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ORIGINAL ARTICLE

Expression of $GABA_A$ α2-, β1- and ε-receptors are altered significantly in the lateral cerebellum of subjects with schizophrenia, major depression and bipolar disorder

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There is abundant evidence that dysfunction of the γ-aminobutyric acid (GABA)ergic signaling system is implicated in the pathology of schizophrenia and mood disorders. Less is known about the alterations in protein expression of GABA receptor subunits in brains of subjects with schizophrenia and mood disorders. We have previously demonstrated reduced expression of $GABA_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) in the lateral cerebella of subjects with schizophrenia, bipolar disorder and major depressive disorder. In the current study, we have expanded these studies to examine the mRNA and protein expression of 12 $GABA_A$ subunit proteins (α1, α2, α3, α5, α6, β1, β2, β3, δ, ε, γ2 and γ3) in the lateral cerebella from the same set of subjects with schizophrenia ($N = 9$–15), bipolar disorder ($N = 10$–15) and major depression ($N = 12$–15) versus healthy controls ($N = 10$–15). We found significant group effects for protein levels of the α2-, β1- and ε-subunits across treatment groups. We also found a significant group effect for mRNA levels of the α1-subunit across treatment groups. New avenues for treatment, such as the use of neurosteroids to promote GABA modulation, could potentially ameliorate GABAergic dysfunction in these disorders.

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INTRODUCTION

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and regulates multiple processes during the brain development. Approximately 20% of all central nervous system neurons are GABAergic.[1] Hypofunction of the GABAergic signaling system has been hypothesized to contribute to the pathologies of schizophrenia, bipolar disorder and major depressive disorder.[2–5] Multiple laboratories have demonstrated a number of dysfunctions of the GABAergic signaling system in these disorders, including: (1) altered expression of glutamic acid decarboxylase 65 and 67 kDa proteins,[6–8] the enzymes that convert glutamate to GABA; (2) microarray results that have demonstrated increased mRNA for a number of GABA(A) ($GABA_A$) receptor subunits in prefrontal cortex (PFC) of subjects with schizophrenia;[9–11] and (3) gene association studies that link GABA receptor subunits to schizophrenia and mood disorders.[12–14]

Structural and functional abnormalities of the cerebellum have been described for schizophrenia, depression and bipolar disorder, including reduced cerebellar volumes.[15–17] Reduced cerebellar activation has also been observed in functional imaging studies of subjects with these disorders.[18–23] There is abundant evidence that the cerebellum has roles in cognition and emotion.[15,20,24] Circuits connecting the cerebellum with other brain regions, such as the cortico-thalamic-cerebellar-cortical circuit, which may monitor execution of mental activity, have also shown disruption in schizophrenia.[24–27]

There is also evidence of GABAergic hypofunction in the cerebella of subjects with schizophrenia and mood disorders.[6] Glutamic acid decarboxylase 65 and 67 kDa proteins have been shown to be reduced in the lateral cerebella of subjects with schizophrenia, bipolar disorder and major depressive disorder.[6] In the granule cell layer of the cerebellum, Bullock *et al.*[28] found reduced mRNA for glutamic acid decarboxylase 65 and 67 kDa along with increased expression of mRNA for $GABA_A$ receptor α6- and δ-subunits. Finally, reduced protein expression of $GABA_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) has been observed in the lateral cerebella of subjects with schizophrenia, bipolar disorder and major depression.[29]

Although there have been some mRNA studies of $GABA_A$ receptor expression in subjects with schizophrenia,[28,30–33] there is a paucity of data regarding $GABA_A$ receptor protein expression in schizophrenia, bipolar disorder and major depressive disorder. Here we expand our previous work on the GABAergic signaling system in these disorders to investigate protein expression of 12 additional $GABA_A$ receptor subunits: GABRα1, GABRα2, GABRα3, GABRα5, GABRα6, GABRβ1, GABRβ2, GABRβ3, GABRδ, GABRε, GABRγ2 and GABRγ3. On the basis of our finding of significantly reduced $GABA_B$ receptor subunits in the lateral cerebella,[29] we hypothesized that we would observe reduced expression of multiple $GABA_A$ receptor subunits in the same brain region of subjects with schizophrenia, bipolar disorder and major depression.

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MATERIALS AND METHODS

Brain procurement

The Institutional Review Board of the University of Minnesota School of Medicine has approved this study. Post-mortem cerebella (lateral posterior lobe) were obtained from the Stanley Foundation Neuropathology Consortium under approved ethical guidelines. Diagnostic and Statistical Manual of Mental Disorders, fourth edition diagnoses were established before death by neurologists and psychiatrists by using information from all available medical records and from family interviews. Details regarding the subject selection, demographics, diagnostic process and tissue processing were collected by the Stanley Medical Research Foundation. The collection consisted of 9–15 subjects with schizophrenia, 10–15 subjects with bipolar disorder, 12–15 with major depression without psychotic features and 10–15 normal controls (Table 1). All groups were matched for age, sex, race, post-mortem interval and hemispheric side (Table 1).

SDS-polyacrylamide gel electrophoresis and western blotting

Brain tissue was prepared as previously described.[29,34–38] Thirty micrograms of the lateral cerebellum was used per lane. For all experiments, we used 10% resolving gels and 5% stacking gels. To minimize interblot variability, we included samples from subjects with schizophrenia, bipolar disorder, major depression and healthy controls on each gel, and all samples were run in duplicate. Samples were electrophoresed for 15 min at 75 V, followed by 60 min at 150 V. Samples were then electroblotted onto nitrocellulose membranes for 2 h at 300 mAmp at 4 °C. Blots were blocked with 0.2% I-Block (Tropix, Bedford, MA, USA) in phosphate-buffered saline supplemented with 0.3% Tween 20 for 1 h at room temperature followed by an overnight incubation in primary antibodies at 4 °C. The primary antibodies used were anti-GABRα1 (06–868; Millipore, Temecula, CA, USA; 1:1000), anti-GABRα2 (GAA21; Alpha Diagnostic International, San Antonio, TX, USA; 1:500), anti-GABRα3 (GAA31; Alpha Diagnostic International; 1:1000), anti-GABRα5 (AB10098; Abcam, Cambridge, MA, USA; 1:500), anti-GABRα6 (AB5610; Millipore; 1:250), anti-GABRβ1 (AB9680; Millipore; 1:500), anti-GABRβ2 (AB5561; Millipore; 1:500), anti-GABRβ3 (ab98968; Abcam; 1:5000), anti-GABRδ (AB37396; Abcam; 1:500), anti-GABRε (ab35971; Abcam; 1:1000), anti-GABRγ2 (NB-300-192; Novus Biologicals, Littleton, CO, USA; 1:500), anti-GABRγ3 (NB100-56662; Novus Biologicals; 1:500), anti-neuronal specific enolase (NSE) (ab16808; Abcam; 1:2000) and anti-β actin (A5441; Sigma Aldrich, St Louis, MO, USA; 1:5000). Following primary antibody incubation, blots were washed for 30 min in phosphate-buffered saline supplemented with 0.3% Tween 20 for 30 min at room temperature, and were subsequently incubated in the proper secondary antibodies. Secondary antibodies were goat anti-rabbit IgG (A9169; Sigma Aldrich; 1:80 000) or goat anti-mouse IgG (A9044; Sigma Aldrich; 1:80 000). Blots were probed together (three to four gels per experiment). Following secondary antibody incubation, blots were washed twice in phosphate-buffered saline supplemented with 0.3% Tween 20 for 15 min each. The immune complexes were then visualized using the ECL Plus detection system (GE Healthcare, Buckinghamshire, UK) and exposed to CL-Xposure film (Thermo Scientific, Rockford, IL, USA). The molecular weights of ~58 kDa (GABRβ3, upper band), 56 kDa (GABRβ3 lower band), 55 kDa (GABRα3, GABRβ1), 52 kDa (GABRα5, GABRβ2), 51 kDa (GABRα1, GABRα2, GABRδ, GABRγ3), 50 kDa (GABRα6), 46 kDa (NSE), 45 kDa (GABRε, GABRγ2) and 42 kDa (β-actin) immunoreactive bands were quantified with background subtraction using a Bio-Rad (Hercules, CA, USA) densitometer and Quantity One software (Bio-Rad). The molecular weight of GABRβ3 has been reported previously as anywhere from 52 kDa to 58 kDa.[34,35,39–43] Using Abcam antibody ab98968, we obtained a doublet of 56 kDa and 58 kDa, similar to results obtained by Bureau and Olsen[40] who identified a doublet of 55 kDa and 58 kDa. For this study, we decided to measure both bands. Sample densities were analyzed, blind to nature of diagnosis. Results obtained are based on at least two independent experiments.

