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Decrease in creatine kinase messenger RNA expression in the hippocampus and dorsolateral prefrontal cortex in bipolar disorder

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Abstract

Objectives—Bipolar disorder (BPD) affects more than 2 million adults in the USA and ranks among the top 10 causes of worldwide disabilities. Despite its prevalence, very little is known about the etiology of BPD. Recent evidence suggests that cellular energy metabolism is disturbed in BPD. Mitochondrial function is altered, and levels of high-energy phosphates, such as phosphocreatine (PCr), are reduced in the brain. This evidence has led to the hypothesis that deficiencies in energy metabolism could account for some of the pathophysiology observed in BPD. To further explore this hypothesis, we examined levels of creatine kinase (CK) mRNA, the enzyme involved in synthesis and metabolism of PCr, in the hippocampus (HIP) and dorsolateral prefrontal cortex (DLPFC) of control, BPD and schizophrenia subjects.

Methods—Tissue was obtained from the Harvard Brain Tissue Resource Center. Real-time quantitative polymerase chain reaction (HIP, DLPFC) and gene expression microarrays (HIP) were employed to compare the brain and mitochondrial 1 isoforms of CK.

Results—Both CK isoforms were downregulated in BPD. Furthermore, mRNA transcripts for oligodendrocyte-specific proteins were downregulated in the DLPFC, whereas the mRNA for the neuron-specific protein microtubule-associated protein 2 was downregulated in the HIP.

Conclusion—Although some of the downregulation of CK might be explained by cell loss, a more general mechanism seems to be responsible. The downregulation of CK transcripts, if translated into protein levels, could explain the reduction of high-energy phosphates previously observed in BPD.

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Keywords

bipolar disorder; creatine kinase; glia; hippocampus; prefrontal cortex; schizophrenia

Bipolar disorder (BPD) is a common and severe mood disorder that affects more than 2 million adults in the USA (1, 2). BPD is associated with increased risk of suicide and a high socioeconomic burden. Although the clinical features of BPD have long been recognized (3), the disease mechanism(s) remain(s) unknown (4), and treatment resistance is high (1, 5).

Recent studies suggest altered energy metabolism and mitochondrial pathology in BPD (6, 7). Spectroscopic studies have shown a decrease in both pH and high-energy phosphates, such as phosphocreatine (PCr) and ATP, in the frontal and temporal lobes of bipolar subjects (8–11); postmortem studies in the human hippocampus (HIP) of BPD subjects showed a decrease in the expression of nuclear genes coding for mRNAs of the mitochondrial respiratory chain (12); and studies in lymphoblastoid cell lines showed a downregulation of the mitochondrial complex I subunit gene, NDUFV2 (24-kDa subunit of the mitochondrial NADH:ubiquinone oxidoreductase), and different haplotype frequencies of four polymorphisms in the upstream region of NDUFV2 (13, 14).

Creatine kinase (CK) is the enzyme responsible for the reversible transfer of the N-phosphoryl group from PCr to ADP to yield ATP and creatine (Cr) (15–17). In tissues with high energy demands, such as the brain, CK serves two purposes: the first is to shuttle phosphate groups from the site of energy production, the mitochondria, to sites of energy consumption, such as ATP-dependent ion pumps and neurotransmitter transporters in neurons and glia (18). The second function is to retrieve ATP from PCr during periods of intense energy demand (16). Four CK isoforms are expressed in humans: the first two, brain (CKB) and muscle (CKM), are the cytosolic CK isoenzymes, which are found as homo- or heterodimers at sites of energy consumption; the other two are the mitochondrial isoforms, mitochondrial 1 ubiquitous (CKMt1), and mitochondrial 2 sarcomeric (17). In the dorsolateral prefrontal cortex (DLPFC) and HIP, the principal CK isoforms are CKB (as the homomer CK-BB) and CKMt1 (19, 20). CKB is expressed at high levels in oligodendrocytes and astroglia and to a lesser extent in neurons, while CKMt1 is expressed in the mitochondria of all cell types, but with the highest levels in neurons (20, 21).

