



Published in final edited form as:

J Autism Dev Disord. 2009 February ; 39(2): 223–230. doi:10.1007/s10803-008-0646-7.

GABA_A receptor downregulation in brains of subjects with autism

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Abstract

Gamma-aminobutyric acid A (GABA_A) receptors are ligand-gated ion channels responsible for mediation of fast inhibitory action of GABA in the brain. Preliminary reports have demonstrated altered expression of GABA receptors in the brains of subjects with autism suggesting GABA/ glutamate system dysregulation. We investigated the expression of four GABA_A receptor subunits and observed significant reductions in GABRA1, GABRA2, GABRA3, and GABRB3 in parietal cortex (Brodmann's Area 40(BA40)), while GABRA1 and GABRB3 were significantly altered in cerebellum, and GABRA1 was significantly altered in superior frontal cortex (BA9). The presence of seizure disorder did not have significant impact on GABA_A receptor subunit expression in the three brain areas. Our results demonstrate that GABA_A receptors are reduced in three brain regions that have previously been implicated in the pathogenesis of autism, suggesting widespread GABAergic dysfunction in the brains of subjects with autism.

Keywords

GABRA1; GABRA2; GABRA3; GABRB3; autism; brain

Autism is a severe neurodevelopmental disorder characterized by social deficits, language abnormalities and repetitive behavior (APA, 1994). A limited number of reports have demonstrated abnormalities involving the glutamate and GABAergic systems in brain, blood, and platelets of subjects with autism (Blatt et al., 2001; Dhossche et al., 2002; Fatemi, 2008). Our laboratory has demonstrated that brain levels of glutamic acid decarboxylase 65 and 67kDa proteins (GAD65/67), the rate limiting enzyme responsible for normal conversion of glutamate to GABA in the brain, were significantly decreased in cerebellum (GAD65) and parietal cortex (GAD67) (Fatemi et al., 2002a). Yip et al. (2007) similarly reported a significant decrease in GAD67 mRNA in autistic cerebellum, thereby confirming our early findings (Fatemi et al., 2002a).

GABA_A receptors are ligand-gated ion channels responsible for mediation of fast inhibitory action of GABA in the brain (Brandon et al., 2000). GABA_A receptors are also the sites for

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There have been no changes in any of the author affiliations subsequent to the time of the study.

clinical action of benzodiazepines, barbiturates, and anesthetics. GABA_A receptors are divided into multiple subunits, e.g., α 1- α 6, β 1- β 4, γ 1- γ 4, δ , ϵ , π , θ , and ρ 1- ρ 2, producing multiple GABA_A receptor isoforms (Ma et al., 2005; Brandon et al., 2000). GABA_A receptor expression varies according to developmental timetables of the brain, suggesting different roles for different types of receptors. Mutations of various subunits cause various phenotypes, e.g., β 3 gene knockouts produce a model of Angelman syndrome (Homanics et al., 1997).

Separate studies by Blatt et al. (2001) and Samaco et al. (2005) have shown a significant decrease in GABA_A receptor binding sites (³H-flunitrazepam-labeled benzodiazepine binding sites and ³H-muscimol-labeled binding sites) and GABA_A receptor β 3 (GABRB3) subunit protein level, respectively, in brain tissues of subjects with autism when compared with controls. Recently, Guptill et al. (2007) extended the earlier work by Blatt et al. (2001) to show that the decrease in ³H-flunitrazepam-labeled benzodiazepine binding sites was due to a decrease in binding site number (B_{max}) rather than altered affinity to ligand binding (K_d). In contrast, Purcell et al. (2001) showed increases in GABA_A protein and GABA_A receptor α 5 mRNA levels in brains of subjects with autism. GABA_A receptor α 2 (GABRA2) has been linked with autism, based on the genotype-pedigree disequilibrium test (Ma et al., 2005) and a case study of an autistic patient, which revealed three copies of the GABRA2 gene (Kakinuma et al., 2007). While no previous reports have linked GABA_A receptor α 1 (GABRA1) or GABA_A receptor α 3 (GABRA3) to autism, both have been linked to schizophrenia (Ohnuma et al., 1999; Hakak et al., 2001; Inada et al., 2008), and it has previously been demonstrated that a number of genes, e.g., glutamic acid decarboxylase 1 and 2, are associated with multiple disorders (Akbarian et al., 1995; Guidotti et al., 2000; Fatemi et al., 2002a, 2005). Thus, these GABA_A receptors may vary differentially in autism.

