Reduced Glutamate Decarboxylase 65 Protein Within Primary Auditory Cortex Inhibitory Boutons in Schizophrenia

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Background: Schizophrenia is associated with perceptual and physiological auditory processing impairments that may result from primary auditory cortex excitatory and inhibitory circuit pathology. High-frequency oscillations are important for auditory function and are often reported to be disrupted in schizophrenia. These oscillations may, in part, depend on upregulation of gamma-aminobutyric acid synthesis by glutamate decarboxylase 65 (GAD65) in response to high interneuron firing rates. It is not known whether levels of GAD65 protein or GAD65-expressing boutons are altered in schizophrenia.

Methods: We studied two cohorts of subjects with schizophrenia and matched control subjects, comprising 27 pairs of subjects. Relative fluorescence intensity, density, volume, and number of GAD65-immunoreactive boutons in primary auditory cortex were measured using quantitative confocal microscopy and stereologic sampling methods. Bouton fluorescence intensities were used to compare the relative expression of GAD65 protein within boutons between diagnostic groups. Additionally, we assessed the correlation between previously measured dendritic spine densities and GAD65-immunoreactive bouton fluorescence intensities.

Results: GAD65-immunoreactive bouton fluorescence intensity was reduced by 40% in subjects with schizophrenia and was correlated with previously measured reduced spine density. The reduction was greater in subjects who were not living independently at time of death. In contrast, GAD65-immunoreactive bouton density and number were not altered in deep layer 3 of primary auditory cortex of subjects with schizophrenia.

Conclusions: Decreased expression of GAD65 protein within inhibitory boutons could contribute to auditory impairments in schizophrenia. The correlated reductions in dendritic spines and GAD65 protein suggest a relationship between inhibitory and excitatory synapse pathology in primary auditory cortex.

Key Words: GAD65, postmortem human tissue, primary auditory cortex, quantitative microscopy, schizophrenia, stereology

Individuals with schizophrenia exhibit basic auditory processing deficits (1) that contribute to debilitating negative and cognitive symptoms. One such deficit is impaired tone frequency discrimination (2,3), assessed either behaviorally or as reduction in the mismatch negativity (MMN) response of the auditory event-related potential (3–5). The inability to properly discriminate between different frequencies may make phoneme identification difficult, translating to impaired speech comprehension in subjects with schizophrenia (6,7). Evidence suggests that features of schizophrenia stemming from disrupted tone processing have a significant impact on patients' quality of life, as tone-matching performance is severely impaired in subjects who require long-term residential care (8). Correlated with tone-matching deficits, subjects with schizophrenia show reduced ability to use pitch-based acoustic cues to recognize vocally expressed emotion (2,9). Consequently, inability to recognize emotional components of speech, a negative symptom of schizophrenia (10), contributes to dysfunction in social interactions (11). Thus, basic impairments in tone frequency discrimination likely impair higher-order functions downstream of auditory stimulus processing, contributing to some of the signs and symptoms of schizophrenia.

Studies in animals suggest that the ability to discriminate frequency depends on auditory cortex function (12,13). The primary auditory cortex is located on Heschl's gyrus, found within the Sylvian fissure on the superior temporal gyrus (STG). Reduction of STG gray matter volume is one of the most consistently reported gray matter volume change findings in schizophrenia subjects (14) and those who are genetically at risk (15). Specifically, findings include reductions of gray matter volume in Heschl's gyrus in both cross-sectional analyses of subjects with schizophrenia (16–18) and longitudinal studies of high-risk individuals (19).

One measure of auditory neurophysiology known to depend on the integrity of the primary auditory cortex is the auditory steady state response (aSSR) (20–22). Many studies have found the aSSR to be abnormal in patients with schizophrenia (23–27). Steady state responses are generated in response to temporally modulated stimuli, are based on the synchronized activity of large populations of neurons, and represent the ability of neural circuits to oscillate at different frequencies (28). Subjects with schizophrenia exhibit abnormal aSSR entrainment to tones and white noise bursts modulated at gamma-range frequencies (30–80 Hz) (23,29). Individuals with schizophrenia also demonstrate reduced power of induced gamma-range oscillatory activity in response to an unmodulated pure tone (24). Altered high-frequency oscillatory activity may reflect a physiological impairment of auditory cortex circuitry that

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Table 1. Summary of Subject Characteristics for Cohorts 1 and 2