Quantitative real-time PCR

Quantitative real-time PCR analysis was performed as previously described.[37] Raw data were analyzed as previously described,[37] using the Sequence Detection Software RQ Manager (ABI, Foster City, CA, USA), whereas relative quantitation using the comparative threshold cycle (C_T method) was performed in a Bioconductor using the ABqPCR package in Microsoft Excel (ABI Technote#2: Relative Gene Expression Quantitation). Calculations were done assuming that 1 delta C_t equals a twofold difference in expression. Significance values were determined using unpaired Student's *t*-tests. The probe IDs used were as follows: (1) GABRA1 (GABRα1): Hs0068058-m1; (2) GABRA2 (GABRα2): Hs00168069-m1; (3) GABRA3 (GABRα3): Hs00968132_m1; (4) GABRA5 (GABRα5): Hs00181291-m1; (5) GABRA6 (GABRα6): Hs00181301_m1; (6) GABRB1 (GABRβ1): Hs00181306_m1; (7) GABRB2 (GABRβ2): Hs00241451_m1; (8) GABRB3 (GABRβ3): Hs00241459-m1; (9) GABRD (GABRδ): Hs00181309_m1; (10) GABRE (GABRε): Hs00608332_m1; (11) GABRG2 (GABRγ2): Hs00168093_m1; (12) GABRG3 (GABRγ3): Hs00264276-m1; (13) β-actin: Hs99999903_m1; and (14) glyceraldehyde 3-phosphate dehydrogenase: Hs99999905_m1.

Table 1. Demographic information for the four diagnostic groups

	Bipolar	*Depression*	*Control*	*Schizophrenia*	*F or* χ^2-test	P-*value*
Age	42.33 (11.72)	46.53 (9.31)	48.07 (10.66)	44.53 (13.11)	0.73	0.54
Sex	6 F, 9 M	6 F, 9 M	6 F, 9 M	6 F, 9 M	0	1.0
Race	14 W, 1 B	15 W	14 W, 1 B	12 W, 3 A	14.7	0.10
PMI	32.53 (16.12)	27.47 (10.73)	23.73 (9.95)	33.67 (14.62)	1.85	0.15
pH	6.18 (0.23)	6.18 (0.22)	6.27 (0.24)	6.16 (0.26)	0.60	0.62
Side of brain	7 L, 8 R	9 L, 6 R	7 L, 8 R	9 L, 6 R	0.85	0.84
Brain weight	1441.2 (171.5)	1462 (142.1)	1501 (164.1)	1471.7 (108.2)	0.42	0.74
Family history	0.93 (0.8)	0.73 (0.46)	0.13 (0.52)	1.13 (0.83)	29.84	**0.0001**
Suicidal death	9 (5 Violent)	9 (4 Violent)	0	6 (2 Violent)	15.9	**0.014**
Drug/alcohol history	0.8 (0.77)	0.4 (0.63)	0.33 (0.72)	0.53 (0.74)	6.42	0.38
Age of onset	21.47 (8.35)	33.93 (13.29)	—	23.2 (7.96)	3.61	**0.001**
Duration	20.13 (9.67)	12.67 (11.06)	—	21.67 (11.24)	0.034	0.86
Severity of substance abuse	1.93 (1.98)	1.07 (1.98)	0.13 (0.52)	1.20 (1.86)	2.82	**0.046**
Severity of alcohol abuse	2.27 (1.98)	1.8 (2.01)	1.07 (1.03)	1.47 (1.59)	1.34	0.27
Fluphenazine (lifetime)	20,826.67 (24,015.96)	—	—	52,266.67 (62,061.57)	3.35	0.078
Alcohol dependence	13.3%	13.3%	0%	0%	4.29	0.23
Alcohol abuse	6.7%	6.7%	0%	0%	1.05	0.79
Substance dependence	6.7%	0%	0%	6.7%	2.07	0.56
Substance abuse	20.0%	6.7%	0%	6.7%	4.15	0.25
Antidepressant use	53.3%	60.0%	0%	33.3%	14.1	**0.003**

Abbreviations: A, Asian; B, Black; F, female; L, left; M, male; PMI, postmortem interval; R, right; W, Whites.
Rating scale for drug/alcohol history: 0, never; 1, current; 2, past. Rating scale for severity of substance abuse: 0, little/none; 1, social; 2, moderate use/past; 3, moderate use/present; 4, heavy use/past; 5, heavy use/present. Rating scale for severity of alcohol abuse: 0, little/none; 1, social (one to two drinks per day); 2, moderate use/past; 3, moderate use/present; 4, heavy use/past; 5, heavy use/present.

Table 2. Western blotting results for GABA$_A$ receptor subunits expressed as a ratio of β-actin in the lateral cerebella

	ANOVA		*Control*		*Schizophrenia*		*Bipolar disorder*		*Major depression*	
Lateral cerebellum	*F-test*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*
GABRα1/β-actin	1.46	NC	0.331 ± 0.127	RG	0.507 ± 0.311	NC	0.474 ± 0.266	NC	0.486 ± 0.252	**0.049**
GABRα2/β-actin	3.35	**0.027**	0.126 ± 0.083	RG	0.370 ± 0.282	**0.0046**	0.321 ± 0.273	**0.017**	0.271 ± 0.154	**0.0047**
GABRα3/β-actin	0.46	NC	0.196 ± 0.076	RG	0.239 ± 0.159	NC	0.236 ± 0.161	NC	0.193 ± 0.14	NC
GABRα5/β-actin	2.02	NC	0.031 ± 0.02	RG	0.063 ± 0.067	NC	0.04 ± 0.03	NC	0.033 ± 0.018	NC
GABRα6/β-actin	1.76	NC	0.013 ± 0.003	RG	0.015 ± 0.007	0.23	0.016 ± 0.009	NC	0.019 ± 0.005	**0.001**
GABRβ1/β-actin	6.03	**0.001**	0.135 ± 0.049	RG	0.066 ± 0.031	**0.0001**	0.092 ± 0.048	**0.026**	0.092 ± 0.047	**0.023**
GABRβ2/β-actin	1.56	NC	0.035 ± 0.013	RG	0.030 ± 0.016	NC	0.025 ± 0.008	**0.025**	0.034 ± 0.016	NC
GABRβ3 (upper) /β-actin	2.01	NC	0.114 ± 0.136	RG	0.058 ± 0.063	NC	0.152 ± 0.084	NC	0.109 ± 0.103	NC
GABRβ3 (lower) /β-actin	0.77	NC	0.57 ± 0.16	RG	0.59 ± 0.14	NC	0.60 ± 0.19	NC	0.50 ± 0.21	NC
GABRδ/β-actin	0.46	NC	0.032 ± 0.017	RG	0.034 ± 0.015	NC	0.027 ± 0.021	NC	0.034 ± 0.017	NC
GABRε/β-actin	8.88	**0.0001**	0.015 ± 0.007	RG	0.035 ± 0.029	**0.044**	0.041 ± 0.018	**0.0006**	0.079 ± 0.046	**0.0003**
GABRγ2/β-actin	0.74	NC	0.016 ± 0.008	RG	0.015 ± 0.009	NC	0.012 ± 0.005	NC	0.012 ± 0.006	NC
GABRγ3/β-actin	1.53	NC	0.099 ± 0.052	RG	0.114 ± 0.059	NC	0.137 ± 0.072	NC	0.141 ± 0.055	**0.047**
β-actin	0.83	NC	25.8 ± 2.07	RG	25.2 ± 2.07	NC	26.9 ± 2.91	NC	26.1 ± 4.38	NC

Abbreviations: ANOVA, analysis of variance; GABA$_A$, γ-aminobutyric acid (A); NC, no change; RG, reference group.
Bold entries are significant *P* values.

Statistical analysis

All protein measurements for each group were normalized against β-actin and NSE (Tables 2 and 3) and were expressed as ratios. Statistical analysis was performed as previously described,[29,36,38] with $P<0.05$ considered significant. Group comparisons were conducted using analysis of variance (ANOVA). Follow-up independent Student's *t*-tests were then conducted as well. Group differences on possible confounding factors were explored using χ^2-tests for categorical variables and ANOVA for continuous variables. Where group differences were found, analysis of covariance was used to explore these effects on group differences for continuous variables and factorial ANOVA with interaction terms for categorical variables. All analyses were conducted using SPSS v.17 (SPSS, Chicago, IL, USA).