Here, we extend our previous investigations of the GABAergic system in autism to measure expression of GABA_A subunits in a well-characterized group of age, sex, and postmortem-interval (PMI) matched brain samples from subjects with autism and matched controls in three brain areas that are involved in the pathology of autism: parietal cortex (Brodmann's area 40 (BA40)), superior frontal cortex (BA9), and cerebellum.

Methods

Tissue Preparation

All experimental procedures were approved by the Institutional Review Board of the University of Minnesota School of Medicine. Postmortem blocks of parietal cortex (Brodmann's BA40), superior frontal cortex (BA9), and cerebellum (lobar origin unknown) were obtained from the Autism Research Foundation and various brain banks (NICHD Brain and Tissue Bank for Developmental Disorders; TARF; the Harvard Brain Tissue Resource Center, which is supported in part by PHS grant number R24 MH068855; the Brain Endowment Bank, which is funded in part by the National Parkinson Foundation, Inc., Miami, Florida; and the Autism Tissue Program). These samples, which have been used by our laboratory previously, are some of the most well-characterized and most-studied brain collections used by multiple groups (for review, see Palmen et al., 2004). Before being frozen, donated brains were sectioned in half, dissected by anatomists and placed in labeled bags. All samples were stored at -80°C until use. Samples were derived from three groups of subjects (cerebellum: $N=5-7$ from subjects with autism, $N=7-9$ from control subjects; BA9: $N=4-6$ from subjects with autism, $N=3$ from control subjects; BA40: $N=6-8$ from subjects with autism, $N=5-6$ from control subjects). Consent from next of kin was given to the respective institutions. DSM-IV diagnoses were established prior to death by neurologists and psychiatrists using information from all available medical records and from family interviews. Details regarding the subject selection, diagnostic process, and tissue processing were collected by the Autism Research Foundation. Samples were matched for age, gender, and postmortem interval (PMI). All demographic information

is listed in Table 1. Seven out of nine subjects with autism had seizure disorder, and all subjects with autism displayed varying degrees of mental retardation (personal communication from Dr. Margaret Bauman). None of the controls had any known history of neuropsychiatric disorders, seizure disorder, or mental retardation.

