Expression of GABA<sub>A</sub> 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 GAB<sub>A</sub> 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 GAB<sub>A</sub> subunit proteins (α1, α2, α3, α5, α6, β1, β2, β3, δ, ε, γ2 and γ3) in the lateral cerebella of 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.

Keywords: bipolar disorder; GABR<sub>α2</sub>; GABR<sub>β1</sub>; GABR<sub>ε</sub>; major depression; schizophrenia

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. Hypofunction of the GABAergic signaling system in schizophrenia, bipolar disorder and major depressive disorder has been hypothesized to contribute to the pathologies of schizophrenia, bipolar disorder and major depressive disorder. 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, the enzymes that convert glutamate to GABA; (2) microarray results that have demonstrated increased mRNA for a number of GABA(A) (GAB<sub>A</sub>) receptor subunits in prefrontal cortex (PFC) of subjects with schizophrenia, and (3) gene association studies that link GABA receptor subunits to schizophrenia and mood disorders.

Structural and functional abnormalities of the cerebellum have been described for schizophrenia, depression and bipolar disorder, including reduced cerebellar volumes. Reduced cerebellar activation has also been observed in functional imaging studies of subjects with these disorders. There is abundant evidence that the cerebellum has roles in cognition and emotion. 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.

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

Although there have been some mRNA studies of GAB<sub>A</sub> receptor expression in subjects with schizophrenia, there is a paucity of data regarding GAB<sub>A</sub> 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 GAB<sub>A</sub> receptor subunits: GABR<sub>α1</sub>, GABR<sub>α2</sub>, GABR<sub>α3</sub>, GABR<sub>γ2</sub>, GABR<sub>β1</sub>, GABR<sub>β2</sub>, GABR<sub>δ</sub>, GABR<sub>ε</sub>, GABR<sub>γ2</sub> and GABR<sub>γ3</sub>. On the basis of our finding of significantly reduced GAB<sub>A</sub> receptor subunits in the lateral cerebellum, we hypothesized that we would observe reduced expression of multiple GAB<sub>A</sub> receptor subunits in the same brain region of subjects with schizophrenia, bipolar disorder and major depression.
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 hemisphere side (Table 1).

SDS-polyacrylamide gel electrophoresis and western blotting

Brain tissue was prepared as previously described.39,43 Thirty milligrams of the lateral cerebellum was used per lane. For all experiments, we used 10% resolving gels and 5% stacking gels. 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

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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,14,26,28 with α < 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 χ²-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 GABRζ2/β-actin (F(3,52) = 3.35, P < 0.027), GABRζ2/β-actin (F(3,52) = 3.49, P < 0.022), GABRζ1/β-actin (F(3,54) = 6.33, P < 0.001), GABRζ1/NSE (F(3,53) = 4.53, P < 0.027), GABRζ2/β-actin (F(3,37) = 8.88, P < 0.0001) and GABRζ2/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 GABRζ2/β-actin (P < 0.0046), GABRζ2/NSE (P < 0.0042) and GABRζ2/β-actin (P < 0.044) (Tables 2 and 3; Figures 1, 2 and 4), and significantly reduced expression of GABRζ1/β-actin (P < 0.0001) and GABRζ1/NSE (P < 0.001) (Tables 2 and 3; Figures 1 and 3).