Given the reduced PCr levels observed in magnetic resonance spectroscopy studies (11, 22), we decided to examine the expression levels of mRNA transcripts coding for CKB and CKMt1 in the DLPFC and HIP in the postmortem brain of BPD, schizophrenia (SZ), and control subjects.

Materials and methods

Sample selection and tissue processing

Gene regulation was examined by real-time quantitative polymerase chain reaction (PCR) (qPCR; HIP, DLPFC) and by microarrays (HIP) in three study groups: healthy controls, subjects with BPD, and subjects with SZ. Brain specimens were obtained from the Harvard Brain Tissue Resource Center (HBTRC; McLean Hospital, Belmont, MA, USA). Subjects

were matched for age, postmortem interval, gender, brain hemisphere and storage time. All diagnoses were established by two psychiatrists at the HBTRC via retrospective review of all available medical records and extensive questionnaires about social and medical history completed by family members of the donors. The criteria of Feighner et al. (23) were applied for the diagnosis of SZ and that of the DSM-IV (24) for the diagnosis of schizoaffective disorder and BPD. Probands with a documented history of substance dependence or neurological illness were excluded from the study.

One hemisphere of each brain underwent a comprehensive neuropathological examination, and only brains with no evidence of stroke, tumor, infection, or neurodegenerative changes were used in the study. Microarray analysis of the HIP was carried out in 10 control subjects, 9 subjects with BPD, and 8 subjects with schizophrenia (12). For qPCR of the HIP, an independent RNA extraction was performed on samples for which tissue was still available at the HBTRC, yielding seven triplets (control, BPD, SZ; Table 1). For qPCR analysis of the DLPFC, nine controls, nine BPD subjects, and eight SZ subjects were obtained (Table 1). For procedures of the HBTRC see <http://www.brainbank.mclean.org/>.

Treatment of rats and processing of rat tissue

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY, USA), 200–225 g, were fed 0.15% lithium carbonate chow or control chow balanced for nutrient content (Harlan Teklad, Madison, WI, USA). The colony room was maintained on a 12-h light–dark cycle. Rats were provided water and 450 mM NaCl solution. On day 15 of lithium chow, rats were decapitated, brains removed and immediately frozen in isopentane/dry ice. Hippocampi were dissected on a freezing microtome, and RNA was extracted and prepared for gene expression microarray analysis applying the same protocol used for the human studies.

Gene array data analysis

Affymetrix HG-U95Av2 arrays were used in the human study, and RAE230A arrays were used in the rat study. (For specifics of tissue and RNA preparation for the Affymetrix GeneChips see 12, 25.) RMAexpress was used for background adjustment, normalization, and computation of gene expression summary values (<http://stat-www.berkeley.edu/users/bolstad/RMAExpress/RMAExpress.html>), (26, 27). The dChip program (<http://www.dchip.org>) (28) and GenMAPP (<http://www.genmapp.org>) (29) were used for further analysis. Recent work on the standardization of microarray experiments indicates that there is good reproducibility across different laboratories, sometimes as good as 90% (30) especially with the Affymetrix platform (30–32).

Real-time quantitative polymerase chain reaction (qPCR)

For qPCR, complementary DNA (cDNA) was synthesized from 1 µg of total RNA (SuperScript First-Strand Synthesis System for real-time quantitative PCR; Invitrogen, Carlsbad, CA, USA) with oligonucleotide deoxythymidine primers. A primer set for each gene was designed with the help of Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplicons were designed to be between 150 and 250 bp in length. Melt Curve analysis and polyacrylamide gel electrophoresis were used to confirm the specificity of each primer pair. Quantitative real-time PCR was carried out with the