SDS-PAGE and Western Blotting

Brain tissue (~40 mg per subject) was cut and placed on ice in lysis buffer (3 μ l/mg of tissue) [20mM Tris pH 8.0, 0.2 mM EDTA, 150 mM NaCl, 3% Igepal.NP40 (v/v), 1% sodium deoxycholate (w/v), 0.1% SDS (w/v), 50 μ l/ml leupeptin, 0.2 mM PMSF, 1 mM sodium orthovanadate, and aprotinin (Sigma, St. Louis, MO; A6279, 30 μ l/ml buffer)]. Tissue samples were homogenized using a Kontes hand pestle (Kimble-Kontes, Vineland, NJ, USA) while the temperature was maintained at 4°C. Following homogenization, an additional 1 μ l of PMSF (0.2 mM) was added to each sample, and the samples were incubated on ice for 30 min. The homogenates were centrifuged for 20 min at 10,000 \times g at 4°C. Supernatants were collected and assayed for total protein using the Bradford method (BioRad, Richmond, CA). Samples were stored at -86°C until used. Samples were mixed with denaturing SDS sample buffer (20% glycerol, 100 mM Tris pH 6.8, 0.05% w/v Bromophenol blue, 2.5% SDS (w/v), 5% β -mercaptoethanol) and denatured by heating at 100°C for 5 minutes. SDS polyacrylamide gels were prepared with standard Laemmli solutions (BioRad) (resolving: 10%, stacking: 5%). Sixty μ g of protein per lane was loaded onto the gel and electrophoresed for 15 min at 75V followed by 55 min at 150V at room temperature (RT). The proteins were electrotransferred onto nitrocellulose membranes for 2 hr at 300mAmp at 4°C. Protein blots were blocked with 0.2% I-Block (Tropix, Bedford, MA, USA) in PBS with 0.3% Tween 20 for 1hr at RT. The blots were then incubated with anti-GABA_A receptor alpha 1 (GABRA1) (06-868, Upstate Biotechnology Inc., (Lake Placid, NY) 1:2,000), anti-GABA_A receptor alpha 2 (GABRA2) (GAA21-P, Alpha Diagnostic International Inc. (San Antonio, TX) 1:1,000), anti-GABA_A receptor alpha 3 (GABRA3) (GAA31-P, Alpha Diagnostic International Inc. (San Antonio, TX) 1:1,000), or anti-GABA_A receptor beta 3 (GABRB3) (NB300-199, Novus Biologicals (Littleton, CO) 1:1,000), for 20 hr at 4°C. Blots were subsequently washed with 0.3% Tween-PBS for 30 minutes, then incubated in secondary antibody for 1 hr at RT (A-9169, Sigma, goat anti-rabbit IgG 1:80,000). Blots were washed twice for 15 minutes with 0.3% Tween-PBS. The immune complexes were visualized using the ECL Plus detection system (Amersham Pharmacia Biotech, Arlington Hts., IL) and exposed to Hyperfilm ECL (Amersham Pharmacia Biotech). Sample densities were analyzed blind to nature of diagnosis using a BioRad densitometer and the BioRad Multi Analyst software. The molecular weights of approximately 56 kDa (GABRB3), 55 kDa (GABRA3), and 51 kDa (GABRA1 and GABRA2) immunoreactive bands were quantified with background subtraction. Results obtained are based on at least two independent experiments.

Statistical Analysis

All protein measurements for subjects with autism and control subjects were normalized against β -actin (Table 2). Possible confounding variables were compared between controls and autistics. Neither age nor PMI were found to be statistically different between groups ($t(40) = 0.94, P = 0.35, t(40) = 0.38, P = 0.71$, respectively) with effect sizes for the differences of 0.33 or smaller. We also examined gender and found no significant difference between the two groups ($\chi^2 = 0.91, P = 0.34$). Given this overall lack of difference on potential confounds, we chose to conduct group comparisons (independent group t-tests) without covariates. Significance criteria was set at $P < 0.05$, and all tests were two-tailed. There was a significant difference on seizure status between groups. None of the controls had seizures whereas 75% of the subjects with autism did ($\chi^2 = 28.6, P < 0.001$). Therefore, we conducted a second analysis examining subjects with autism comorbid with seizure disorder vs. controls using

independent group t-tests. Significance criteria was again set at $P < 0.05$, and all tests were two-tailed.

Results

All GABA_A western-blotting experiments were normalized against β -actin and are shown as ratios of the various GABA_A subunits to β -actin. GABRA1 and GABRB3 were decreased in cerebella obtained from subjects with autism vs. matched controls: GABRA1 (51 kDa) was decreased by 63%, $P < 0.007$; and GABRB3 (56 kDa) was decreased by 51%, $P < 0.008$ (Figure 1, Table 2).

In BA40, we found all GABA_A subunits significantly decreased in subjects with autism when compared with controls. GABRA1 was decreased by 50%, $P < 0.018$; GABRA2 (51 kDa) was decreased by 39%, $P < 0.033$; GABRA3 (55 kDa) was decreased by 57%, $P < 0.005$; and GABRB3 was decreased by 52%, $P < 0.006$ (Figure 1, Table 2). In contrast, only GABRA1 was reduced significantly in BA9 (65%, $P < 0.012$) (Figure 1, Table 2).

