll AL *et al* (2004). Lithium and valproic acid treatment effects on brain chemistry in bipolar disorder. *Biol Psychiatry* **56**: 340–348.

Frye MA, Ketter TA, Kimbrell TA, Dunn RT, Speer AM, Osuch EA *et al* (2000). A placebo-controlled study of lamotrigine and gabapentin monotherapy in refractory mood disorders. *J Clin Psychopharmacol* **20**: 607–614.

Frye MA, Tsai G, Huggins T, Coyle JT, Post RM (2007). Low cerebrospinal fluid glutamate and glycine in refractory affective disorder. *Biol Psychiatry* **61**: 162–166.

Haase A, Frahm J, Hänicke W, Matthaei D (1985). 1H NMR chemical shift selective (CHESS) imaging. *Phys Med Biol* **30**: 341–344.

Hamakawa H, Kato T, Murashita J, Kato N (1998). Quantitative proton magnetic resonance spectroscopy of the basal ganglia in patients with affective disorders. *Eur Arch Psychiatry Clin Neurosci* **248**: 53–58.

Hamakawa H, Kato T, Shioiri T, Inubushi T, Kato N (1999). Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. *Psychol Med* **29**: 639–644.

Hirayasu Y, Shenton ME, Salisbury DF, Kwon JS, Wibble CG, Fischer IA *et al* (1999). Subgenual cingulate cortex volume in first episode psychosis. *Am J Psychiatry* **156**: 1091–1093.

Ketter TA, Manji HK, Post RM (2003). Potential mechanisms of action of Lamotrigine in the treatment of bipolar disorders. *J Clin Psychopharmacol* **23**: 484–495.

Kugaya A, Sanacora A (2005). Beyond monoamines: Glutamatergic Function in Mood Disorders. *CNS Spectrums* **10**: 808–818.

Lee WT, Shen YZ, Chang C (2000). Neuroprotective effect of Lamotrigine and MK-801 on rat brain lesions induced by 3-nitroproprionic acid: evaluation by magnetic resonance imaging and *in vivo* proton magnetic resonance spectroscopy. *Neuroscience* **1**: 89–95.

Liebsch G, Landgraf R, Engelmann M, Lorscher P, Holsboer F (1999). Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. *J Psychiatr Res* **33**: 153–163.

Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M *et al* (1995). Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdale reduced anxiety-related behavior in socially defeated rats. *Regul Pept* **59**: 229–239.

Littell RC, Millikeen GA, Stroup WW, Wolfingere RD (1996). *The SAS System for Mixed Models*. SAS Institute: Cary, NC. p 175.

Lopez-Larson MP, DelBello MP, Zimmerman ME, Schwiers ML, Strakowski SM (2002). Regional prefrontal gray and white matter abnormalities in bipolar disorder. *Biol Psychiatry* **52**: 93–100.

Liu J, Yu B, Neugebauer V, Grigoriadis DE, Rivier J, Vale WW *et al* (2004). Corticotropin-releasing factor and Urocortin I modulate excitatory glutamatergic synaptic transmission. *J Neuroscience* **16**: 4020–4029.

Magistretti PJ, Sorg O, Yu N, Martin J-L, Pellerin L (1993). Neurotransmitters regulate energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells. *Dev Neurosci* **15**: 306–312.

Mathews DC, Diamond JS (2003). Neuronal glutamate uptake contributes to GABA synthesis and inhibitory synaptic strength. *J Neuroscience* **23**: 2040–2048.

Mai JK, Assheuer J, Paxinos G (1997). *Atlas Hum Brain*. Academic Press: San Diego viii: 328.

Michael N, Erfurth A, Ohemann P, Gossling M, Arolt V, Weindel W *et al* (2003). Acute mania is accompanied by elevated glutamate/glutamine levels within the left dorsolateral prefrontal cortex. *Psychopharmacology* **168**: 344–346.

Mirza Y, Tang J, Russell A, Banerjee S, Bhandari R, Ivey J *et al* (2004). Reduced anterior cingulate cortex glutamatergic concentrations in childhood major depression. *JAACAP* **43**: 341–348.

Montgomery SA, Asberg M (1979). A new depression scale designed to be sensitive to change. *Br J Psychiatry* **134**: 382–389.

Moore GJ, Bebchuk JM, Wilds IB, Chen G, Menji HK (2000b). Lithium–induced increase in human brain grey matter. *Lancet* **356**: 1241–1242.

Moore CM, Breeze JL, Bruber SA, Babb SM, Frederick B deB, Villafuerte RA *et al* (2000c). Choline, myo-inositol, and mood in bipolar disorder: a proton magnetic resonance spectroscopic study of the anterior cinguate cortex. *Bipolar Disord* **2**: 207–216.

Moore GJ, Cortese BM, Glitz DA, Zajac-Benitez, Quiroz JA, Uhde TW *et al* (2005). Lithium increases gray matter in the prefrontal and subgenual prefrontal cortices in treament responsive bipolar disorder patients. 44th Annual Meeting of the American College of Neuropsychopharmacology, New Research December 13, 2005 Kona Hawaii.