DyNAmo HS SYBR Green real-time quantitative PCR kit (Finnzymes, Espoo, Finland) in a volume of 20 μ L, with cDNA equivalent to $\approx$ 40 ng total RNA, 10 μ L DyNAmo HS master mix, 0.3 μ M each of forward and reverse primer and ultrapure water (Invitrogen). The PCR cycling conditions were 95°C for 7 min followed by 39 cycles of 94°C for 10 s, 55°C for 15 s, 72°C for 20 s, and 73–82°C (depending on product melt temperature) for 2 s. A melt curve analysis was performed at the end of each PCR experiment to verify product specificity for each reaction. Dilution curves were generated for each experiment by diluting cDNA four times from a control sample with a ratio of 1:5, yielding a dilution series of 1.00, 0.2, 0.04, 0.008, and 0.0016. The logarithm of the dilution value was plotted against the cycle threshold value to give a standard curve. Blanks were included in each dilution curve to control for cross-contamination. Dilution curves, blanks, and samples were assayed in duplicates on the same plate. Reported values were normalized to the internal control human filamin A alpha (Table 2), an actin binding protein that was not regulated in the gene array experiment or the qPCR analysis.

Results

Microarray data revealed a significant decrease ($p < 0.05$) of the CKB and CKMt1 transcript in the HIP in BPD (Fig. 1A) but not in SZ (Fig. 1B). To further examine the expression levels of the CK isoforms in BPD and SZ, qPCR was performed on postmortem tissue from the DLPFC and HIP. A subset of the same samples used to generate the microarray data was used for qPCR analysis, and downregulations of CKB and CKMt1 ($p = 0.07$; not significant) was confirmed in the HIP in BPD (Fig. 2A), in contrast to SZ (Fig. 2B). Moreover, similar downregulations of CKB and CKMt1 were observed in the DLPFC in BPD (Fig. 2C), but not in SZ (Fig. 2D). However, the data indicate that SZ brains might follow a similar, albeit weaker, trend. Power analysis revealed that a sample size of 26 (CKMt1) to 44 (CKB) samples could potentially yield significant downregulations of CK isoforms in the HIP in SZ, and a sample size of 33 (CKB) to 43 (CKMt1) in the DLPFC.

CKMt1 is expressed predominantly in neurons, while CKB is expressed predominantly in oligodendrocytes and astrocytes (20, 21, 33). Because some studies of BPD have shown a reduction in glial markers and glial density (34–36) and others have shown a reduction in neuronal size and number (36–38), the downregulation of CKB might be a consequence of glial loss, or, conversely, the downregulation of CKMt1 might indicate neuronal cell loss. Therefore, we used qPCR to examine expression levels of markers for astrocytes (glial fibrillary acidic protein), oligodendrocytes (myelin-associated glycoprotein; gelsolin, v-erb-b2 erythroblastic leukemia viral oncogene homolog 3, ERBB3; and myelin basic protein) and neurons (doublecortin and microtubule-associated protein 2, MAP2) in the HIP (Fig. 3A) and DLPFC (Fig. 3B). Doublecortin was similar to control in both brain areas, whereas in the HIP, MAP2 was downregulated. The data imply that the downregulation of CKMt1 in the DLPFC is not due to neuronal cell death, but leaves the question open for the HIP (Fig. 4). In agreement with previous observations, several oligodendrocyte markers were significantly downregulated in the DLPFC in BPD (Fig. 3; 34). Thus, it cannot be ruled out that the downregulation of CKB in the DLPFC in BPD was augmented by a decrease of the glial cell population.

In order to examine the potential effect of mood stabilizers on the expression of transcripts of CKB, CKMt1 and neuronal and glial markers, we fed rats lithium chow for 15 days and performed a gene expression microarray analysis (Table 3). Except for ERBB3, no significant changes were found, suggesting that treatment might not be responsible for the observed findings.

Discussion

The Cr shuttle plays a critical role in cellular energy storage and regulation (15, 16, 39), and research suggests that BPD might be accompanied by reduced PCr levels and altered mitochondrial function. Spectroscopic imaging studies have shown reductions of PCr in BPD and depression (8, 11, 22, 40–43). In a study of geriatric depression, remission was accompanied by an increase in PCr concentration (40). The decreased PCr concentration observed in BPD was interpreted to be a result of mitochondrial dysfunction (11). This interpretation was based on increased lactate levels observed in BPD subjects, as increases in lactate levels are associated with mitochondrial myopathies and other syndromes of energy impairment (44). Concordant with this interpretation, a downregulation of nuclear mRNAs coding for proteins of the mitochondrial respiratory chain was observed in the HIP in BPD subjects (12). In this study we demonstrate that transcripts of CK isoforms are downregulated in the same patients that showed a downregulation of genomic mitochondrial transcripts (12), raising the possibility that the reductions in PCr levels and downregulations of CK and mitochondrial respiratory chain transcripts are linked.

