Differential Hippocampal Expression of Glutamic Acid Decarboxylase 65 and 67 Messenger RNA in Bipolar Disorder and Schizophrenia

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Background: Expression of messenger RNA (mRNA) for the γ-aminobutyric acid (GABA)–synthesizing enzyme, glutamic acid decarboxylase (GAD), in the prefrontal cortex and the number of GABAergic neurons in the hippocampus are reduced in schizophrenia and bipolar disorder. We tested the hypothesis that the expression of the 2 isoforms, one 65 kd (GAD65) and the other 67 kd (GAD67), is differentially affected in the hippocampus in schizophrenia and bipolar disorder.

Methods: Hippocampal sections from 15 subjects in 3 groups (control subjects and subjects with schizophrenia and bipolar disorder) were studied using an in situ hybridization protocol with sulfur 35–labeled complementary riboprobes for GAD65 and GAD67 mRNA. Emulsion-dipped slides were analyzed for the density of GAD mRNA–positive neurons in 4 sectors of the hippocampus and for the cellular expression level of both GAD mRNAs.

Results: The density of GAD65 and GAD67 mRNA–positive neurons was decreased by 45% and 43%, respectively, in subjects with bipolar disorder, but only 14% and 4%, respectively, in subjects with schizophrenia. The decreased density of GAD65 mRNA–positive neurons in subjects with bipolar disorder was significant in sectors CA2/3 and dentate gyrus, and that of GAD67 mRNA–positive neurons was significant in CA4, but not other hippocampal sectors. Cellular GAD65 mRNA expression was significantly decreased in subjects with bipolar disorder, particularly in CA4, but not in schizophrenic subjects. Cellular GAD67 mRNA expression was normal in both groups.

Conclusion: We have found a region-specific deficit of GAD65 and GAD67 mRNA expression in bipolar disorder.

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SUBJECTS AND METHODS

SUBJECTS

Brain specimens were obtained from the Harvard Brain Tissue Resource Center, Belmont, Mass, and included 15 control subjects, 15 subjects with bipolar disorder (bipolar subjects), and 15 subjects with schizophrenia (schizophrenic subjects) (Table). Each control was matched with 1 schizophrenic and 1 bipolar subject based on age and postmortem interval (PMI) to form 15 triplets. The mean (±SD) differences within each triplet were less than 3.16 (±2.50) years for age and 3.23 (±2.34) hours for PMI. The female-male ratios were 3:10 for the controls and 6:9 for both patient groups.

During the microscopic analysis of the study material, 1 schizophrenic subject (S3346, triplet 9) was found to have pathologic changes in the hippocampal CA1 sector consistent with a diagnosis of hippocampal sclerosis. A neuropathologist had studied the contralateral hippocampus, and no signs of hippocampal abnormality had been present. This subject was removed from the analysis.

Diagnoses were made by means of retrospective review of medical records and of an extensive questionnaire about social and medical history completed by family members of the donor. Two psychiatrists (S.H. and F.M.B.) reviewed all records and applied the criteria of Feighner et al19 for the diagnosis of schizophrenia and DSM-III-R criteria20 for the diagnosis of schizoaffective and bipolar disorders. During the course of the study, documentation for 1 bipolar subject (BP3256, triplet 3) was not sufficient to verify the diagnosis. We therefore decided to exclude this subject from the study. The removal of 2 subjects resulted in 15 controls, 14 schizophrenic subjects, and 14 bipolar subjects in the study.

TISSUE PREPARATION AND IN SITU HYBRIDIZATION

One hemisphere of each brain specimen was dissected fresh, and 3-mm-thick blocks of a central portion of the hippocampus were removed. The hippocampal blocks were fixed in 4% paraformaldehyde in ice-cold 0.1M phosphate buffer (pH, 7.4) for 90 minutes, immersed in 30% sucrose in the same buffer overnight, then frozen in medium (Tissue Tek H11003, H11003, or H11003/3, H11003/3, or H11003/3) at −70°C. In 27 of the 45 cases, a piece of frozen cerebellar tissue was homogenized in 15 volumes of distilled and deionized water, and the acidity was measured using pH meter (Corning Inc, Acton, Mass). These measurements were used to assess whether brain pH may have affected the integrity of GAD mRNA during the PMI.