RESULTS

All protein measurements were normalized against β-actin and NSE (Figure 1). ANOVA identified group differences for GABRα2/β-actin ($F(3,52)=3.35$, $P<0.027$), GABRα2/NSE ($F(3,52)=3.49$, $P<0.022$), GABRβ1/β-actin ($F(3,54)=6.03$, $P<0.001$), GABRβ1/NSE ($F(3,54)=4.53$, $P<0.007$), GABRε/β-actin ($F(3,37)=8.88$, $P<0.0001$) and GABRε/NSE ($F(3,37)=7.26$, $P<0.001$) (Tables 2 and 3; Figures 1–4). In subjects with schizophrenia, follow-up Student's *t*-tests found significantly increased expression of GABRα2/β-actin ($P<0.0046$), GABRα2/NSE ($P<0.0042$) and GABRε/β-actin ($P<0.044$) (Tables 2 and 3; Figures 1, 2 and 4), and significantly reduced expression of GABRβ1/β-actin ($P<0.0001$) and GABRβ1/NSE ($P<0.001$) (Tables 2 and 3; Figures 1 and 3).

In subjects with bipolar disorder, follow-up Student's *t*-test found significantly increased expression of GABRα2/β-actin ($P<0.017$), GABRα2/NSE ($P<0.011$), GABRε/β-actin ($P<0.0006$) and GABRε/NSE ($P<0.0013$) (Tables 2 and 3; Figures 1 and 4), and significantly reduced expression of GABRβ1/β-actin ($P<0.026$) and GABRβ1/NSE ($P<0.034$) (Tables 2 and 3; Figures 1 and 3). We also observed significantly reduced expression of GABRβ2/β-actin ($P<0.025$) and GABRβ2/NSE ($P<0.022$) in the cerebella of subjects with bipolar disorder (Tables 2 and 3; Figures 1 and 3).

In subjects with major depressive disorder, follow-up Student's *t*-tests found significantly upregulated expression of GABRα2/β-actin ($P<0.0047$), GABRα2/NSE ($P<0.0063$), GABRε/β-actin ($P<0.0003$) and GABRε/NSE ($P<0.0012$) (Tables 2 and 3; Figures 1, 2, and 4), and significantly reduced expression of GABRβ1/β-actin ($P<0.023$) and GABRβ1/NSE ($P<0.03$) (Tables 2 and 3; Figures 1 and 3). We found that GABRα1/β-actin expression was significantly increased in the cerebella of subjects with major depression ($P<0.049$) (Table 2; Figures 1 and 2). In addition, there were significantly increased expression for GABRγ3/β-actin ($P<0.047$; Table 2; Figures 1 and 5), GABRα6/β-actin ($P<0.001$) and GABRα6/NSE ($P<0.0023$) (Tables 2 and 3; Figures 1 and 2).

No significant differences were found between diagnostic groups on hemisphere side, ethnicity, history of substance abuse, gender, severity of alcohol abuse, brain weight, post-mortem interval, age, or pH. We did find that subjects with bipolar disorder had significantly higher levels of severity of substance use than did normal controls ($P<0.046$). We also compared the three diagnostic groups on family history and suicide, and found a significant increased rate of suicide among the psychiatric groups when compared with controls ($P<0.0001$ and $P<0.014$, respectively). Age of onset was significantly later (33.9 years) for depressed subjects when compared with schizophrenics (23.2) and bipolar subjects (21.5), ($P<0.001$). Finally, antidepressant use was significantly different between the four groups ($P<0.003$). ANOVAs controlling for hemisphere side, ethnicity, history of substance abuse, severity of substance abuse, gender, severity of alcohol abuse, brain weight, post-mortem interval, age or pH found no meaningful or significant impact on the results reported above.

When we controlled for antidepressant use, we lost significance for GABRα2/β-actin in subjects with major depression ($P<0.068$); GABRβ1/β-actin in subjects with bipolar disorder ($P<0.46$); GABRβ2/β-actin in subjects with bipolar disorder ($P<0.061$); GABRγ3/β-actin in subjects with major depression ($P<0.25$); and GABRβ1/NSE in subjects with major depression ($P<0.15$) (Supplementary Table 1). However, we found no significant differences between values for individuals taking antidepressants versus those not taking antidepressants within each diagnostic group for GABRα2/β-actin, GABRβ2/β-actin, GABRγ3/β-actin and GABRβ1/NSE ($P<0.74$, $P<0.98$, $P<0.68$; and $P<0.76$, respectively), suggesting that antidepressant use had no real impact on these measures (Supplementary Table 2). Subjects with bipolar disorder, who took antidepressants, had significantly lower protein levels of GABRβ1/β-actin when compared with subjects with bipolar disorder, who did not take antidepressants ($t(12)=2.47$, $P<0.030$), suggesting that in this case antidepressant use was partially responsible for the reduction in GABRβ1/β-actin (Supplementary Table 2). However, the GABRβ1/NSE ratio continued to be significantly lower in the bipolar group ($P<0.034$) and was not affected by the antidepressant confound.

Table 3. Western Blotting Results for GABA$_A$ receptor subunits expressed as a ratio of NSE in lateral cerebella

	ANOVA		*Control*		*Schizophrenia*		*Bipolar disorder*		*Major depression*	
Lateral cerebellum	*F-test*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*	*Protein*	P-*value*
GABRα1/NSE	1.19	NC	0.45 ± 0.19	RG	0.60 ± 0.28	NC	0.60 ± 0.28	NC	0.56 ± 0.21	NC
GABRα2/NSE	3.49	**0.022**	0.14 ± 0.09	RG	0.32 ± 0.19	**0.0042**	0.28 ± 0.17	**0.011**	0.31 ± 0.19	**0.0063**
GABRα3/NSE	0.81	NC	0.25 ± 0.11	RG	0.25 ± 0.17	NC	0.29 ± 0.16	NC	0.21 ± 0.10	NC
GABRα5/NE	2.00	NC	0.036 ± 0.023	RG	0.071 ± 0.076	NC	0.043 ± 0.029	NC	0.037 ± 0.020	NC
GABRα6/NSE	1.79	NC	0.026 ± 0.007	RG	0.037 ± 0.023	NC	0.031 ± 0.018	NC	0.040 ± 0.011	**0.0023**
GABRβ1/NSE	4.53	**0.007**	0.17 ± 0.07	RG	0.084 ± 0.048	**0.001**	0.11 ± 0.064	**0.034**	0.11 ± 0.066	**0.03**
GABRβ2/NSE	1.35	NC	0.039 ± 0.015	RG	0.036 ± 0.020	NC	0.028 ± 0.007	**0.022**	0.035 ± 0.013	NC
GABRβ3 (upper) /NSE	1.97	NC	0.11 ± 0.13	RG	0.063 ± 0.069	NC	0.16 ± 0.10	NC	0.11 ± 0.098	NC
GABRβ3 (lower) /NSE	1.80	NC	0.63 ± 0.13	RG	0.65 ± 0.13	NC	0.57 ± 0.11	NC	0.55 ± 0.13	NC
GABRδ/NSE	1.46	NC	0.038 ± 0.022	RG	0.046 ± 0.026	NC	0.028 ± 0.016	NC	0.038 ± 0.019	NC
GABRε/NSE	7.26	**0.001**	0.017 ± 0.009	RG	0.037 ± 0.032	NC	0.041 ± 0.017	**0.0013**	0.084 ± 0.056	**0.0012**
GABRγ2/NSE	0.40	NC	0.03 ± 0.014	RG	0.029 ± 0.018	NC	0.024 ± 0.010	NC	0.029 ± 0.021	NC
GABRγ3/NSE	1.33	NC	0.12 ± 0.06	RG	0.13 ± 0.08	NC	0.17 ± 0.11	NC	0.16 ± 0.07	NC
NSE	0.93	NC	20.2 ± 2.34	RG	20.1 ± 1.67	NC	20.2 ± 3.34	NC	21.6 ± 2.87	NC

Abbreviations: ANOVA, analysis of variance; GABA$_A$, γ-aminobutyric acid (A); NC, no change; NSE, neuronal specific enolase; RG, reference group.
Bold entries represent significant *P* values.