It has been demonstrated that mice lacking CKB exhibit diminished open-field habituation and delayed acquisition of spatial tasks (45), while mice lacking CKMt display a reduced acoustic startle response and lack of prepulse inhibition in addition to the deficits seen in CKB knockout mice (46, 47). These mice did not show any gross abnormalities of brain structure or of mitochondrial ultrastructure; in fact, the mice were ‘overtly normal’ and ‘rather sophisticated analysis and challenging conditions were needed to reveal (these deficits)’ (48). Thus, while the complete removal of a CK isoform does produce measurable behavioral deficits, it does not create overt symptoms, and a downregulation of CK transcripts, if translated into lower protein levels, might produce a subtle pathology that could be compatible with BPD.

In contrast to the decreased expression of CK mRNA observed in our study in the brain, *increased* levels of CK protein were observed in cerebrospinal fluid and serum of BPD patients immediately after an acute episode (49–51). Increased serum levels of CK protein are likely the muscle isoform and may indicate muscle damage (52, 53); however, studies have shown that isometric muscle tension cannot account for the large spike in serum CK protein levels following an elevated state in BPD and SZ (54). Increased CNS levels of CK protein might indicate cell death in the brain (49, 55–57). Our data cannot refute a hypothesis of neuronal cell death in the HIP or loss of glia in the DLPFC leading to increased CK protein during an acute episode, and decreased levels of CK mRNA due to lower cell numbers after the episode and in the euthymic state. A reduction in glial cell population would also have an adverse effect on the Cr shuttle system, since only

oligodendrocytes and astroglia express the enzyme responsible for Cr synthesis, suggesting that glia supplies neurons with Cr (20).

However, cell death is not a satisfactory explanation for reduced CK mRNA levels. First, the particular distribution of CKB and CKMt1 between neurons and glia would suggest that a loss of neurons should be mostly evident as a loss of CKMt1 transcripts, whereas a loss of glia would be reflected in a loss of CKB transcripts. Loss of one cell type would lead to a relative increase in the remaining cell type in a given sample, thus decrease in one CK type should lead to an increase in the other. We did see both isoforms reduced in both brain areas. Second, the level of only one of two neuron-specific genes, MAP2, was reduced in the HIP, thus providing weak support for cell death in this brain area. While we cannot exclude that cell death might be contributing to the reduced transcript levels of CK isoforms, the data point more likely to a generalized disturbance of energy regulation in BPD.

Although we took great care in matching the samples, human postmortem studies offer only limited experimental control. We excluded subjects who died under respiratory distress, were on a ventilator or had otherwise prolonged agonal events, but variability in mode of death beyond our control could have influenced the results. Disease-specific treatment is another concern: because BPD patients were treated with mood stabilizers, we examined in a rat model if lithium treatment affects transcript levels of CK isoforms in the HIP. Lithium treatment did not affect CK transcript levels nor transcript levels of neuronal or glial genes, although we did find a downregulation of ERBB3. However, ERBB3 was represented by two different probe sets on the array, only one of which was changed. Because of this discrepancy, because ERBB3 was only one of five glial-specific transcripts, and because ERBB3 was not altered in the human HIP, we conclude that treatment with mood stabilizers is not responsible for the observed downregulations of glial- and neuron-specific transcripts, nor of CK.

Our data lend further support to altered energy systems in BPD. Whether this change is cause or consequence of the disorder cannot be deduced from the results. However, the question of cause and consequence might not be of major significance for treatment strategies, since a negative impact, be it primary or secondary, would always benefit from treatment. In conclusion, the 'energy hypothesis of BPD' deserves closer scrutiny and further examination as it has the potential to alter therapeutic approaches to the disease.