Moore GJ, Bebchuk JM, Hasanat K, Chen G, Seraji-Bozorgzd N, Wilds IB *et al* (2000a). Lithium increases *N*-Acetyl-Aspartate in the human brain: *in vivo* evidence in support of bcl-2's neuroprotective effects? *Biol Psychiatry* **48**: 1–8.

Moore GJ, Galloway M (2002). Magnetic resonance spectroscopy: neurochemistry and treatment effects in affective disorders. *Psychopharm Bull* **36**: 5–23.

Nonaka S, Chuang D (1998). Neuroprotective effects of chronic lithium on focal cerebral ischemia in rates. *Neuroreprot* **9**: 2081–2084.

Obrocea GV, Dunn RM, Frye MA, Ketter TA, Luckenbaugh DA, Leverich GS *et al* (2002). Clinical predictors of response to lamotrigine and gabapentin monotherapy in refractory affective disorders. *Biol Psychiatry* **3**: 253–260.

Öngur D, Drevets WC, Price JL (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA* **95**: 13290–13295.

Osuch E, Ketter TA, Kimbrell TA, George MS, Benson B, Willis MW *et al* (2000). Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. *Biol Psychiatry* **48**: 1020–1023.

Papez JW (1937). A proposed mechanism of emotion. *Arch Neurol Psychiatry* **38**: 725.

Patel TB, Clark JB (1979). Synthesis of *N*-acetyl-L-aspartate by rat brain mitochondria and its involvement in mitochondrial/cytosolic carbon transport. *Biochem J* **184**: 539–546.

Petroff OAC, Errante LD, Rothman DL, Kim JH, Spencer DD (2002). Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia* **43**: 703–710.

Pfleiderer B, Michael N, Erfurth A, Ohrmann P, Hohmann U, Wolgast M *et al* (2003). Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left anterior cingulum of unipolar depressed patients. *Psychiatry Res* **3**: 185–192.

Pouwels PJ, Frahm J (1998). Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. *Magn Reson Med* **39**: 53–60.

Provencher SW (1993). Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. *Magn Reson Med* **30**: 672–679.

Provencher SW (2001). Automatic quantitation of localized *in vivo* 1H spectra with LCModel. *NMR Biomed* **14**: 260–264.

Rosenberg DR, MacMaster FP, Mirza Y, Smith JM, Easter PC, Banerjee SP *et al* (2005). Reduced anterior cingulate glutamate in pediatric major depression: a magnetic resonance spectroscopy study. *Biol Psychiatry* **58**: 700–704.

Rubinsztein JS, Fletcher PC, Rogers RD, Ho LW, Aigbirhio FL, Paykel ES *et al* (2001). Decision making in mania: a PET study. *Brain* **124**: 2550–2563.

Sanacora G, Gueorguieva R, Epperson N, Wu YT, Appel M, Rothman DL *et al* (2004). Subtype-specific alternations of gamaminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry* **61**: 705–713.

Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA *et al* (1999). Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* **11**: 1043–1047.

Sassi RB, Brambilla P, Hatch JP, Nicoletti MA, Mallinger AG, Frank E *et al* (2004). Reduced left anterior cingulate volumes in untreated bipolar patients. *Biol Psychiatry* **56**: 467–475.

Shegeri Y, Seal RP, Shimamoto K (2004). Molecular pharmacology of glutamate transporters EAAT's and VGLUTs. *Brain Res Rev* **45**: 150–165.

Shen J, Rothman DL (2002). Magnetic resonance spectroscopic approaches to studying neuronal: glial interactions. *Biol Psychiatry* **52**: 694–700.

Speer AM, Kimbrell TA, Wassermann EM, Repella JD, Willis MW, Herscovitch P *et al* (2000). Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. *Biol Psychiatry* **48**: 1133–1141.

Stanley JA (2002). *In vivo* magnetic resonance spectroscopy and its application to neuropsychiatric disorders. *Can J Psychiatry* **47**: 315–326.

Strakowski SM, Adler CM, Holladn SK, Mills N, DelBello M (2004). A preliminary fMRI study of sustained attention in euthymic, unmedicated bipolar disorder. *Neuropsychopharmacology* **29**: 1734–1740.

Strakowski SM, DelBello MP, Adler CM (2005). The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. *Mol Psychiatry* **1**: 105–116.

Stork C, Renshaw PF (2005). Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol Psychiatry* **1**: 1–20.

Theberge J, Al-Seamaan Y, Williamson PC, Menon RS, Neufeld RW, Rajakumar N *et al* (2003). Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. *Am J Psychiatry* **12**: 2231–2233.

Thomas MA, Yue K, Binesh N, Davanzo P, Kumar A, Siegel B *et al* (2001). Localized two-dimensional shift correlated MR spectroscopy of human brain. *Magn Reson Med* **46**: 58–67.

Ventura J, Liberman RP, Green MF, Shaner A, Mintz J (1998). Training and quality assurance with the Structured Clinical Interview for DSM-IV I/P (SCID). *Psychiatry Res* **79**: 163–173.

Vogt BA (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci* **7**: 533–544.

Vogt BA, Berger GR, Derbyshire SWG (2003). Structural and functional dichotomy of human midcingulate cortex. *E J Neurosci* **18**: 3134–3144.

Young RC, Biggs JT, Ziegler VE, Meyer DA (1978). A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry* **133**: 429–435.