	Cohort 1		Cohort 2		Total	
	Control	Schizophrenia	Control	Schizophrenia	Control	Schizophrenia
n	15	15	12	12	27	27
Mean Age (SD)	46.8 (8.3)	47.6 (5.5)	45.1 (12.9)	47.3 (13.4)	46.0 (10.4)	47.4 (9.6)
Range	27–64	38–63	19–65	25–71	19–65	25–71
Sex (F/M)	6/9	6/9	4/8	4/8	10/17	10/17
Handedness (R/L/A/U)	10/4/0/1	7/3/1/4	11/1/0/0	6/2/1/3	21/5/0/1	13/5/2/7
PMI (SD)	13.9 (5.5)	15.9 (6.6)	18.0 (6.6)	17.9 (8.8)	15.7 (6.3)	16.8 (7.6)
Storage Time, Months (SD)	168 (29)	167 (26)	111 (27)	102 (30)	142 (40)	138 (43)
Illness Duration, Years (SD)		24.9 (5.6)		22.1 (14.6)		23.7 (10.6)
Range		14–34		3–50		3–50
Suicide, *n* (%)		3 (20%)		2 (17%)		5 (19%)
Schizoaffective, *n* (%)		3 (20%)		4 (33%)		7 (26%)
Living Independently ATOD, *n* (%)		7 (47%)		2 (17%)		9 (33%)
Alcohol/Substance Abuse ATOD, *n* (%)		9 (60%)		7 (58%)		16 (59%)
History of Cannabis Use, *n* (%)		3 (20%)		5 (42%)		8 (30%)
Antipsychotic ATOD, *n* (%)		13 (87%)		11 (92%)		24 (89%)
Benzodiazepine ATOD, *n* (%)	1 (7%)	5 (33%)		1 (8%)	1 (4%)	6 (22%)
Anticonvulsant ATOD, *n* (%)		3 (20%)		4 (33%)		7 (26%)
Antidepressant ATOD, *n* (%)		5 (33%)		4 (33%)		9 (33%)

Each subject in cohorts 1 and 2 was previously matched to a normal comparison subject based on sex and as closely as possible for age and postmortem interval and group matched for handedness. There were no diagnostic group differences in age [$t(52) = .517$, $p = .608$] or postmortem interval [$t(52) = .584$, $p = .561$] or in the distribution of handedness between diagnostic groups ($\chi_1^2 = 1.46$, $p = .314$). Mean storage time did not differ between diagnostic groups [cohort 1: $t(28) = .040$, $p = .968$; cohort 2: $t(22) = .596$, $p = .557$].

A, ambidextrous; ATOD, at time of death; F, female; L, left-handed; M, male; PMI, postmortem interval; R, right-handed; U, unknown.

contributes to reduced ability to discriminate the features of auditory stimuli (30).

Inhibitory γ-aminobutyric acid (GABA)-ergic interneurons contribute to the generation of neural oscillatory activity through the production of rhythmic inhibitory postsynaptic potentials in excitatory neurons, inducing synchronization of their firing (31,32). It has been shown that rapid adjustments to levels of inhibition are required for controlling changes in oscillation frequency (33). This necessity of rapid adjustments to levels of inhibition suggests that modulation of the amount of GABA synthesized for release might be crucial for the ability of interneurons to mediate oscillatory activity. The GABA-producing activity of the 65 kDa isoform of glutamate decarboxylase (GAD65) is rapidly upregulated via binding to its cofactor pyridoxal-5′-phosphate under conditions of increased neural activity (34,35). Mice lacking GAD65 demonstrate reduced GABA release during sustained activation of inhibitory neurons (36). Glutamate decarboxylase 65 may therefore be particularly important for rapidly modulating GABA synthesis to maintain gamma-range oscillatory activity in the cortex during conditions of sustained high interneuron firing rates (37). Thus, impaired gamma-range oscillatory activity in auditory cortex in schizophrenia could indicate that GAD65-mediated GABA synthesis in inhibitory boutons is impaired in such a way as to be unable to keep up with the necessary high firing rates.

In the present study, we asked whether the relative level of GAD65 protein is reduced in the auditory cortex of subjects with schizophrenia. As auditory cortex circuitry is thought to participate in the generation of both aSSRs and the MMN component of the auditory event-related potential (20–22,38,39) and MMN reflects activity of the supragranular cortical layers (4,40), we wanted to determine whether GAD65 protein was reduced in this subregion of auditory cortex, specifically at its site of action: the bouton. To address this, we used quantitative fluorescence microscopy to assess levels of GAD65 protein within deep cortical layer 3 inhibitory boutons. We found that whereas the number and density of GAD65-expressing inhibitory boutons in deep layer 3 of primary auditory cortex were unaltered, these boutons contained less GAD65 protein, suggesting that the amount of GABA available for release when auditory cortex interneurons are firing at high rates may be reduced in subjects with schizophrenia.

Methods and Materials

Subjects and Animals

We studied two cohorts (Table 1 and Table S1 in Supplement 1) of subjects diagnosed with schizophrenia or schizoaffective disorder and matched control subjects included in our previous studies (41–44). We also studied a cohort of four male macaque monkeys (*Macaca fascicularis*) chronically exposed to haloperidol decanoate and four control macaques matched for sex and weight (43). See Supplemental Methods in Supplement 1 for further description.

Immunohistochemistry

Auditory cortex containing tissue sections from matched pairs were processed together in immunohistochemistry runs. Glutamate decarboxylase 65 was detected using a mouse anti-GAD65 primary antibody (MAB351; Millipore, Billerica, Massachusetts), the specificity of which was assessed by Western blot and immunohistochemistry (see Supplemental Methods and Figure S1 in Supplement 1).

Quantification of GAD65-Immunoreactive Puncta

GAD65-immunoreactive (IR) boutons within deep cortical layer 3 of primary auditory cortex were quantified in this study using confocal microscopy. Stereologic sampling was conducted as shown in Figure 1. Sections were coded so that the experimenter was blind to diagnostic or drug exposure group, and sections were organized into sets so that sections from paired subjects were imaged during the same imaging session. Images were collected and