In subjects with bipolar disorder, follow-up Student’s t-tests found significantly increased expression of GABRζ2/β-actin (P < 0.017), GABRζ2/NSE (P < 0.011), GABRζ2/β-actin (P < 0.0006) and GABRζ2/NSE (P < 0.0013) (Tables 2 and 3; Figures 1 and 4), and significantly reduced expression of GABRζ1/β-actin (P < 0.026) and GABRζ1/NSE (P < 0.034) (Tables 2 and 3; Figures 1 and 3). We also observed significantly reduced expression of GABRζ2/β-actin (P < 0.025) and GABRζ2/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 GABRζ2/β-actin (P < 0.0047), GABRζ2/NSE (P < 0.0063), GABRζ1/β-actin (P < 0.0003) and GABRζ2/NSE (P < 0.0012) (Tables 2 and 3; Figures 1, 2, and 4), and significantly reduced expression of GABRζ1/β-actin (P < 0.023) and GABRζ1/NSE (P < 0.03) (Tables 2 and 3; Figures 1 and 3). We found that GABRζ1/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 GABRζ3/β-actin (P < 0.047; Table 2; Figures 1 and 5), GABRζ6/β-actin (P < 0.001) and GABRζ6/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 significantly 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 (32.3) 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 GABRζ2/β-actin in subjects with major depression (P < 0.068); GABRζ1/β-actin in subjects with bipolar disorder (P < 0.46); GABRζ2/β-actin in subjects with bipolar disorder (P < 0.061); GABRζ3/β-actin in subjects with major depression (P < 0.25); and GABRζ1/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 GABRζ2/β-actin, GABRζ2/β-actin, GABRζ3/β-actin and GABRζ1/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 GABRζ1/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 GABRζ1/β-actin (Supplementary Table 2). However, the GABRζ1/NSE ratio continued to be significantly lower in the bipolar group (P < 0.034) and was not affected by the antidepressant confound.
and GABR
dependence were removed, none of the values lost significance (Supplementary Table 3). When individuals with substance abuse were removed, all values for individuals with alcohol dependence and substance abuse did not impact any of our data. When individuals with alcohol dependence/abuse or subjects with substance abuse were removed, all values for individuals with alcohol dependence, alcohol abuse, substance abuse did not impact any of our data. When individuals with alcohol dependence/abuse or subjects with substance abuse were removed, significance was lost for GABRβ1/β-actin (P < 0.063), GABRβ1/NSE (P < 0.063) and GABRγ3/β-actin (P < 0.061) (Supplementary Table 4). 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<sub>A</sub> receptor subunits (Table 4). ANOVA found a significant group difference for GABRA1 (GABRβ1; P < 0.012) with significantly reduced mRNA for GABRA1 in the lateral cerebellum of subjects with schizophrenia (P < 0.011) and major depression (P < 0.001; 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 α- and β2-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-, α2- 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 GABRB1/β-actin in subjects with bipolar disorder.

In rat brain, GABRα2 mRNA is distributed in multiple regions, including the neocortex, hippocampus, hypothalamus and cerebellum.43 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 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).
schizophrenia versus matched controls, greater ‘aberrant’ fronto-temporal modulation observed in patients with schizophrenia was correlated with polymorphisms of the \( \text{GABRA2} \) gene. Moreover, recent studies have shown that positive modulators of \( \text{GABR} \alpha_2 \) can improve working memory in a monkey model of schizophrenia and in humans. However, a separate study found no benefit of the \( \text{GABR} \alpha_2 \)-positive modulator MK-0777 for patients with schizophrenia on tests of working memory. The \( \text{GABR} \alpha_2 \) gene (\( \text{GABRA2} \)), which is localized to 4q13–p12, has been associated with risk for alcohol dependence and drug abuse. 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 \( \text{GABR} \alpha_2 \) may help explain comorbid substance abuse in subjects with schizophrenia. To date, there are no reports of an association between \( \text{GABRA2} \) with bipolar disorder or major depression. However, a recent set of experiments comparing \( \text{GABRA2} \) heterozygous and homozygous knockout mice with wild-type mice have found that males lacking the \( \alpha_2 \)-subunit displayed depressive symptoms during the forced swimming test, the novelty suppressed-feeding test and the tail suspension test. These results have led the authors to conclude that \( \text{GABA} \)ergic inhibition acting through receptors that include the \( \alpha_2 \)-subunit has a potential antidepressant-like effect. The gene for \( \text{GABR} \alpha_3 \) clusters at Xq28 (Table 5) with genes for the \( \alpha_3 \) - and \( \theta \)-subunits. mRNA for the \( \varepsilon \)-subunit has been identified in the septum, thalamus, hypothalamus and amygdala in rat brain and was often coexpressed with mRNA for the \( \theta \)-subunit; however, it was not found in the cerebellum. \( \text{GABA} \alpha_3 \) receptors