RESULTS DISTRIBUTION OF GAD65 AND GAD67 mRNA-POSITIVE NEURONS IN THE HUMAN HIPPOCAMPUS

The hippocampus of each subject was sectioned at 10 µm and mounted on slides (Superfrost Plus; Fisher Scientific, Pittsburgh, Pa). Two sections were used per subject, and sections were mounted (2 per slide) such that the following 3 slides were used for each matched triplet: 1 slide containing control and schizophrenic tissue; 1, control and bipolar tissue; and 1, schizophrenic and bipolar tissue. This method of mounting sections was designed to control for slide-to-slide variability associated with the in situ hybridization procedure.

The other hemisphere of each brain specimen was cut in serial sections for a complete neuropathological examination. Results of gross and microscopic examination did not reveal any evidence of Alzheimer disease, cerebrovascular accident, or tumors.

The complementary RNA probes were transcribed in vitro from full-length human complementary DNA (cDNA) clones inserted into bluescript vector (2.01-kilobase [kb] human GAD65 and 2.7-kb human GAD67, provided by Allan Tobin, PhD, University of California–Los Angeles). The cDNA probes have been characterized previously and were found to hybridize with human brain RNA.41 Probes were synthesized using sulfur 35–labeled uridine triphosphate (NESS Life Sciences, Boston, Mass). The probe specificity for GAD65 and GAD67 was demonstrated by means of a control experiment in which sense probes failed to reveal any specific hybridization signal. To ensure full penetration into tissue, the 2.01-kb GAD65 cRNA and 2.7-kb human GAD67 probes were hydrolyzed with an equal volume of sodium carbonate buffer (pH, 10.2; 40mM sodium bicarbonate and 60mM sodium carbonate) at 60°C for a resultant average fragment size of 0.8 kb. The reaction was stopped by adding 0.08 volume of 2M sodium acetate in 6.25% glacial acetic acid. Probes were then reconstituted in a hybridization buffer consisting of 30% formamide, 0.1% yeast transfer RNA, 10% dextran sulfate, 1× Dehardt solution, 0.5M EDTA, 0.02% sodium dodecyl sulfate, 4% saline sodium citrate buffer (Sigma-Aldrich Corp, St Louis, Mo), 10mM dithiothreitol, and 0.1% single-stranded DNA, at a final probe concentration of 0.4 ng/µL of hybridization buffer.

Slides were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH, 7.4) for 15 minutes, treated in proteinase K solution for 15 minutes, and then incubated in 0.1M triethanolamine (pH, 8.0) for 5 minutes, followed by 0.1M triethanolamine and 0.25% acetic anhydride (pH, 8.0) for 10 minutes. Slides were then dehydrated using a graded series of ethyl alcohol solutions (50%-100%). Sections were hybridized for 3 hours at 55°C. After hybridization, slides were incubated in 0.025 mg/mL RNase A in 0.3M sodium chloride and 0.05M phosphate buffer for 1 hour at 37°C, and washed in 50% formamide, 0.5M sodium chloride, 0.1M triethanolamine (pH, 7.4) for 90 minutes, immersed in 30% sucrose in the same buffer overnight, then frozen in medium (Tissue Tek H11003, H11003, or H11003/3, H11003/3, or H11003/3) at −70°C. In 27 of the 45 cases, a piece of frozen cerebellar tissue was homogenized in 15 volumes of distilled and deionized filtered water, and the acidity was measured using a pH meter (Corning Inc, Acton, Mass). These measurements were used to assess whether brain pH may have affected the integrity of GAD mRNA during the PMI.

GAD67 mRNA in a subset of prefrontal cortex neurons in schizophrenia16-18 and bipolar disorder.18

Here we present, to our knowledge, the first comprehensive study of GAD mRNA expression in the human hippocampus. The relative density of GAD mRNA–positive neurons has been determined in all subsectors of the human hippocampus, and the relative cellular expression within identified GAD mRNA–positive neurons has been estimated. This approach has allowed us to test the hypothesis that the expression of the 2 GAD mRNAs may be differentially affected in schizophrenia and/or bipolar disorder.