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Fig. 1.

Gene expression microarray data from the human hippocampus of (A) bipolar disorder (BPD) and (B) schizophrenia (SZ) subjects. CKB is represented twice on the HG-U95Av2 array and identified by the respective probe set number. Relative mRNA levels in percentage of control are shown as mean $\pm$ SEM. * $p < 0.05$.

Fig. 2.

Real-time quantitative polymerase chain reaction data verified the gene expression microarray findings in the hippocampus in (A) bipolar disorder (BPD) and (B) schizophrenia (SZ) subjects. The downregulation of CK isoforms was furthermore extended to the dorsolateral prefrontal cortex (DLPFC) in BPD subjects (C). No significant downregulation was observed in the DLPFC in SZ subjects (D) with the sample size available. Relative mRNA levels of experiment (BPD or SZ corrected for internal standard, filamin A) over control (corrected for internal standard) are shown as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

Fig. 3.

Real-time quantitative polymerase chain reaction experiments show that (A) the level of the MAP2 transcript, a gene specifically expressed in neurons, is downregulated in the hippocampus in bipolar disorder (BPD) subjects. Doublecortin, another neuron-specific transcript, is unchanged. (B) Neuronal markers are expressed at control level in the dorsolateral prefrontal cortex (DLPFC). The shaded table in (A) shows the gene expression data from the microarray study in the hippocampus. MAP2 was represented twice on the array, and one probe set was significantly downregulated. Relative mRNA levels of BPD (corrected for the internal standard, filamin A) over control (corrected for internal standard) are shown as mean $\pm$ SEM. * $p < 0.05$.

Fig. 4.

Real-time quantitative polymerase chain reaction experiments were carried out for glial transcripts. (A) A downregulation of gelsolin was observed in the hippocampus, but other markers were unchanged. (B) Three of four markers were significantly downregulated in the dorsolateral prefrontal cortex (DLPFC) in bipolar disorder (BPD) subjects. The shaded table in (A) shows the gene expression data from the microarray study in the hippocampus. Relative mRNA levels of BPD (corrected for the internal standard, filamin A) over control (corrected for internal standard) are shown as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