Figure 2. Expression of \( \text{GABR} \alpha_1/\beta \)-actin (a), \( \text{GABR} \alpha_1/\text{NSE} \) (b), \( \text{GABR} \alpha_2/\beta \)-actin (c), \( \text{GABR} \alpha_2/\text{NSE} \) (d), \( \text{GABR} \alpha_3/\beta \)-actin (e), \( \text{GABR} \alpha_3/\text{NSE} \) (f), \( \text{GABR} \alpha_5/\beta \)-actin (g) and \( \text{GABR} \alpha_5/\text{NSE} \) (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 GABR\textsubscript{e} have been shown to be insensitive to benzodiazepines\textsuperscript{63,64} and overexpression of GABR\textsubscript{e} has shown to result in insensitivity to anesthetics.\textsuperscript{65} Our finding of increased expression of GABR\textsubscript{e} 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 \textgreek{e}-receptors in all three disorders. The altered expression may change the pharmacological properties of GABA\textsubscript{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 GABR\textsubscript{b1} protein in brains of subjects diagnosed with schizophrenia, bipolar disorder or major depression. GABR\textsubscript{b1} mRNA localizes to multiple brain regions, with strong expression in the hippocampus of rat, as well as in the amygdala and cerebellar granular cells.\textsuperscript{45} Previous studies have found no changes in mRNA for GABR\textsubscript{b1} in PFC of subjects with schizophrenia when compared with controls.\textsuperscript{30,66} Our observed reduction may signify regional changes in the \textgreek{b1}-subunit expression. Moreover, recent genetic studies have implicated GABRB1 (\textgreek{b1}) in bipolar disorder, schizoaffective disorder and major depression.\textsuperscript{67–72} Finally, GABRB1 has been associated with the risk of alcohol dependence.\textsuperscript{73,74} Again, as no mRNA effects were seen, all \textgreek{b1} protein changes may be due to posttranslational processing intracellularly.

GABRA6, the gene that codes for the GABR\textsubscript{o6} subunit is localized to 5q31.1–q35.\textsuperscript{75} In studies from the rat brain, GABR\textsubscript{o6} mRNA was found to localize exclusively to the cerebellar granule

Figure 3. Expression of GABR\textsubscript{o6}/\textgreek{a}-actin (a), GABR\textsubscript{o6}/NSE (b), GABR\textsubscript{\textgreek{b}1}/\textgreek{a}-actin (c), GABR\textsubscript{\textgreek{b}1}/NSE (d), GABR\textsubscript{\textgreek{b}2}/\textgreek{a}-actin (e), GABR\textsubscript{\textgreek{b}2}/NSE (f), GABR\textsubscript{\textgreek{b}3} upper band/\textgreek{a}-actin (g) and GABR\textsubscript{\textgreek{b}3} 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., *\textit{P} < 0.05.
We observed increased expression of GABRA6/β-actin and GABRA6/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. Moreover, a single-nucleotide polymorphism of GABRA6 (rs1992647) has been associated with antidepressant response in a Chinese population sample. A study by Petryshen et al. associated a variant of GABRA6 with schizophrenia, whereas a separate study found no association. Interestingly, a recent study identified a single-nucleotide polymorphism of GABRA6 (rs3219151) that is associated with decreased risk of schizophrenia. 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. The α1-subunit is expressed in a majority of GABA receptors and has a wide distribution, including the neocortex, hippocampus, globus pallidus, medial septum, thalamus and cerebellum. Within the cerebellum, mRNA for the α1-subunit is localized to the stellate/basket cells, Purkinje cells and granule cells. We observed a significant decrease in mRNA levels for GABRα1 in the cerebella of subjects with schizophrenia, whereas a separate study found no change. Glausier and Lewis further identified selective reduction of GABRα1 mRNA in pyramidal cells located in layer 3

Figure 4. Expression of GABRα3 lower band/β-actin (a), GABRα3 lower band/NSE (b), GABRα3/β-actin (c), GABRα/NSE (d), GABRα/β-actin (e), GABRα/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.
of the PFC, whereas there was no change in GABR\textsubscript{a1} 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;\textsuperscript{83} however, other studies have associated this gene with bipolar disorder and schizophrenia.\textsuperscript{33}

The gene that codes for GABR\textsubscript{g3} (GABRG3) localizes to the 15q11.2–q13 site, where it clusters with the genes for GABR\textsubscript{a5} (GABRA5) and GABR\textsubscript{b3} (GABRB3).\textsuperscript{85} mRNA for the g3-subunit localizes to the cerebellar granule cells, as well as the neocortex, caudate putamen and nucleus accumbens among other regions.\textsuperscript{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.\textsuperscript{86} Similar to GABRA2 and GABRB1, GABRG3 may be associated with alcohol dependence.\textsuperscript{87}

Our finding of a significant increase in expression of GABR\textsubscript{g3}/\beta-actin in the lateral cerebella of subjects with major depression is thus novel and potentially interesting in light of g3 being a potential risk gene for suicide.