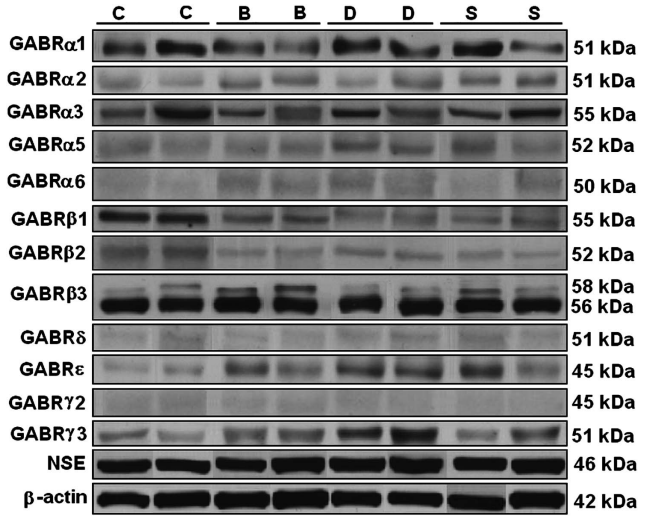

Figure 1. Representative bands for GABRα1, GABRα2, GABRα3, GABRα5, GABRα6, GABRβ1, GABRβ2, GABRβ3, GABRδ, GABRε, GABRγ2, GABRγ3, NSE and β-actin in the lateral cerebellum of subjects with schizophrenia and mood disorders.

We found that alcohol dependence, alcohol abuse, substance dependence and substance abuse did not impact any of our data (Table 1). However, as an additional analysis, we removed subjects with alcohol dependence/abuse or subjects with substance dependence/abuse, and reanalyzed the data. When individuals with substance abuse were removed, significance was lost for GABRγ3/β-actin ($P<0.072$) in subjects with major depression and GABRβ1/NSE ($P<0.063$) in subjects with bipolar disorder (Supplementary Table 3). When individuals with substance dependence were removed, none of the values lost significance (Supplementary Table 4). When individuals with alcohol abuse were removed, significance was lost for GABRα1/β-actin ($P<0.076$) and GABRγ3/β-actin ($P<0.072$) in subjects with major depression and GABRβ1/NSE ($P<0.061$) in subjects with bipolar disorder (Supplementary Table 5). Finally, when individuals with alcohol dependence were removed, all values for individuals with schizophrenia and bipolar disorder remained significant, whereas in subjects with major depression significance was lost for GABRα1/β-actin ($P<0.063$), GABRβ1/β-actin ($P<0.061$), GABRβ1/NSE ($P<0.063$) and GABRγ3/β-actin ($P<0.061$) (Supplementary Table 6). However, as none of the above confound effects were significant, the above changes are not deemed meaningful.

We performed quantitative real-time PCR to investigate changes in mRNA for the 12 GABA$_A$ receptor subunits (Table 4). ANOVA found a significant group difference for GABRA1 (GABRα1; $P<0.012$) with significantly reduced mRNA for GABRA1 in the lateral cerebella of subjects with schizophrenia ($P<0.011$) and major depression ($P<0.009$; Table 4). In subjects with schizophrenia, we also observed a significant reduction in mRNA for GABRA2 (GABRα2) ($P<0.017$) and a significant increase in mRNA for GABRB3 (GABRβ3; Table 4) ($P<0.044$).

DISCUSSION

The salient, significant findings for this work include the following: (1) novel significant increases in protein levels for ε- and α2-receptors in schizophrenia, bipolar disorder and major depression; (2) novel significant decreases in protein levels for β1-receptor in all three disorders; (3) significant increases in protein levels for α1-, α6- and γ3-receptors in major depression; (4) significant decrease in protein level for β2-receptor in bipolar disorder; (5) significant decrease in mRNA for α2 and increase in β3 in schizophrenia; (6) significant decrease in mRNA for α1 in subjects with schizophrenia and major depression; and (7) absence of any major confound effects on obtained protein and mRNA results, with the exception of antidepressant use on protein levels of GABRβ1/β-actin in subjects with bipolar disorder.

In rat brain, GABRα2 mRNA is distributed in multiple regions, including the neocortex, hippocampus, hypothalamus and cerebellum.[44,45] In the cerebellum, GABRα2 mRNA was identified on Bergman glial cells.[44–46] We identified significantly increased expression of GABRα2 protein in all three groups, whereas subjects with schizophrenia displayed decreased expression of GABRα2 mRNA. The decreased expression of GABRα2 mRNA may indicate a potential feedback loop effect. Consistent with our findings, GABRα2 mRNA and protein has been observed to be upregulated in postsynaptic pyramidal cell membranes in the dorsolateral PFC (DLPFC) of subjects with schizophrenia.[47] It has been hypothesized that this increased expression is a compensatory response to deficits in GABA synthesis in presynaptic chandelier subclass of GABAergic neurons.[48] In a recent study of cross-frequency modulation in subjects with

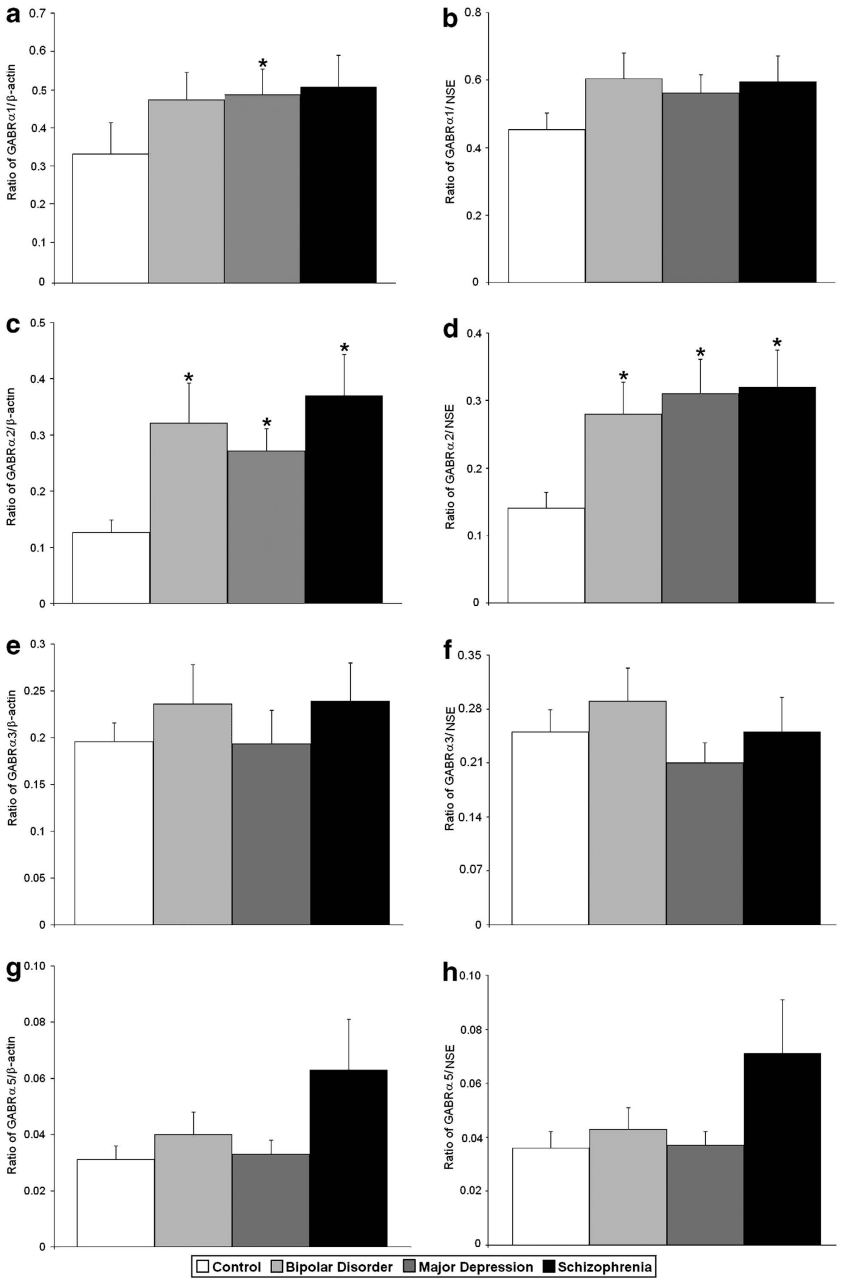

Figure 2. Expression of GABRα1/β-actin (**a**), GABRα1/NSE (**b**), GABRα2/β-actin (**c**), GABRα2/NSE (**d**), GABRα3/β-actin (**e**), GABRα3/NSE (**f**), GABRα5/β-actin (**g**) and GABRα5/NSE (**h**) in the lateral cerebella of healthy control subjects versus subjects with bipolar disorder, major depressive disorder and schizophrenia. Histogram bars shown as mean ± s.e., $*P < 0.05$.