Light microscopic analysis of the emulsion-dipped sections showed a characteristic clustering of silver grains over Nissl-stained cell bodies (Figure 1). Grain clusters were traced within the DG, CA4, CA2/3, and CA1 hippocampal subdivisions (Figure 2). The GAD65 and GAD67 mRNA–positive neurons were found in all 4 subdivisions (Figure 2). The GAD65 mRNA–positive neurons were more...
0.05M phosphate buffer at 63°C for 30 minutes, followed by an overnight wash in 0.25 × silver sulfadiazine and chlorhexidine at room temperature. Slides were then covered with NTB2 emulsion (Eastman Kodak Co, Rochester, NY) and exposed for 21 days for cellular analysis. After development, slides were counterstained with cresyl violet and dehydrated through a graded series of ethanol and xylene. A coverslip was then applied.

**QUANTIFICATION OF GAD mRNA EXPRESSION**

All slides were coded to conceal subject identity throughout the study. All sections undergoing in situ hybridization were used for analysis, which was performed using a bright-field microscope (Leitz Laborlux; Leitz, Wetzlar, Germany) interfaced with an image analysis system (Bioquant MEG IV; R & M Biometrics, Nashville, Tenn). The microscopic analysis was conducted in the following 3 stages. First, we outlined 4 sectors, ie, the dentate gyrus (DG) and the cornu ammonis sectors CA4, CA2/3, and CA1, in each hippocampus. Second, we counted all grain clusters within the sectors using an XYZ encoder to establish the density of GAD mRNA–positive neurons in the hippocampus. Finally, we counted grains in all or a subsample of the previously identified clusters to assess the cellular expression of GAD mRNA in individual neurons.

All sections were inspected at low power using ×4 and ×10 objectives to outline the boundaries of the 4 hippocampal sectors. The DG sector included the molecular and granule cell layers, but not the polymorph cell layer. Sector CA4 was defined as the area encapsulated by the granule cell layer, but included the polymorph cell layer of the DG. Because the granule-polymorph cell layer border is very distinct, whereas the polymorph-hilus border is rather ambiguous, this approach made possible the performance of the analysis in a reproducible manner. The medial boundary of sector CA2/3 was defined by a straight line that connected the 2 ends of the C-shaped granule cell layer of the DG, because the anatomical CA2/3-CA4 border was difficult to define in our material. The lateral boundary of the CA2/3 sector was defined by a decrease in cell density and lighter counterstaining, indicating the beginning of sector CA1. Pilot studies demonstrated a homogeneous density of grain clusters in sector CA1, which allowed us to sample a part of the large CA1 sector.

The position of each grain cluster was plotted using a ×25 objective within each area outlined for the various sectors. All clearly identifiable clusters overlying a neuronal profile were counted. One section from each triplet was analyzed twice for test-retest reliability of the cluster counting. These 15 pairs demonstrated a high reliability of the grain clustering in all 4 sectors (Spearman ρs, 0.94, 0.95, 0.99, and 0.92 for DG, CA4, CA2/3, and CA1, respectively).

Grain counting within individual clusters was performed using a ×40 objective. All clusters were analyzed if a given sector contained up to 20 grain clusters. If a sector contained more than 20 clusters, the samples were taken in a systematic fashion in the following regions: 20 clusters at the polymorph-granule cell layer border for the CA4 sector and 10 clusters each at the medial and lateral borders of the CA2/3 sector. For sector CA1, 20 clusters were sampled in a columnar fashion through the full width of the pyramidal cell layer. First, we outlined each cluster using a cursor displayed on the computer monitor. The cluster area was then assigned a threshold, and the area covered by the grains within each cluster was determined as a pixel count. Before each sector was evaluated, the light intensity was adjusted to be consistent throughout the study. After each sector was evaluated, 2 areas within the sectors that were free of any grain clusters were sampled to determine the local background level of grains. This local background level was subtracted from the mean area covered by grains to give a corrected grain count for each sector.

Overall, 37373 clusters indicating GAD mRNA–positive neurons were counted (2135 in DG, 9613 in CA4, 6510 in CA2/3, and 19115 in CA1); 11641 clusters (31% of all clusters) were sampled for grain counting (1404 in DG, 3632 in CA4, 3242 in CA2/3, and 3363 in CA1).

