Differential Distribution of Proteins Regulating GABA Synthesis and Reuptake in Axon Boutons of Subpopulations of Cortical Interneurons

Kenneth N. Fish¹, Robert A. Sweet¹,²,³ and David A. Lewis¹,⁴

¹Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA; ²Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15213, USA; ³VISN 4 Mental Illness Research, Education and Clinical Center (MIRECC), VA Pittsburgh Healthcare System, Pittsburgh, PA, 15206, USA and ⁴Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213, USA

Address correspondence to Kenneth N. Fish, Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh, Biomedical Science Tower, Room E1652, 3811 O’Hara Street, Pittsburgh, PA 15213, USA. Email: fishkn@upmc.edu

Subclasses of γ-aminobutyric acid (GABA) interneurons differentially influence cortical network activity. The contribution of differences in GABA synthesis and reuptake in axon boutons to cell type-specific functions is unknown. GABA is synthesized within boutons by glutamic acid decarboxylase 65 (GAD65) and GAD67, while GAT1 is responsible for GABA reuptake. Using an imaging methodology capable of determining the colocalization frequency of different immunocytochemical labels in the same bouton and the quantification of the fluorescence intensity of each label in these same structures, we assessed the bouton levels of GAD65, GAD67, and GAT1 in parvalbumin--expressing chandelier (PVᵦ) and basket (PVᵦ) neurons and cannabinoid 1 receptor--expressing basket (CB₁ᵦ) neurons in the monkey prefrontal cortex. We show that PVᵦ boutons almost exclusively contained GAD67, relative to GAD65, whereas CB₁ᵦ boutons contained mostly GAD65. In contrast, both GAD65 and GAD67 were easily detected in PVᵦ boutons. Furthermore, in comparison with PVᵦ boutons, CB₁ᵦ boutons expressed low to undetectable levels of GAT1. Our findings provide a new basis for the distinctive functional roles of these perisomatic-innervating interneurons in cortical circuits. In addition, they strongly suggest that altered levels of GAD67 or GAD65, as seen in some psychiatric diseases, would have cell type-specific consequences on the modulation of GABA neurotransmission.

Keywords: cannabinoid 1 receptor, GABA, GABA membrane transporter 1, glutamic acid decarboxylase, parvalbumin

Introduction

Altered γ-aminobutyric acid (GABA) neurotransmission in subpopulations of interneurons in the prefrontal cortex (PFC) appears to contribute to the pathophysiology of schizophrenia and has also been suggested to contribute to the pathobiology of major depression and autism (Akbarian and Huang 2006). The affected GABA neurons include parvalbumin--containing chandelier (PVᵦ) and basket (PVᵦ) neurons, and cholecystokinin/ cannabinoid 1 receptor--expressing basket (CB₁ᵦ) neurons. The axon boutons of PVᵦ neurons form distinctive vertical arrays (“cartridges”) that synapse exclusively onto the axon initial segment (AIS) of pyramidal cells. In contrast, PVᵦ and CB₁ᵦ cells innervate pyramidal cell bodies and proximal dendrites. PV cells are fast-spiking and strongly coupled to gamma oscillations (30–80 Hz), whereas CB₁ᵦ neurons are adapting and more strongly coupled to slower theta oscillations (3–8 Hz) (Klausberger and Somogyi 2008). Thus, over a similar period of activity, the demand for GABA synthesis would be higher in PV boutons than in CB₁ᵦ boutons.

GABA is synthesized locally in boutons by the 67- and the 65-kDa isoforms of glutamic acid decarboxylase (GAD), which are products of separate genes (Erlander et al. 1991). Within the cortex, GAD67 is distributed throughout interneurons, accounts for the majority of GABA synthesis (Asada et al. 1997), and is activity-regulated mainly by transcription (Kaufman et al. 1991; Esclapez et al. 1994; Battaglia et al. 2003). In contrast, GAD65 is mostly concentrated in boutons; its co-factor-dependent activity is highly regulated in response to GABA concentration and neuronal activity (Kaufman et al. 1991; Esclapez et al. 1994; Kleppner and Tobin 2002; Battaglia et al. 2003).