There was a significant difference on seizure status between subjects with autism and controls (chi-square=28.6, $P < 0.001$). However, presence of seizure disorder in subjects with autism did not have an impact on the observed significant reductions in protein levels of GABA_A receptor subunits (Table 3). Subjects with autism and seizure disorder showed significant reductions in protein levels of GABRA1 (63%, $P < 0.007$) and GABRB3 (51%, $P < 0.015$) in the cerebellum (Table 3) when compared to controls. In BA40, there were significant reductions in protein levels of GABRA1 (52%, $P < 0.018$), GABRA2 (45%, $P < 0.017$), GABRA3 (56%, $P < 0.010$), and GABRB3 (38%, $P < 0.009$) in subjects with autism and seizure disorder when compared to controls (Table 3). Finally, in BA9, there was a significant reduction in GABRA1 protein (65%, $P < 0.012$) in subjects with autism and seizure disorder when compared to controls (Table 3).

Discussion

We observed that GABRA1–3 and GABRB3 were significantly reduced in BA40, while GABRA1 and GABRB3 were significantly reduced in cerebellum, and GABRA1 was significantly reduced in BA9 of subjects with autism. These results were specific for GABA_A subunits, as β -actin was unchanged. While there was a significant difference between subjects with autism and control subjects regarding presence of seizure, it did not impact the observed reductions of GABA_A subunits in the three brain areas studied. All subjects with autism had varying degrees of mental retardation, which may also have had an impact on levels of GABA_A subunits.

Neuroanatomical studies have revealed structural abnormalities throughout the brain of subjects with autism including frontal (BA9) and parietal (BA40) cortices and cerebellum (for review, see Bauman and Kemper, 1994, 2005). Cerebellar structural abnormalities include loss of granular and Purkinje cells (Ritvo et al., 1986; Bauman and Kemper, 1994) and atrophy of Purkinje cells (Fatemi et al., 2000, 2002b). The cerebellar abnormalities may be responsible for the dysfunctions within the motor system associated with autism (reviewed in Nayate et al., 2005). Several studies also indicate that the parietal cortex may be abnormal in autism (Courchesne et al., 1993; Saitoh and Courchesne, 1998). Courchesne et al. (1993) reported on the reduction in volumes of the parietal lobes in some autistic subjects. Abnormalities of the parietal cortex in autism may be associated with disturbances of visuospatial-integration, impaired language, and slowed attention shift between and within modalities (Townsend et al., 1996; Haas et al., 1996). Abnormalities of the frontal cortex, including early growth abnormalities (Carper et al., 2002; Carper and Courchesne, 2005) and minicolumn maldevelopment (Casanova et al., 2002; Buxhoeveden et al., 2004), are likely to contribute to

the serious deficiencies in cognition, language, and emotional functions associated with autism (reviewed in Courchesne and Pierce, 2005). Our results demonstrate reductions in GABA_A subunits in these three regions, suggesting widespread GABAergic dysfunction in the brains of individuals with autism. Interestingly, GABRB3 knockout mice display significant decreases in surface area of cerebellar vermal lobules II - VII compared to control mice (Delorey et al., 2008). It may be that the observed changes in GABA_A subunits may contribute to similar gross abnormalities in subjects with autism.

While there is evidence of a strong genetic component to autism (Persico et al., 2006; Steffenburg et al., 1989), to date there have not been consistent findings for any specific gene. However, abnormalities in the 15q11-q13 locus are present in 1–4% of subjects with autism (McCauley et al., 2004; Schroer et al., 1998). There are several potential gene targets in this locus, including the GABA_A receptor gene cluster, which consists of three genes for the receptor's subunits: GABRB3, GABRA5, and GABRG3. Investigations of these genes has yielded mixed results, although GABRB3 seems to yield the most potential. While an association with a GABRB3 has been demonstrated in the Korean population (Kim et al., 2006), no association was found in the Japanese population (Tochigi et al., 2007). It has previously been shown that mice that are deficient in GABRB3 display epilepsy, as well as learning and memory deficits (DeLorey et al., 1998, 2008). Samaco et al. (2005) have demonstrated reduced GABRB3 expression in BA9 (Samaco et al., 2005). Samaco et al. (2005) also observed reductions in GABRB3 protein in mice deficient for MECP2, which codes methyl-CpG-binding protein 2 (MeCP2). This protein acts as a transcriptional repressor for methylated gene constructs, suggesting that epigenetic changes could lead to a reduction in GABRB3 (Samaco et al., 2005). More recently, Hogart et al. (2007) observed that while GABRB3 is normally biallelically expressed, in some samples from subjects with autism, GABRB3 was monoallelically expressed, and this correlated with a reduction of GABRB3 protein (Hogart et al., 2007). Moreover, chromatin immunoprecipitation experiments revealed two locations within the 5' end of the GABRB3 gene, where MeCP2 binds and these regions are methylated, suggesting that MeCP2 is a positive regulator of GABRB3 (Hogart et al., 2007). Taken together, these results suggest epigenetic dysregulation for GABRB3 in subjects with autism. Further studies are required to investigate possible epigenetic regulation of GABA_A receptor expression outside of the 15q11-q13 locus. While we did not observe decreased GABRB3 in BA9, we did observe reductions in BA40 and cerebellum, again suggesting a potential role for this subunit in the etiology and pathogenesis of autism.