**STATISTICAL ANALYSIS**

For each subject, the numerical density of clusters and grain counts per cluster within each of the 4 hippocampal sectors were averaged across the 2 sections studied. The mean cluster and grain densities were entered into a repeated-measures analysis of variance (ANOVA), with diagnosis (2 levels, control-bipolar or control-schizophrenia) and sector (repeated measure with 4 levels) as main effects and the confounding variables (hemisphere, age, sex, medication type, chlorpromazine equivalents, brain pH, PMI, and storage time) as covariates. The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3).

The numerical density of GAD65 mRNA–positive neurons was decreased from 34% (CA1) to 55% (DG) in bipolar subjects and from 1% (CA1) to 21% (CA4) in schizophrenic subjects (Figure 3), relative to controls. The decrease of hippocampal GAD65 mRNA–positive neurons was significant for bipolar subjects (main effect of group, F1,14=17.4; P=.001) but not for schizophrenic subjects (F1,14=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3). The changes in the bipolar subjects did not affect all hippocampal subdivisions (region-by-diagnosis interaction, F1,4=1.57; P=.23) (Figure 3).
polar subjects was compared with their matched controls in sector CA2/3, the region with the most significant change, 11 of the 13 bipolar subjects had a lower density of GAD65 mRNA–positive neurons (Figure 4).

The numerical density of GAD67 mRNA–positive neurons was decreased from 33% (CA1) to 50% (CA4) in bipolar subjects relative to controls (main effect of group, $F_{1,14}=7.73; P=.02$), but did not show an overall decrease in schizophrenic subjects ($F_{1,14}=0.16; P=.70$) (Figure 3). The changes in the bipolar subjects were regionally specific (region-by-diagnosis interaction, $F_{1,3}=6.13; P=.002$), with the decrease in sector CA4 being significant ($t=2.76; P=.04$; Cohen $d=−1.02$), but not in CA2/3 ($t=2.47; P=.08$), CA1 ($t=1.93; P=.26$), or DG ($t=1.31; P=.80$).
CELLULAR EXPRESSION OF GAD65 AND GAD67 mRNA

The area covered by grains within each cluster was a measure of the cellular expression of GAD mRNA. The controls, but not the 2 patient groups, showed the highest GAD65 mRNA expression in sector CA4 (Figure 5). Expression of GAD65 mRNA was decreased, relative to controls, from 21% (CA1) to 37% (CA4) in bipolar subjects (main effect of group, \( F_{1,14}=5.38; P = .04 \)) and from 2% (CA2/3) to 24% (CA4) in schizophrenic subjects (\( F_{1,14}=1.65; P = .22 \)) (Figure 5). The decrease of cellular GAD65 mRNA expression in bipolar subjects was regionally specific (region × diagnosis interaction, \( p < .05 \)).
Expression of GAD67 mRNA was not significantly decreased in the bipolar (F₁,₁₄=1.36; P=.28) or the schizophrenic (F₁,₁₄=.005; P=.95) group (Figure 5).

The 8 confounding variables were evaluated with respect to the numerical density of GAD mRNA–positive neurons and expression of GAD mRNA. There were no significant correlations (P> .05 for all sectors) of the 2 dependent variables with PMI, storage time, or chlorpromazine equivalents. Furthermore, patients treated with atypical antipsychotic medication did not differ from those treated with typical antipsychotic medication.

The overall effects of hemisphere and sex were evaluated by comparing all female (n=16) and male (n=27) subjects and all left (n=28) and right (n=15) hemisphere specimens. We found no effect of hemisphere. Male subjects had a higher density of GAD₆₅ mRNA–positive neurons in CA1 (t=2.51; P=.02) and a higher expression of GAD mRNA expression in CA4 neurons (t=2.05; P=.047), but all other parameters showed no effect of sex. The 3 diagnostic groups had similar male-female and left-right hemisphere ratios, and all of our findings from the ANOVA using diagnosis and sector as main effects were confirmed by means of ANCOVAs that included sex and hemisphere as single or dual covariates.

Age was negatively associated with the density of GAD₆₅ mRNA–positive neurons (P< .05) and the cellular expression levels of GAD₆₅ mRNA in all sectors (P< .02) and GAD₇ mRNA in sector CA4 (P< .02). Because the 3
subjects of each triplet were matched by age, we controlled for the effect of age by including triplet as a blocking effect in our ANOVA.