Studies in brain regions other than the PFC suggest that the GAD67/GAD65bouton ratio is mechanistically linked to the function of GABA neurons (Esclapez et al. 1994; Wilson and Kawaguchi 1996; Sogomonian and Martin 1998). For example, GAD67 tends to be highly expressed in tonically firing neurons (e.g., neurons of the reticular nucleus of the thalamus), whereas GAD65 is more abundant in neurons whose activation is highly dependent upon synaptic inputs and that fire phasically (e.g., striatal projection neurons; Wilson and Groves 1981; Contreras et al. 1992; Mercugliano et al. 1992; Feldblum et al. 1993, 1995; Esclapez et al. 1994; Wilson and Kawaguchi 1996). Considering that expression and activity of GAD65 and GAD67 are differentially regulated, neurons that are more dependent on one isoform might have less flexibility in the regulation of GABA synthesis than those that express both isoforms in their boutons. In addition, they might be more vulnerable to the reductions in GAD expression associated with psychiatric diseases such as schizophrenia (Lewis et al. 2005).

Subpopulations of GABA neurons may also regulate bouton expression levels of GAT1 in distinctive ways to enhance their specific functional roles. For example, the close proximity of boutons within PVᵦ cartridges suggests that high levels of GAT1 in these boutons might be needed to preserve synaptic independence (Gonzalez-Burgos et al. 2009). In contrast, GABA released from CB₁ᵦ-expressing boutons acts on extrasynaptic GABA_A receptors (Alle and Geiger 2007; Karson et al. 2009), which would be facilitated by low bouton levels of GAT1. Taken together, the above findings led us to hypothesize that CB₁ᵦ axon boutons express higher levels of GAD65, but lower levels of GAD67 than PVᵦ and PVᵦ boutons, and that CB₁ᵦ boutons have lower levels of GAT1 than PVᵦ boutons do. Consequently, we assessed the relative levels of these proteins within the axon boutons of PVᵦ, PVᵦ, and CB₁ᵦ in monkey PFC.

Materials and Methods

All monkey tissue was obtained from 3- to 4-year-old, male, long-tailed macaque monkeys (Macaca fascicularis). Monkeys were anesthetized with ketamine (20 mg/kg) and pentobarbital (30 mg/kg) and then
perfused transcardially with 1% paraformaldehyde in phosphate buffer (pH 7.4) at 4 °C followed by 4% paraformaldehyde in phosphate buffer, as previously described (Oeth and Lewis 1993). Brains were immediately removed, and coronal blocks (5- to 6-mm-thick) were postfixed in buffered 4% paraformaldehyde, then immersed in increasing gradients of sucrose solutions, and stored in a cryoprotectant solution at −30 °C (Cruz et al. 2003). Sections (40 μm) were cut along the rostral-caudal axis from coronal blocks containing the principal sulcus (area 46 of PFC) from the left hemisphere. Sections were taken from the middle one-third of the principal sulcus (Fig. 1) and processed as previously described (Fish et al. 2008). For Figures 4 and 6, the quantitative analyses were performed using tissue sections from 3 adult monkeys (3 sections per monkey). For all other experiments, tissue from at least 2 adult monkeys (2 sections per monkey) was assessed.

Sampling
For the presented studies, analyses were confined to PFC layer 4, which contains the highest density of PV- and CB1r-IR boutons in monkey PFC area 46 (Erickson and Lewis 2002; Eggan et al. 2010). Layer 4 of the principal sulcus (area 46) was defined as extending from 50% to 60% of the cortical traverse from the pial surface to white matter (shaded region on the medial bank of the PS; Woo et al. 1998; Pierri et al. 1999). (CS, cingulated sulcus; A, Analyses were confined to the medial bank of the PS, where layer 1 was parallel to the layer 6-white matter border. PFC layer 4 was defined as extending from 50% to 60% of the cortical traverse from the pial surface to white matter (shaded region on the medial bank of the PS; Woo et al. 1998; Pierri et al. 1999). (CS, cingulated sulcus; RS, rostral sulcus; MO, middle orbital sulcus; and LO, lateral orbital sulcus).
GAD67 that the signal could not be used for masking; and 3) in contrast, the vast majority of cartridge GAD-IR puncta, which all expressed GAD67, could only be masked using the GAD67 channel. Note that although PVb boutons could have been masked using either the GAD65 or GAD67 channel, without there being any statistical difference in the GAD65 and GAD67 mean fluorescence intensity per bouton (data not shown), GAD65 was used to prevent false positive because it is primarily found in axon terminals (Kaufman et al. 1991). For this same reason, the GAD65 channel was used to assess GAT1 mean fluorescence intensity per GAD65+/GAD67+ bouton (Fig. 6). During postprocessing, channel alignment was performed to correct for alignment issues between wavelengths that were determined using TetraSpeck 0.1 μm microspheres (fluorescent blue/green/orange/dark red; Invitrogen). For assessing relative levels of GAD67 and GAD65, a total of 7632 boutons (605 PVch, 6526 PVb, and 501 CB1rb) were analyzed. For assessing relative levels of GAT1, 1214 boutons (438 PVch and 776 CB1rb) were analyzed.