schizophrenia versus matched controls, greater 'aberrant' fronto-temporal modulation observed in patients with schizophrenia was correlated with polymorphisms of the *GABRA2* (α2) gene.[49] Moreover, recent studies have shown that positive modulators of GABRα2 can improve working memory in a monkey model of schizophrenia[50] and in humans.[51] However, a separate study found no benefit of the GABRα2-positive modulator MK-0777 for patients with schizophrenia on tests of working memory.[52]

The GABRα2 gene (*GABRA2*), which is localized to 4q13–p12,[53] has been associated with risk for alcohol dependence[54,55] and drug abuse.[56–58] Although we did not find a significant effect of severity of alcohol abuse, or history of alcohol or substance abuse, we did observe a significant difference for severity of substance abuse in subjects with bipolar disorder. Others have suggested that altered expression of GABRα2 may help explain comorbid substance abuse in subjects with schizophrenia.[2] To date, there are no reports of an association between *GABRA2* with bipolar disorder or major depression. However, a recent set of experiments comparing *GABRA2* heterozygous and homozygous knockout mice with wild–type mice have found that males lacking the α2-subunit displayed depressive symptoms during the forced swimming test, the novelty suppressed-feeding test and the tail suspension test.[59] These results have led the authors to conclude that GABAergic inhibition acting through receptors that include the α2-subunit has a potential antidepressant-like effect.[59]

The gene for GABRε clusters at Xq28 (Table 5) with genes for the α3- and θ-subunits.[60] mRNA for the ε-subunit has been identified in the septum, thalamus, hypothalamus and amygdala in rat brain and was often coexpressed with mRNA for the θ-subunit;[61] however, it was not found in the cerebellum.[62] GABA$_A$ receptors

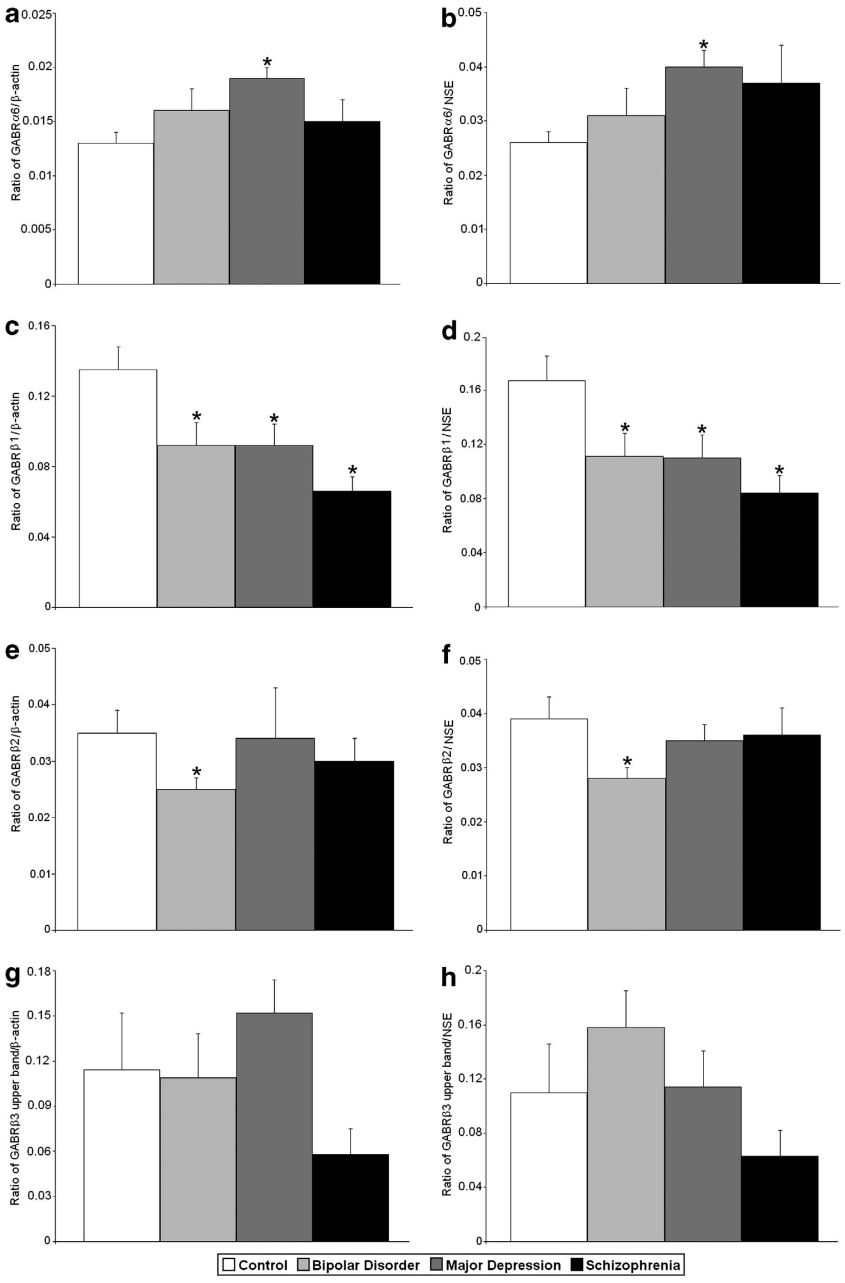

Figure 3. Expression of GABRα6/β-actin (**a**), GABRα6/NSE (**b**), GABRβ1/β-actin (**c**), GABRβ1/NSE (**d**), GABRβ2/β-actin (**e**), GABRβ2/NSE (**f**), GABRβ3 upper band/β-actin (**g**) and GABRβ3 upper band/NSE (**h**) in the lateral cerebella of healthy control subjects versus subjects with bipolar disorder, major depressive disorder and schizophrenia. Histogram bars shown as mean ± s.e., $*P < 0.05$.

that include GABRε have been shown to be insensitive to benzodiazepines[63,64] and overexpression of GABRε has shown to result in insensitivity to anesthetics.[65] Our finding of increased expression of GABRε in the lateral cerebella of subjects with schizophrenia, bipolar disorder and major depressive disorder represents the first such protein data on this subunit in these disorders. In addition, the absence of any mRNA changes indicate that the altered receptor protein expression is likely secondary to posttranslation deficits in processing of ε-receptors in all three disorders. The altered expression may change the pharmacological properties of GABA$_A$ receptors in this region, leading to altered neurotransmission.

To the best of our knowledge, we are the first laboratory to observe significant reduction of GABRβ1 protein in brains of subjects diagnosed with schizophrenia, bipolar disorder or major depression. GABRβ1 mRNA localizes to multiple brain regions, with strong expression in the hippocampus of rat, as well as in the amygdala and cerebellar granular cells.[45] Previous studies have found no changes in mRNA for GABRβ1 in PFC of subjects with schizophrenia when compared with controls.[30,66] Our observed reduction may signify regional changes in the β1-subunit expression. Moreover, recent genetic studies have implicated *GABRB1* (β1) in bipolar disorder, schizoaffective disorder and major depression.[67–72] Finally, *GABRB1* has been associated with the risk of alcohol dependence.[73,74] Again, as no mRNA effects were seen, all β1 protein changes may be due to posttranslational processing deficits intracellularly.