The gene for GABR\textsubscript{b2} (GABRB2) is located at 5q34–q35, where it clusters with the genes for GABRA1 and GABR\textsubscript{g2} (GABRG2).\textsuperscript{88} The b2-subunit mRNA has been found in the olfactory bulb, neocortex, globus pallidus, thalamus and cerebellar granule cells.\textsuperscript{44,45} Recently, GABRB2 has been associated with both bipolar disorder and schizophrenia.\textsuperscript{89–91} Two novel isoforms of GABRB2, \(\beta(2S1)\) and \(\beta(2S2)\) have also been associated with male subjects with bipolar disorder.\textsuperscript{89} Moreover, Zhao \textit{et al.}\textsuperscript{89} by using quantitative real-time PCR, found that in post-mortem brain, there was significantly increased mRNA for \(\beta(2S1)\) in the DLPFC of subjects with bipolar disorder and significantly reduced mRNA for \(\beta(2S2)\) in DLPFC of subjects with bipolar disorder and schizophrenia. A separate group has also

**Table 4.** mRNA expression for 12 GABA\textsubscript{A} receptor subunits in the lateral cerebella of subjects with schizophrenia and mood disorders

| Subunit  | Schizophrenia | Bipolar disorder | Major depression |
|----------|---------------|-----------------|-----------------|
| GABRA1   | 0.012         | 0.573           | 0.011           |
| GABRA2   | 0.126         | 0.563           | 0.017           |
| GABRA3   | 0.505         | 0.531           | 0.139           |
| GABRA5   | 0.118         | 0.754           | 0.127           |
| GABRA6   | 0.385         | 0.994           | 0.967           |
| GABRB1   | 0.684         | 0.997           | 0.989           |
| GABRB2   | 0.866         | 1.113           | 0.644           |
| GABRB3   | 0.232         | 1.473           | 0.044           |
| GABRD    | 0.233         | 0.678           | 0.051           |
| GABRE    | 0.400         | 1.046           | 0.859           |
| GABRG2   | 0.238         | 1.118           | 0.419           |
| GABRG3   | 0.188         | 0.785           | 0.373           |

Abbreviations: ANOVA, analysis of variance; C, control; GABA\textsubscript{A}, \(\gamma\)-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.
recently observed a reduction in mRNA for the β2-subunit in DLPFC of subjects with schizophrenia.30

As previously mentioned, the gene that codes GABRB3 (GABRβ3) clusters at 15q11.2–q13, with GABRAS5 and GABR3. GABRB3 mRNA has been found in multiple brain areas, including the olfactory bulb, neocortex, hippocampus, hypothalamus and cerebellum.44,45 In the cerebellum, the mRNA for the β3-subunit has been found in both Purkinje cells and granule cells.44,45 An association between GABRB3 and schizophrenia has been documented in two recent studies.92,93 A previous study has shown that mRNA for GABRB3 is not changed in DLPFC of subjects with schizophrenia.30 However, we found increased mRNA for GABRB3 in the lateral cerebella from subjects with schizophrenia, suggesting regional differences. Although we did not find any changes in GABRB3 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 β-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 GABRB1 and GABRB2 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 GABRB2 displayed increased expression (Figures 6–10; 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 GABAα 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 GABAα and GABAβ 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—GABRB2, GABRB3, GABRB1 and GABRB2—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,30,37,47,66,89 less is known of the cerebellum.28,29 We found no change in mRNA expression for GABRB6 and GABR0 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
tissue used for both sets of experiments. Bullock et al.\textsuperscript{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 10),\textsuperscript{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\textsubscript{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\textsubscript{A} receptors.\textsuperscript{102} In a rat model, injection of allopregnanolone into the hippocampus improved prepulse inhibition.\textsuperscript{103} Pregnenolone, the biosynthetic precursor of allopregnanolone has also been shown to increase concentrations of allopregnanolone in patients with schizophrenia.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsubscript{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 γ2-receptor subtypes in some of these disorders. These results, combined with our previous findings of reductions in GABA\textsubscript{B} receptor subunits, provide further

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

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

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

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