Statistics
In all cases, diagnostic statistics were used to confirm that the data were normally distributed prior to analysis. For the comparisons between GAD levels in Figure 4, 1-way analysis of variance with post hoc comparison via Tukey’s honestly significant difference was used. For the comparisons of GAD67/GAD65 ratio, post hoc comparison was performed via Dunnett T3 test to account for the unequal variances between groups. The analysis of the GAT1 mean fluorescence data was performed using the paired t-test. In all analyses, the statistics were performed on the mean values for individual monkeys (N = 3). The average mean fluorescence intensity of each bouton population studied per monkey was determined in the following way: 1) bouton intensities were averaged within stack; 2) the stack averages were averaged within section; 3) the section averages were used to generate the mean bouton intensity per monkey.

Results

GAT1- and PV-IR Cartridges Colocalize with GAD67 but not GAD65
Double-label fluorescence confocal microscopy was used to determine qualitatively the level of colocalization between GAT1-IR cartridges and the 2 GABA synthesizing enzymes GAD65 and GAD67. GAT1-IR cartridges highly colocalized with GAD67 (Fig. 2A–C). In contrast, there was little to no colocalization between GAT1-IR cartridges and GAD65 immunoreactivity (Fig. 2D–F). The same observations were made in double-labeled experiments using a different GAT1 antibody (Supplementary Fig. 1A–F) and in triple-label experiments using different GAD65 and GAD67 antibodies (Supplementary Fig. 1G–J).

To determine if the same was true for cartridges identified by PV immunoreactivity, triple-labeled fluorescence confocal microscopy was used. Similar to the findings in Figure 2, only GAD67 colocalized with PV-IR cartridges. These findings were confirmed in double-labeled fluorescence confocal microscopy experiments that used different antibodies against PV, GAD65, and GAD67 (see Supplementary Fig. 2). In contrast, in the triple- and double-label experiments, noncartridge PV boutons, which were considered to be from PVb, colocalized with both PV and GAD65 but not GAD67.
GAD65 and GAD67 (open arrowheads Fig. 3A–D and Supplemen-
tary Fig. 2; also see Fig. 4).

**CB1r Boutons Colocalize with GAD65 but not GAD67**

Triple-label fluorescence confocal microscopy was used to qualitatively assess the level of colocalization between CB1r, axon boutons, GAD65, and GAD67. The projection images in Figure 3E–H show several CB1r boutons that colocalize with GAD65 but have little to no GAD67 immunoreactivity (open arrowheads). Taken together with the above findings, these data show that in the monkey PFC layer 4, PVch, PVb, and CB1r differentially express GAD65 and GAD67 in their boutons.

**Quantitative Assessment of the Relative Levels of GAD65 and GAD67 Protein in PVch, PVb, and CB1r Boutons**

Quadruple-label fluorescence confocal microscopy was used to quantitatively assess the relative levels of GAD67 and GAD65 protein within the axon boutons of PVch, PVb, and CB1r neurons in tissue sections from monkey PFC (Fig. 4). The ability to quadruple-label sections for PV, CB1r, GAD65, and GAD67