GABRA6, the gene that codes for the GABRα6 subunit is localized to 5q31.1–q35.[75] In studies from the rat brain, GABRα6 mRNA was found to localize exclusively to the cerebellar granule

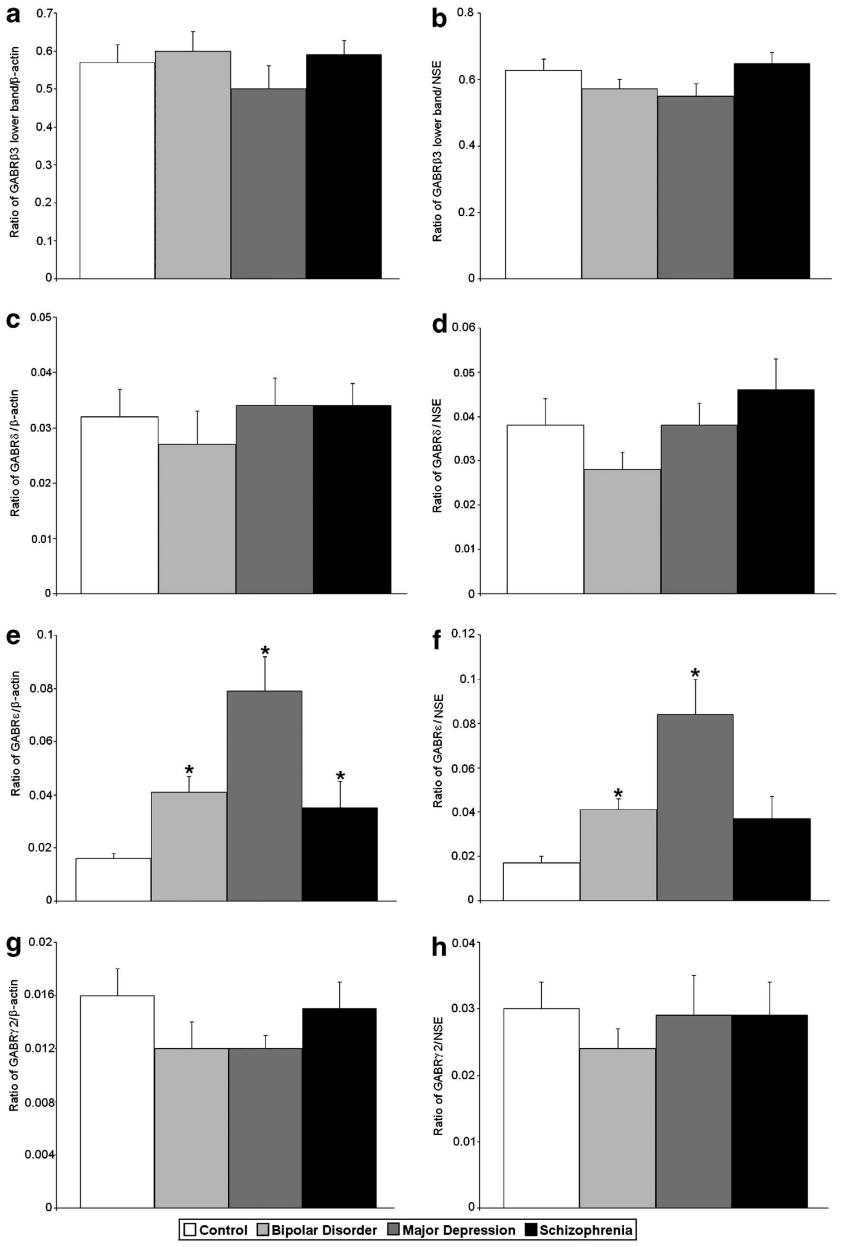

Figure 4. Expression of GABRβ3 lower band/β-actin (**a**), GABRβ3 lower band/NSE (**b**), GABRδ/β-actin (**c**), GABRδ/NSE (**d**), GABRε/β-actin (**e**), GABRε/NSE (**f**), GABRγ2/β-actin (**g**) and GABRγ2/NSE (**h**) in the lateral cerebella of healthy control subjects versus subjects with bipolar disorder, major depressive disorder and schizophrenia. Histogram bars shown as mean ± s.e., $^*P < 0.05$.

neurons.[44,76] We observed increased expression of GABRα6/β-actin and GABRα6/NSE protein in the lateral cerebella of subjects with major depression only, with no changes in either schizophrenia or bipolar disorder. Polymorphisms of *GABRA6* have been shown to have significant associations with mood disorders in females.[77] Moreover, a single-nucleotide polymorphism of *GABRA6* (rs1992647) has been associated with antidepressant response in a Chinese population sample.[78] A study by Petryshen *et al.*[14] associated a variant of *GABRA6* with schizophrenia, whereas a separate study found no association.[79] Interestingly, a recent study identified a single-nucleotide polymorphism of *GABRA6* (rs3219151) that is associated with decreased risk of schizophrenia.[80] Thus, significant α6 protein expression in major depression may signify a specific marker for this disorder.

The gene for GABRα1 is located at 5q34–q35.[53] The α1-subunit is expressed in a majority of GABA$_A$ receptors and has a wide distribution, including the neocortex, hippocampus, globus pallidus, medial septum, thalamus and cerebellum.[45] Within the cerebellum, mRNA for the α1-subunit is localized to the stellate/basket cells, Purkinje cells and granule cells.[44–46] We observed a significant decrease in mRNA levels for GABRα1 in the cerebella of subjects with schizophrenia and major depression, whereas we found a significant increase in the GABRα1/β-actin protein in subjects with major depression. Several groups have identified reduced expression of GABRα1 in PFC from subjects with schizophrenia,[30,31,66] whereas a separate study found no change.[81] Glausier and Lewis[82] further identified selective reduction of GABRα1 mRNA in pyramidal cells located in layer 3

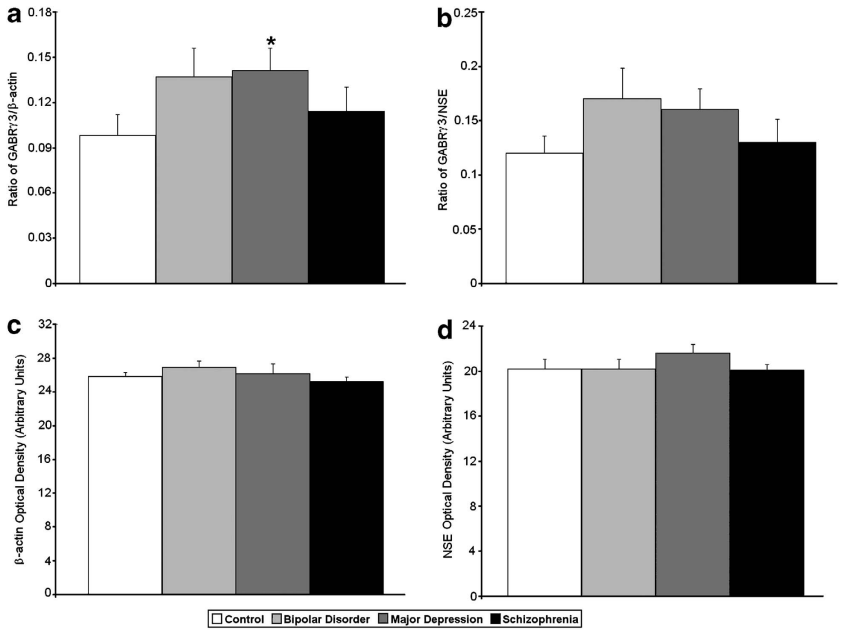

Figure 5. Expression of GABRγ3/β-actin (**a**), GABRγ3/NSE (**b**), β-actin (**c**) and NSE (**d**) in the lateral cerebella of healthy control subjects versus subjects with bipolar disorder, major depressive disorder and schizophrenia. Histogram bars shown as mean ± s.e., $*P < 0.05$.

Table 4. mRNA expression for 12 GABA$_A$ receptor subunits in the lateral cerebella of subjects with schizophrenia and mood disorders

		Schizophrenia		*Bipolar disorder*		*Major depression*	
	ANOVA	*Fold change*	P-*value*	*Fold change*	P-*value*	*Fold change*	P-*value*
GABRA1	**0.012**	**0.713**	**0.011**	0.949	0.707	**0.618**	**0.009**
GABRA2	0.126	**0.563**	**0.017**	0.688	0.111	0.688	0.099
GABRA3	0.505	0.531	0.139	0.589	0.289	0.865	0.599
GABRA5	0.118	0.754	0.127	1.094	0.621	0.735	0.206
GABRA6	0.385	0.994	0.967	0.674	0.210	0.789	0.217
GABRB1	0.684	0.997	0.989	0.781	0.319	0.937	0.743
GABRB2	0.866	1.113	0.644	0.915	0.784	1.128	0.583
GABRB3	0.232	**1.473**	**0.044**	1.317	0.115	1.375	0.071
GABRD	0.233	0.678	0.051	0.602	0.101	0.887	0.632
GABRE	0.400	1.046	0.859	1.320	0.196	0.905	0.639
GABRG2	0.238	1.118	0.419	0.758	0.168	0.888	0.461
GABRG3	0.188	0.785	0.373	0.877	0.625	0.450	0.081