Demographics of the subjects used in the study

Table 1

Case no.	Sex /age	Diagnosis	Hemisphere	Postmortem interval (h)	Cause of death	Chlorpromazine equivalents (mg)	Psychoactive medication use	qPCR brain area	pH cerebellum	Hippocampus microarray data				
										3'/5' GAPDH	3'/5' B-Actin	Present calls	Scaling factor	Back-ground level 28S /18S
4605	M/29	NA (control)	L	18.2	Renal failure	NA	NA	HIP, PFC	7.1	2	2.5	43.4	2.5	51
4737	F/74	NA (control)	L	12.5	Renal failure	NA	NA	HIP, PFC	6.3	3.4	3.6	37.3	3.82	49
4751	M/54	NA (control)	L	24.2	Cardiac arrest	NA	NA	HIP, PFC	6.5	2.3	2.7	47.1	1.85	56
5074	M/79	NA (control)	R	20.9	Pancreatic cancer	NA	NA	HIP, PFC	6.7	1.8	2.6	48.3	2	46
3806	F/70	NA (control)	R	15	Cardiac arrest	NA	NA	HIP	6.6	2.2	3.2	45.1	2.27	49
3898	F/78	NA (control)	R	14.1	Myocardial infarction	NA	NA	HIP, PFC	6.2	3.1	3.9	45	2.16	50
5082	F/78	NA (control)	R	23.9	Breast cancer	NA	NA	HIP, PFC	6.7	1.9	2.1	45.4	2.18	65
4853	F/70	NA (control)	L	22.5	Liver cancer	NA	NA	PFC	6.3	2	2.6	43.3	3.1	45
4932	M/67	NA (control)	R	22.3	Cardiac arrest	NA	NA	PFC	6.4	1.5	2	47.4	1.74	47
4810	F/62	NA (control)	L	16.4	Lung cancer	NA	NA	PFC	nd	1.6	2.1	45.9	2.39	46
4069	F/80	Bipolar disorder	L	11.6	Cerebrovascular accident	67	Perphenazine, benzotropine, valproate	HIP, PFC	6.5	2	2.6	44.9	1.98	49
4403	F/76	Bipolar disorder	L	22.8	Cardiopulmonary failure	0	Lithium carbonate	HIP, PFC	6.6	2.5	3	41.3	3.49	46
4462	M/50	Bipolar disorder	R	30.5	Cardiac arrest	NA	?	HIP, PFC	6.4	3.4	3.8	42	2.7	50
4661	M/25	Bipolar disorder	L	12.6	Pulmonary edema	0	Sertraline, trazodone, gabapentin, lithium	HIP, PFC	6.7	2.6	2.7	39	3.01	52
3817	F/64	Bipolar disorder	R	11	Respiratory failure	800	Trifluoperazine	HIP, PFC	6.7	2.1	2.5	46.3	2.25	46
4464	M/74	Bipolar disorder	L	24.8	Pneumonia	285	Divalproex sodium, quetiapine	HIP, PFC	6.5	2.8	3.3	39.1	5.61	39
4961	M/74	Bipolar disorder	R	14.3	Pneumonia	0	Lithium, divalproex sodium	HIP, PFC	6.3	2.6	3.3	42.2	2.55	44
4914	F/73	Bipolar disorder	R	20.8	Sepsis	33	Risperidone, carbamazepine	PFC	6.3	2.2	2.5	42.9	2.85	48
5044	F/73	Bipolar disorder	R	17	Renal failure	133	Lithium	PFC	6.4	2.7	3.5	28.6	3.43	52
4190	F/78	Schizoaffective disorder	L	13.4	Sinus node disease	1066	Lithium, haloperidol	HIP, PFC	6.8	2.7	3.6	44.1	2.02	46
4875	F/55	Schizoaffective disorder	R	18	Cancer	200	Divalproex sodium, intramuscular fluphenazine	HIP, PFC	6.5	1.7	1.7	47.7	2.22	46
5047	M/63	Schizophrenia	R	22.3	Cardiac arrest	532	Clozapine, clonazepam	HIP, PFC	nd	2.4	3.8	40	3.12	52
5100	F/72	Schizophrenia	R	21.7	Cancer	267	Risperidone, benzotropine, paroxetine	HIP, PFC	6.7	1.5	2	47.4	2.1	49
4469	M/80	Schizophrenia	L	11	Cardiopulmonary failure	10	Thioridazine	HIP, PFC	6.4	2.5	3.4	47.1	1.96	49
4907	F/73	Schizoaffective disorder	R	24	Lung cancer	600	Prolixin	HIP, PFC	6.1	3	2.8	38	3.82	47
4238	M/26	Schizophrenia	R	16	Suicide by hanging	357	Prolixin decanoate	HIP, PFC	6.8	1.6	1.6	47.4	1.36	51