Brain tissue suitable for pH measurements was available in 27 of the 43 subjects studied (Table). Mean (±SD) brain pH did not differ among the 3 groups (F2,24= .06; P = .94) that included 10 controls (6.47±0.3), 9 bipolar subjects (6.51±0.26), and 8 schizophrenic subjects (6.50±0.37). The pH was positively associated with the mean density of GAD65 and GAD67 mRNA–positive neurons in DG and CA4 (P < .04), but not in CA2/3 and CA1 (P > .05). Brain pH also correlated with the expression of GAD65 and GAD67 mRNA in all 4 hippocampal subdivisions (P < .01).

Since pH could explain a significant variance component for the 2 variables of interest, ANCOVAs were performed in the subset of 27 subjects using pH and age as covariates. The decreased density of GAD mRNA– positive neurons in bipolar disorder was confirmed for GAD65 mRNA–positive neurons in CA2/3 (P = .002), CA4 (P = .006), and DG (P < .001), and for GAD65 mRNA–positive neurons in CA4 (P = .02) and CA2/3 (P = .03). Furthermore, the trend of decreased expression of GAD65 mRNA in CA4 in bipolar subjects also became significant (P = .01). All other results from the main analysis remained unchanged.

The findings reported herein indicate that bipolar disorder, but not schizophrenia, is associated with a significant decrease of GAD mRNA–positive neurons and of GAD65 mRNA expression in the hippocampus. The loss of GAD mRNA–positive neurons in bipolar disorder was confirmed for GAD65 mRNA–positive neurons in CA2/3 (P = .002), CA4 (P = .006), and DG (P < .001), and for GAD65 mRNA–positive neurons in CA4 (P = .02) and CA2/3 (P = .03). Furthermore, the trend of decreased expression of GAD65 mRNA in CA4 in bipolar subjects also became significant (P = .01). All other results from the main analysis remained unchanged.

**COMMENT**

The findings reported herein indicate that bipolar disorder, but not schizophrenia, is associated with a significant decrease of GAD mRNA–positive neurons and of GAD65 mRNA expression in the hippocampus. The loss of GAD mRNA–positive neurons in bipolar disorder was confirmed for GAD65 mRNA–positive neurons in CA2/3 (P = .002), CA4 (P = .006), and DG (P < .001), and for GAD65 mRNA–positive neurons in CA4 (P = .02) and CA2/3 (P = .03). Furthermore, the trend of decreased expression of GAD65 mRNA in CA4 in bipolar subjects also became significant (P = .01). All other results from the main analysis remained unchanged.

vious studies demonstrated abnormalities of hippocampal interneurons and of GAD67 mRNA expression in schizophrenia. On the basis of studies in rat, approximately 95% of GABAergic neurons express both GAD genes. However, the relative expression of GAD65 and GAD67 mRNA and the translation into protein differ at the regional and cellular levels. This study is, to our knowledge, the first description of the distribution of GAD65 and GAD67 mRNA–positive neurons in the human hippocampus. The highest density of GAD mRNA–positive neurons was found in the polymorph cell layer (included in the CA4 sector in this study), a pattern similar in the human and nonhuman primate hippocampus.

The mechanism responsible for the differential expression of the 2 GAD mRNA isoforms is not fully understood, but most likely involve separate mechanisms, since the regulatory regions for their respective genes show...
Figure 5. Bar graphs showing the mean (±SEM) area covered by grains over 65-kd glutamic acid decarboxylase (GAD65) (A) and 67-kd GAD (GAD67) (B) messenger RNA (mRNA)-positive neurons in control subjects (n=15), subjects with schizophrenia (n=14), and subjects with bipolar disorder (n=13 in hippocampal sectors dentate gyrus [DG] and CA2/3; n=14 in sectors CA4 and CA1).

less than 25% homology. Previous studies in rat striatum demonstrated a preferential modulation of GAD65 and GAD67 mRNA expression via D1 and D2 dopamine receptors, respectively. Previous studies also suggested that GAD67 is more concentrated in neurons that fire tonically, whereas GAD65 is more concentrated in neurons with a low basal firing rate, whose activation is under strong synaptic control. The most prominent decrease in GAD65 mRNA expression per cell was found in subdivision CA4, whereas that for GAD67 was not different. The GABAergic neurons in this region are known to provide local inhibitory control in the sector DG. Thus, decreased expression of GAD65 mRNA in this subset of neurons could indicate a decreased control over information arriving via the perforant pathway, resulting in increased activity conducted along the trisynaptic pathway.