Abbreviations: ANOVA, analysis of variance; C, control; GABA$_A$, γ-aminobutyric acid (A); S, schizophrenia; B, bipolar disorder; D, major depression.
Note: ANOVA based on six comparisons: C versus S, C versus B, C versus D, S versus B, S versus D and B versus D.
Bold entries represent significant fold changes and *P* values.

of the PFC, whereas there was no change in GABRα1 mRNA levels in interneurons in the same layer. Our results are the first to show a similar reduction in the cerebella from subjects with schizophrenia or major depression. A previous study has failed to find a linkage between *GABRA1* variants and major depression;[83] however, other studies have associated this gene with bipolar disorder[84] and schizophrenia.[33]

The gene that codes for GABRγ3 (*GABRG3*) localizes to the 15q11.2–q13 site, where it clusters with the genes for GABRα5 (*GABRA5*) and GABRβ3 (*GABRB3*).[85] mRNA for the γ3-subunit localizes to the cerebellar granule cells, as well as the neocortex, caudate putamen and nucleus accumbens among other regions.[44,45] Although no genetic associations between *GABRG3* and schizophrenia and bipolar disorders have been identified, a single-nucleotide polymorphism of *GABRG3* (rs2376481) has been linked to female suicide attempters.[86] Similar to *GABRA2* and *GABRB1*, *GABRG3* may be associated with alcohol dependence.[87] Our finding of a significant increase in expression of GABRγ3/β-actin in the lateral cerebella of subjects with major depression is thus novel and potentially interesting in light of *γ3* being a potential risk gene for suicide.

The gene for GABRβ2 (*GABRB2*) is located at 5q34–q35, where it clusters with the genes for GABRα1 (*GABRA1*) and GABRγ2 (*GABRG2*).[88] The β2-subunit mRNA has been found in the olfactory bulb, neocortex, globus pallidus, thalamus and cerebellar granule cells.[44,45] Recently, *GABRB2* has been associated with both bipolar disorder and schizophrenia.[89–91] Two novel isoforms of *GABRB2*, β(2S1) and β(2S2) have also been associated with male subjects with bipolar disorder.[89] Moreover, Zhao *et al.*,[89] by using quantitative real-time PCR, found that in post-mortem brain, there was significantly increased mRNA for β(2S1) in the DLPFC of subjects with bipolar disorder and significantly reduced mRNA for β(2S2) in DLPFC of subjects with bipolar disorder and schizophrenia. A separate group has also

Table 5. Summary of mRNA and protein levels for selected GABA$_A$ and GABA$_B$ receptor subunits in cerebella from subjects with three major psychiatric disorders

		Schizophrenia		*Bipolar disorder*		*Major depression*	
Subunit	*Chromosomal location*	*M*	*P*	*M*	*P*	*M*	*P*
GABRα1	5q34–q35	↓	NC	NC	NC	↓	↑
GABRα2	4q13–p12	↓	↑	NC	↑	NC	↑
GABRα3	Xq28	NC	NC	NC	NC	NC	NC
GABRα5	15q11.2–q13	NC	NC	NC	NC	NC	NC
GABRα6	5q31.1–q35	NC	NC	NC	NC	NC	↑
GABRβ1	4q13–p12	NC	↓	NC	↓	NC	↓
GABRβ2	5q34–q35	NC	NC	NC	↓	NC	NC
GABRβ3	15q11.2–q13	↓	NC	NC	NC	NC	NC
GABRδ	1p36.3	NC	NC	NC	NC	NC	NC
GABRε	Xq28	NC	↑	NC	↑	NC	↑
GABRγ2	15q31.1–q33.1	NC	NC	NC	NC	NC	NC
GABRγ3	15q11.2–q13	NC	NC	NC	NC	NC	↑
GABBR1[1]	6p21.3	NC	↓	NC	↓	NC	↓
GABBR2[1]	6q12–q21	NC	↓	↑	↓	NC	↓

Abbreviations: GABBR1, GABA$_B$ receptor subunit 1; GABBR2, GABA$_B$ receptor subunit 2; M, mRNA; NC, no change; P, protein. GABBR1 and GABBR2 data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

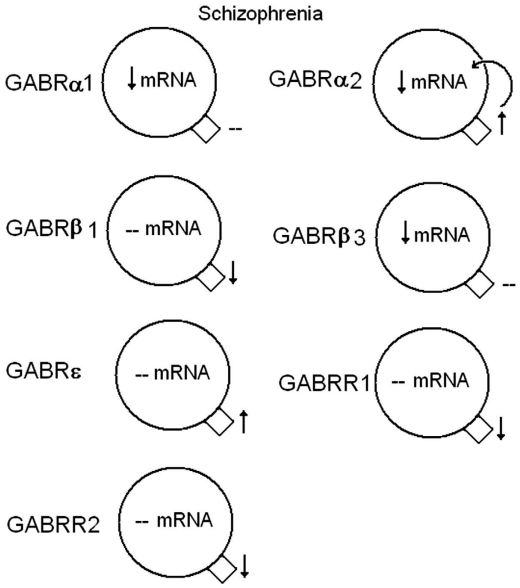

Figure 6. Summary of significant mRNA and protein expression for γ-aminobutyric acid A and B (GABA$_A$ and GABA$_B$) receptors in the lateral cerebella of subjects with schizophrenia. Increased expression of GABRα2 protein may lead to a negative feedback loop decreasing the mRNA expression. ↑, increased expression; ↓, reduced expression, --, no change. GABA$_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

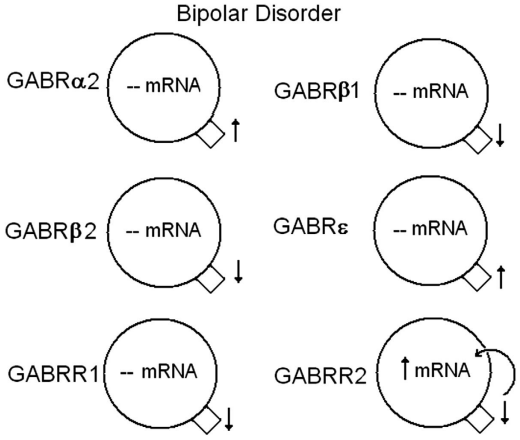

Figure 7. Summary of significant mRNA and protein expression for γ-aminobutyric acid A and B (GABA$_A$ and GABA$_B$) receptors in the lateral cerebella of subjects with bipolar disorder. Decreased expression of GABA$_B$ receptor subunit 2 (GABRR2) protein may lead to a positive feedback loop increasing the mRNA expression. ↑, increased expression; ↓, reduced expression, --, no change. GABA$_B$ receptor subunit 1 (GABBR1) and GABBR2 data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

recently observed a reduction in mRNA for the β2-subunit in DLPFC of subjects with schizophrenia.[30]

As previously mentioned, the gene that codes GABRβ3 (*GABRB3*) clusters at 15q11.2–q13, with *GABRA5* and *GABRG3*. GABRβ3 mRNA has been found in multiple brain areas, including the olfactory bulb, neocortex, hippocampus, hypothalamus and cerebellum.[44,45] In the cerebellum, mRNA for the β3-subunit has been found in both Purkinje cells and granule cells.[44,46] An association between *GABRB3* and schizophrenia has been documented in two recent studies.[92,93] A previous study has shown that mRNA for GABRβ3 is not changed in DLPFC of subjects with schizophrenia.[30] However, we found increased mRNA for GABRβ3 in the lateral cerebella from subjects with schizophrenia, suggesting regional differences. Although we did not find any changes in GABRβ3 protein expression, we have previously found reduction of GABRβ3 protein levels in the cerebella of subjects with autism.[34,94]

Overall, we observed significant increased expression of α2- and ε-subunit protein levels in all three disorders. In addition, we observed decreased protein expression for β1 in all three disorders and α1 mRNA in schizophrenia and major depression. (Figures 6–9; Table 5). We have previously shown reduced expression of GABBR1 and GABBR2 in the lateral cerebella of subjects with schizophrenia, bipolar disorder and major depression (Table 5; Figures 6–10).[29] With regard to mRNA expression, α- and β-subunits showed reduced expression, whereas GABBR2 displayed increased expression (Figures 6–8 and 10; Table 5). These changes may result in improper GABAergic transmission, both within the cerebellum and in circuits connecting the cerebellum with other parts of the brain, including the PFC. Functional consequences of impaired GABAergic transmission are likely to include dysregulated states of anxiety, panic and deficits in learning.[95–97] Deficits in GABA$_B$ receptor expression may contribute to deficits in information processing in schizophrenia, including abnormalities in prepulse inhibition and P50 suppression.[98–101] Moreover, altered expression of GABA$_A$ and GABA$_B$ subunits may affect the pharmacological properties of the receptors, altering their ability to respond to drugs, such as anesthetics, benzodiazepines and neurosteroids. Taken together, the changes that were consistent across the three diagnostic groups—GABRα2, GABRβ1, GABRε, GABBR1 and GABBR2—may help explain similarities between these disorders.