The occurrence of seizure disorders with autism has been estimated anywhere from 4% to 44% (Tuchman and Rapin, 2002). This wide range is thought to be due to the heterogeneity of clinical populations (Canitano, 2007). Eplieptiform activity interferes with cognition by causing disturbances of vigilance, shifting attention, and sudden language difficulties (Binnie, 1993); phenomena that may also occur in children with autism and epilepsy. Alterations in all GABA receptors may partially explain the seizure disorders associated with autism. One of our subjects with autism died from seizures, and, in total, seven of our subjects with autism were comorbid with seizure disorders (Table 1). Whether the reduction in GABA_A subunits is due to seizure or whether reduced levels of GABA_A subunits contribute to seizure is not yet known. Future studies should include additional brain areas associated with seizure disorder, including the thalamus.

There are multiple ways in which the observed reductions GABA_A receptor protein may have occurred: 1) Reduction in GABA_A receptor mRNA, resulting in reduction of GABA_A receptor protein; 2) Posttranslational modification, leading to a reduction in GABA_A receptor protein; or 3) Epigenetics. It has been demonstrated that Reelin expression is reduced following hypermethylation of the *Reln* gene promoter (Abdolmaleky et al., 2005; Grayson et al., 2005, 2006). As described above, there is strong evidence of epigenetic control of GABRB3

expression in autism (Samaco et al., 2005; Hogart et al., 2007), and it is possible that other epigenetic mechanisms may play a role in expression of GABA_A subunit genes.

Our results are the first to demonstrate systematic changes in GABA_A subunit expression in superior frontal cortex, parietal cortex, and cerebellum of subjects with autism. Limitations of the study include: 1) Limited sample size; 2) Presence of mental retardation in subjects with autism; 3) Presence of seizure disorder in the majority of subjects with autism; 4) Lack of adequate samples from younger patients; and 5) Heterogeneity of samples, indicating presence of different biological etiologies for various subtypes of autism. Future studies should expand the number of brain regions to include other areas involved in the pathology of autism, including hippocampus and amygdala, use of techniques such as qRT-PCR to verify our results, and include data from children with autism and matched controls in order to account for neuroanatomical changes in childhood that are associated with autism.

Acknowledgments

Human tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders; the Harvard Brain Tissue Resource Center, which is supported in part by PHS grant number R24 MH068855; the Brain Endowment Bank, which is funded in part by the National Parkinson Foundation, Inc., Miami, Florida; and the Autism Tissue Program and is gratefully acknowledged. Grant support by National Institute of Child Health and Human Development (#5R01HD052074-01A2) to SHF is gratefully acknowledged.