One possible explanation for the decrease of GAD mRNA in bipolar disorder is an overt loss of GABAergic interneurons, rather than a decrease of expression in an otherwise intact neuron. Hippocampal cell loss in psychosis appears to occur preferentially in the population of interneurons, since studies of total cell number or pyramidal cell density have not found differences in schizophrenia or bipolar disorder. We found a selective decrease of GAD67 mRNA-positive neurons in sector CA2 in bipolar disorder, but a similar decrease in schizophrenia was not observed. The more pronounced decrease of GAD65 mRNA-positive neurons compared with GAD67 mRNA-positive neurons in bipolar disorder suggests a preferential decrease in GAD65 expression. Furthermore, the pronounced decrease of GAD65 mRNA expression per cell in sector CA4 is consistent with the notion that decreased GAD mRNA expression is, in part, due to abnormal regulation in existing GABAergic interneurons in bipolar disorder.

We found no correlation of GAD mRNA expression and the dosage of antipsychotic medication taken by all of the schizophrenic subjects and some of the bipolar subjects before death. Therefore, the observed changes in GAD mRNA expression in bipolar disorder are unlikely to be due to the treatment with antipsychotic drugs. Previous studies, however, have demonstrated that treatment with haloperidol can increase GABA-immunoreactive axon terminals in rat medial prefrontal cortex and that the number of GAD65-immunoreactive terminals correlates positively with the dose of typical antipsychotic drugs in schizophrenic subjects. Since the average chlorpromazine equivalent dose in schizophrenic subjects was double that of bipolar subjects, these drugs may have stimulated the hippocampal GABA system more in the schizophrenic subjects. Schizophrenic subjects showed a marked up-regulation of the GABA A receptor in the hippocampus, particularly in the sectors CA4, CA3, and CA2, which is consistent with the possibility that an inherent defect exists in this system in schizophrenia, possibly one that is partially compensated by antipsychotic drugs.

Our finding that GAD67 mRNA expression is not significantly decreased in schizophrenia differs from a preliminary report of decreased hippocampal GAD67 mRNA expression in schizophrenia. Our study also suggests that a decrease of GAD65 mRNA expression, previously documented in subsets of neurons in the prefrontal cortex, is not ubiquitously found in schizophrenia. Although details of the experimental protocols could explain some of the differences between the studies, the decrease of GAD67 mRNA expression in schizophrenia likely affects isocortex more than limbic allocortex, including the hippocampal formation. This finding is consistent with the differential modulation of GAD expression, which varies across brain regions and most likely involves multiple mechanisms.

The lack of marked changes of hippocampal GAD mRNA expression in schizophrenia is of interest for the interpretation of recent neuroimaging studies that have demonstrated increased hippocampal activity during rest, during the experience of auditory hallucinations, and during word retrieval in schizophrenia. Our study makes it less likely that the increased baseline activity and the lack of normal modulation of hippocampal activity seen in schizophrenia are due to a decreased inhibitory tone, as assessed by the expression of GAD mRNA. The GABAergic activity might still be decreased, even with a normal expression of GAD mRNA, if the translation into GAD protein is abnormal. However, the number of GAD65 immunoreactive puncta...
on hippocampal neurons was found to be normal in schizophrenia, although schizophrenic subjects free of neuroleptic drugs showed a significant reduction.20

A recent study of GAD65 immunoreactive terminals in the cingulate and prefrontal cortices found decreases in bipolar disorder but not in schizophrenia.36 With our finding of decreased GAD65 mRNA expression in bipolar disorder, this finding seems to indicate that abnormalities of hippocampal GAD expression are more prominent in bipolar disorder than in schizophrenia. Studies of correlates of this GABAergic abnormality at the level of neural circuitry and cognition would be of interest. Such studies could provide insights into the link between GABAergic abnormalities of the hippocampus and the clinical features of bipolar disorder, potentially leading to new targets for pharmacological intervention.

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REFERENCES


17. Volk DW, Austin MG, Pierré JS, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase(65) messenger RNA expression in a subset of prefrontal cortical γ-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry. 2000;57:237-245.


27. Feldblum S, Erlander MG, Tobin AJ. Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. J Neurosci. 1993:34:689-706.