Our findings build upon previous work to examine GABA receptor subunit expression in brains of subjects with psychiatric disorders. Although most of the previously discussed findings are from the PFC,[30,31,47,66,89] less is known of the cerebellum.[28,29] We found no change in mRNA expression for GABRα6 and GABRδ in the cerebella of subjects with schizophrenia, which are in contrast to the findings of Bullock *et al.*[28] who found increased mRNA expression for both subunits. A potential explanation for this discrepancy may be due to the anatomic location of the cerebellar

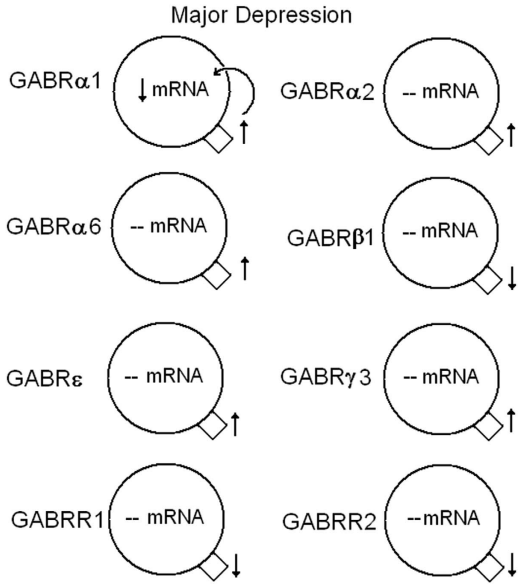

Figure 8. Summary of significant mRNA and protein expression for γ-aminobutyric acid A and B (GABA$_A$ and GABA$_B$) receptors in the lateral cerebella of subjects with major depression. Increased expression of GABRα1 protein may lead to a negative feedback loop decreasing the mRNA expression. ↑, increased expression; ↓, reduced expression, --, no change. GABA$_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

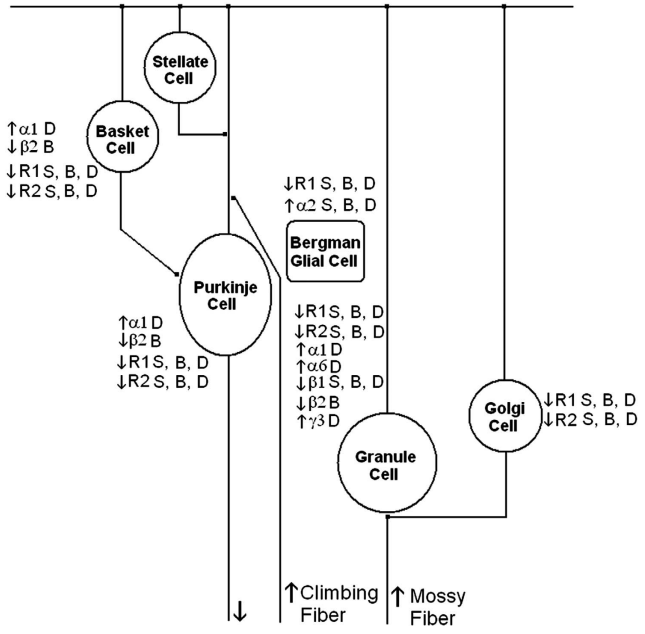

Figure 9. Altered protein expression of γ-aminobutyric acid A and B (GABA$_A$ and GABA$_B$) receptor subunits in various cells of the cerebellar circuitry of subjects with schizophrenia (S), bipolar disorder (B) or major depression (D). R1, GABA$_B$ receptor 1; R2, GABA$_B$ receptor 2. GABA$_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

tissue used for both sets of experiments. Bullock *et al.*[28] describe their tissue as being from the lateral cerebellar hemisphere corresponding to crus I of lobule VIIa, whereas ours is described as a 'lateral posterior lobe'. Just as each subunit has a unique distribution among the cell types in the cerebellum (Figures 9 and

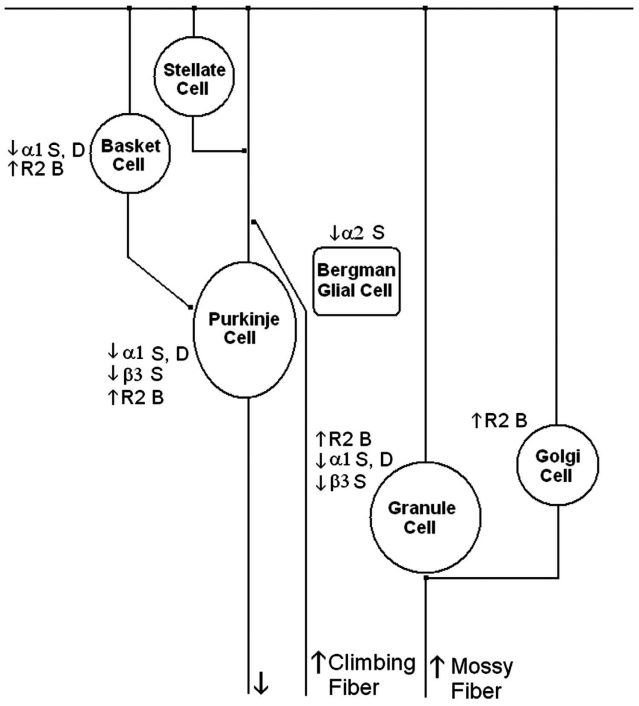

Figure 10. Altered mRNA expression of γ-aminobutyric acid A (GABA$_A$) receptor subunits in various cells of the cerebellar circuitry of subjects with schizophrenia (S), bipolar disorder (B) or major depression (D). R2, GABA$_B$ receptor 2. GABA$_B$ receptor subunits 1 and 2 (GABBR1 and GABBR2) data reprinted from Fatemi *et al.*,[29] with permission from Elsevier.

10),[44,45] there may be regional differences in GABA subunit expression throughout the cerebellum.

As targets of several psychotropic agents including benzodiazepines and neurosteroids, GABA$_A$ receptors are sites of potential therapeutic intervention. Experiments in rodents have shown that chronic treatment with atypical antipsychotic drugs clozapine and olanzapine result in increased levels of the neurosteroid allopregnanolone to a concentration large enough to stimulate GABA$_A$ receptors.[102] In a rat model, injection of allopregnanolone into the hippocampus improved prepulse inhibition.[103] Pregnenolone, the biosynthetic precursor of allopregnanolone has also been shown to increase concentrations of allopregnanolone in patients with schizophrenia.[104] Schizophrenic patients with higher levels of allopregnanolone displayed significantly improved cognition as measured by the Brief Assessment of Cognition in Schizophrenia and significantly improved negative symptoms as measured by Scale for the Assessment of Negative Symptoms.[104] Treatment with antidepressants fluoxetine and fluvoxamine has also been shown to increase levels of allopregnanolone in the cerebrospinal fluid of patients diagnosed with major depression.[105] Neurosteroid treatment may provide new means of treating GABA deficits in these disorders.

CONCLUSION

The examination of mRNA and protein levels for 12 GABA$_A$ receptor gene families in the lateral cerebella of subjects with schizophrenia and mood disorders showed significant increases in α2-and ε-, and decreases in β1-receptor protein expression in schizophrenia, bipolar disorder and major depression. In addition, several important alterations were observed in mRNA or protein levels for α1-, α6-, β2-, β3- and γ3-receptor subtypes in some of these disorders. These results, combined with our previous findings of reductions in GABA$_B$ receptor subunits, provide further

evidence of GABAergic dysfunction in schizophrenia and mood disorders, which could ultimately underlie some of the cognitive, psychotic and mood dysfunctions associated with these disorders. Our findings may also open the door to new, targeted, therapeutic treatments, such as the use of neurosteroids.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)