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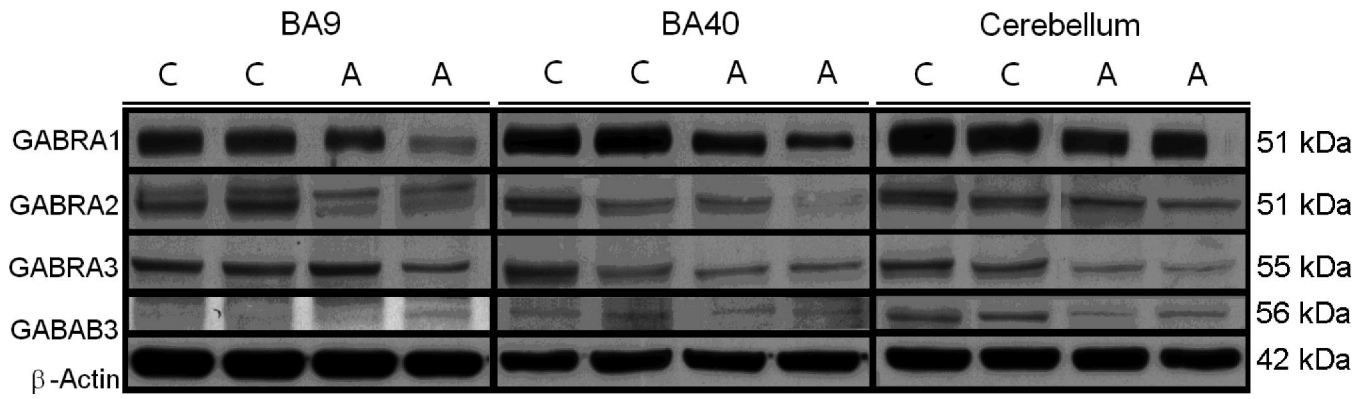


Figure 1. Representative samples of GABRA1 (51 kDa), GABRA2 (51 kDa), GABRA3 (55 kDa), GABRB3 (56 kDa), and β-Actin (42 kDa) in BA9, BA40, and cerebellum of subjects with autism (A) and matched controls (C).

Table 1
Demographic Data for Subjects with Autism and Controls

Case	Dx	Sex	Age	PMI (Hrs.)	Ethnicity	Medication History	Cause of Death	MR*	Seizure*	Brain Areas
B1078	Autistic	M	22	14.3	Caucasian	Dilantin, Tegretol, Phenobarbital, Theodure	Asphyxia	Yes	Yes	A40
B1045	Autistic	M	28	16.3	Caucasian	Cefobid, Urecholine, Duracef	Cardiac arrest	Yes	Yes	Cer, A40
B5000	Autistic	M	27	8.3	Caucasian	Synthroid	Drowning	Yes	No	Cer
B1401	Autistic	F	21	20.6	Caucasian	Tetracycline	Pneumonia, sepsis	Yes	Yes	Cer, A9, A40
B1664	Autistic	M	20	15	Caucasian	Vitamins B, C	Perforation of ulcer; asphyxia	Yes	Yes	Cer, A9, A40
B2825	Autistic	M	19	9.5	Caucasian	None	Seizure	Yes	Yes	Cer, A9, A40
B3511	Autistic	M	29	15	Caucasian	None	Hit by train	Yes	Yes	Cer, A9, A40
B3845	Autistic	M	30	28.4	Caucasian	Mellaril, Phenobarbital, Dilantin	Shock; acute pancreatitis	Yes	Yes	A9, A40
B1484	Autistic	M	19	15	Caucasian	None	Burns	Yes	No	A9, A40
B3829	Control	M	22	24.3	Caucasian	None	MVA	No	No	Cer
B4267	Control	M	26	20	African-American	None	MVA	No	No	Cer
B4268	Control	M	30	22	African-American	None	Cardiomyopathy	No	No	Cer, A40
B4269	Control	M	28	24	Caucasian	Lidocaine 12.0 mg/L found in blood	Arteriosclerotic cardiovascular disease	No	No	Cer, A9, A40
B4272	Control	M	19	17	Caucasian	None	Accident; chest injuries	No	No	Cer
B4275	Control	M	20	16	Caucasian	None	Accident	No	No	Cer, A9, A40
B4279	Control	F	20	21	Caucasian	None	MVA	No	No	Cer
B4362	Control	M	30	20	African-American	None	MVA	No	No	Cer, A9
B4101	Control	M	24	5	Unknown	None	Gun shot wound	No	No	Cer, A40
B4271	Control	M	19	21	African-American	EtOH, Advil, Amoxapine	Epiglottitis	No	No	A40
B4756	Control	M	56	23	Unknown	None	Myocardial infarction	No	No	Cer
B4363	Control	M	21	9	Caucasian	None	MVA	No	No	Cer, A40

Dx, diagnosis; Hrs, hours; PMI, postmortem interval; M, male; F, female; EtOH, alcohol; MVA, motor vehicle accident; MR, Mental retardation

* Communication from Dr. M. Bauman.