Case no.	Sex /age	Diagnosis	Hemisphere	Postmortem interval (h)	Cause of death	Chlorpromazine equivalents (mg)	Psychoactive medication use	qPCR brain area	Hippocampus microarray data						
									pH cerebellum	3'/5' GAPDH	3'/5' B-Actin	Present calls	Scaling factor	Back-ground level	28S /18S
5115	M/49	Schizophrenia	L	24.5	Acute respiratory failure	1066	Haloperidol decanoate	PFC	nd	1.5	1.6	47.1	1.8	48	1.22
Summary															
Experiment	Sex/age	Hemisphere	Postmortem interval (h)*	Storage time (W)*	pH cerebellum*	3'/5' GAPDH*	3 '/5/ B-Actin *	Present calls*	Scaling factor*	Background level*	28S/18S*				
PFC qPCR															
Control	4M, 5F/66 ± 5	5L, 4R	19.4 ± 1.4	219.6 ± 17.5	6.53 ± 0.10										
Bipolar disorder	4M, 5F/65 ± 6	5L, 4R	18.4 ± 2.3	253.0 ± 20.8	6.49 ± 0.05										
Schizophrenia	4M, 4F/62 ± 6	3L, 5R	18.9 ± 1.8	228.4 ± 19.9	6.55 ± 0.11										
HIP qPCR															
Control	3M, 4F/66 ± 7	3L, 4R	18.4 ± 1.8	246.5 ± 28.9	6.59 ± 0.11										
Bipolar disorder	4M, 3F/63 ± 7	4L, 3R	18.2 ± 2.9	272.7 ± 21.1	6.53 ± 0.06										
Schizophrenia	3M, 4F/64 ± 7	2L, 5R	18.1 ± 1.8	237.6 ± 20.4	6.55 ± 0.11										
Microarray															
Control	4M, 6F/66 ± 5	5L, 5R	19.0 ± 1.4	129.1 ± 20.9	6.53 ± 0.09	2.2 ± 0.2	2.7 ± 0.2	44.8 ± 1.0	2.4 ± 0.2	50.4 ± 1.9	1.1 ± 0.1				
Bipolar disorder	4M, 5F/65 ± 6	5L, 4R	18.4 ± 2.3	148.6 ± 20.8	6.49 ± 0.05	2.5 ± 0.1	3.0 ± 0.2	40.7 ± 1.7	3.1 ± 0.4	47.3 ± 1.4	0.8 ± 0.1				
Schizophrenia	4M, 4F/62 ± 6	3L, 5R	18.9 ± 1.8	124.0 ± 19.9	6.55 ± 0.11	2.1 ± 0.2	2.6 ± 0.3	44.9 ± 1.4	2.3 ± 0.3	48.5 ± 0.8	1.1 ± 0.1				

HIP = hippocampus; PFC = prefrontal cortex; qPCR = quantitative real-time PCR.

* Average ± SEM.

Table 2

Accession numbers of human genes evaluated in the study

Common name	Abbreviation	Accession number
Creatine kinase, brain	CKB	NM_001823
Creatine kinase, mitochondrial 1	CKMt1	NM_020990
Doublecortin	DCX	NM_000555
Erythroblastic leukemia viral oncogene homolog 3	ERBB3	NM_001982
Gelsolin	GSN	NM_000177
Glial fibrillary acidic protein	GFAP	NM_002055
Filamin A, alpha (actin-binding protein 280)	FLNA	NM_001456
Microtubule-associated protein 2	MAP2	NM_031845
Myelin-associated glycoprotein	MAG	NM_002361
Myelin-associated oligodendrocyte basic protein	MOBP	NM_006501

Table 3

Gene expression microarray results in rat HIP with lithium chow for 15 days. Transcripts with more than one probe set on the array (RAE230A) are shown in all their representations.

Locus Link ID	Gene name	Affymetrix ID	Fold change	p-value	P call % (control)	P call % (lithium)
24264	Creatine kinase, brain	1367740_at	1.1	0.239497	100	100
29593	Creatine kinase, mitochondrial 1, ubiquitous	1390566_a_at	1	0.991371	100	100
84394	Doublecortin	1374966_at	-1.04	0.651172	12	0
84394	Doublecortin	1387601_at	-1.04	0.319053	0	0
25595	Microtubule-associated protein 2 (MAP2)	1368411_a_at	-1.22	0.202223	100	100
25595	Microtubule-associated protein 2 (MAP2)	1388152_at	-1.15	0.149025	100	100
296654	Gelsolin	1371414_at	1.04	0.630259	100	100
24387	Glial fibrillary acidic protein (GFAP)	1368353_at	1.06	0.439748	100	100
24547	Myelin basic protein (MBP)	1368810_a_at	-1.09	0.110849	100	100
24547	Myelin basic protein (MBP)	1387341_a_at	-1.11	0.413766	100	100
29409	Myelin-associated glycoprotein (MAG)	1368861_a_at	-1.14	0.213108	100	100
29496	ERBB3	1369088_at	-1.07	0.367392	25	0
29496	ERBB3	1377821_at	-1.3	0.003336	100	100