Table 2

Expression of GABRA1-GABRA3 and GABRB3 in Cerebellum, BA40, and BA9 in Subjects with Autism vs. Controls

Cerebellum	Control	Autistic	Change	<i>P</i> [*]
GABRA1 / β -Actin	1.350 \pm 0.470	0.504 \pm 0.356	↓ 63%	0.007
GABRA2 / β -Actin	0.073 \pm 0.028	0.053 \pm 0.025	↓ 27%	0.21
GABRA3 / β -Actin	0.216 \pm 0.100	0.142 \pm 0.073	↓ 34%	0.17
GABRB3 / β -Actin	0.061 \pm 0.022	0.030 \pm 0.013	↓ 51%	0.008

BA40	Control	Autistic	Change	<i>P</i>
GABRA1 / β -Actin	0.955 \pm 0.305	0.458 \pm 0.266	↓ 52%	0.018
GABRA2 / β -Actin	0.214 \pm 0.054	0.131 \pm 0.060	↓ 39%	0.033
GABRA3 / β -Actin	0.166 \pm 0.039	0.072 \pm 0.049	↓ 57%	0.005
GABRB3 / β -Actin	0.039 \pm 0.011	0.024 \pm 0.006	↓ 38%	0.006

BA9	Control	Autistic	Change	<i>P</i>
GABRA1 / β -Actin	0.260 \pm 0.050	0.090 \pm 0.062	↓ 65%	0.012
GABRA2 / β -Actin	0.159 \pm 0.041	0.092 \pm 0.042	↓ 42%	0.057
GABRA3 / β -Actin	0.127 \pm 0.006	0.193 \pm 0.083	↑ 52%	0.236
GABRB3 / β -Actin	0.040 \pm 0.002	0.041 \pm 0.009	↑ 2.5%	0.853

* two-tailed independent group t-test

Table 3

The Impact of Seizure on Expression of GABRA1-GABRA3 and GABRB3 in Cerebellum, BA40, and BA9 in Subjects with Autism vs. Controls

Cerebellum	Control	Autistic	Change	<i>P</i> [*]
GABRA1 / β -Actin	1.350 \pm 0.470	0.504 \pm 0.356	↓ 63%	0.007
GABRA2 / β -Actin	0.073 \pm 0.028	0.052 \pm 0.029	↓ 29%	0.240
GABRA3 / β -Actin	0.216 \pm 0.100	0.138 \pm 0.083	↓ 36%	0.200
GABRB3 / β -Actin	0.061 \pm 0.022	0.030 \pm 0.014	↓ 51%	0.015

BA40	Control	Autistic	Change	<i>P</i>
GABRA1 / β -Actin	0.955 \pm 0.305	0.458 \pm 0.266	↓ 52%	0.018
GABRA2 / β -Actin	0.214 \pm 0.054	0.118 \pm 0.055	↓ 45%	0.017
GABRA3 / β -Actin	0.166 \pm 0.039	0.073 \pm 0.053	↓ 56%	0.010
GABRB3 / β -Actin	0.039 \pm 0.011	0.024 \pm 0.006	↓ 38%	0.009

BA9	Control	Autistic	Change	<i>P</i>
GABRA1 / β -Actin	0.260 \pm 0.050	0.090 \pm 0.062	↓ 65%	0.012
GABRA2 / β -Actin	0.159 \pm 0.041	0.096 \pm 0.045	↓ 40%	0.098
GABRA3 / β -Actin	0.127 \pm 0.006	0.193 \pm 0.083	↑ 52%	0.236
GABRB3 / β -Actin	0.040 \pm 0.002	0.043 \pm 0.008	↑ 7.5%	0.597

* two-tailed independent group t-test