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**Figure 3.** PV-IR cartridges are GAD67+/GAD65−, while CB1r-IR boutons are GAD65+/GAD67−. Monkey PFC cryostat sections (40 μm) triple labeled for PV, GAD67, and GAD65. Single channel (A–C) and merged (D) projection images of deconvolved image stacks (13 z-planes taken 0.22 μm apart). Arrows point to PV-IR cartridges, and open arrowheads point to PV-IR puncta not in cartridges, considered to be from PVb. (E–H) Monkey PFC cryostat sections (40 μm) triple labeled for GAD65, GAD67, and CB1r. Single channel (E–G) and merged (H) projection images of deconvolved image stacks (5 z-planes 0.25 μm apart). Open arrowheads point to GAD65+/CB1r+ and GAD67− boutons and arrows point to a GAD65+/GAD67− (CB1r−) bouton that is in close proximity to CB1r-IR boutons. Arrows and arrowheads point to the same place in each associated panel. Bars = 10 μm.
allowed us to directly compare the relative levels of GAD65 and GAD67 protein between the perisomatic-innervating interneurons studied. Figure 4A–F is a representative image that shows PV<ch> (arrows), PV<cb> (open arrowheads), and CB1<rb> (solid arrowheads) boutons and their GAD immunoreactivities. The GAD67 and GAD65 mean fluorescence intensities of individual PV<ch>, PV<cb>, and CB1<rb> boutons are shown in Figure 4G. The scatter plot in Figure 4H shows the mean fluorescence intensities of PV<ch>, PV<cb>, and CB1<rb> boutons, and the ANOVA results are presented in Table 1. Figure 4I is a schematic drawing summarizing the findings presented in A–H.

**Figure 4.** Relative levels of GAD65 and GAD67 in PV<ch>, PV<cb>, and CB1<rb> boutons. Monkey PFC cryostat sections (40 μm) were quadruple-label for GAD65, GAD67, CB1<rb>, and PV. Single channel (A,B; D,F) and merged (C,E) projection images of deconvolved image stacks (5 z-planes 0.25 μm apart). Since 4 cannot be displayed together in a single image, they have been separated into 2 RGB images that contain GAD67 (red), GAD65 (green), and PV (blue; C) or CB1<rb> (blue; F). Arrows—PV cartridge; solid arrowheads—CB1<rb>+/GAD65+ and GAD67−boutons; and open arrowheads—GAD65+/GAD67+/PV+ boutons. Bar = 10 μm. (G) A scatter plot of the GAD65 and GAD67 mean fluorescence intensities of PV<ch>, PV<cb>, and CB1<rb> boutons (100 each) selected randomly from the entire data set. (H) Assessment of the GAD65 and GAD67 mean fluorescence intensity in PV<ch>, PV<cb>, and CB1<rb> boutons. Data shown are the average fluorescence mean intensities per IR bouton (SD) across 3 monkeys. See main text for additional statistical information. (I) A schematic drawing summarizing the findings presented in A–H.
and CB1r, boutons (100 each) selected randomly from the entire data set are shown in Figure 4G. Quantitative analysis revealed similar GAD67 fluorescence intensity levels in both populations of PV boutons, which were significantly greater than in CB1r boutons. In contrast, PVr and CB1rb boutons had similar levels of GAD65 fluorescence, with both having significantly greater levels than PVch boutons (Fig. 4H). The GAD67/65 ratio in PVch boutons (15.42 [standard deviation (SD) 2.03]) was significantly larger (P = 0.015) than in PVr boutons (1.49 [SD 0.06]), which in turn was significantly larger (P < 0.001) than the ratio in CB1rb boutons (0.18 [SD 0.06]; Fig. 4C).

**GAT1 Colocalizes with PV-IR Cartridges and Puncta but not CB1r Boutons**

A qualitative examination of GAT1 colocalization with PV and CB1r was performed by double-label fluorescence confocal microscopy (Fig. 5A–F). Although GAT1 highly colocalized with PV-IR cartridges (arrows) and noncartridge PV-IR puncta (open arrowheads; Fig. 5A–C), little to no colocalization was seen with CB1r (solid arrowheads; Fig. 5D–F). To specifically assess the level of GAT1 colocalization in the boutons of CB1rb neurons, sections were triple labeled (GAT1, CB1r, and GAD65; Fig. 5G–J). As demonstrated in panels Figure 5G–J, GAT1 did not appear to colocalize with CB1r+/GAD65+ boutons (arrows).

**Quantitative Assessment of the Relative Levels of GAT1 in PVch and CB1rb**

In order to quantitatively compare the level of GAT1 in CB1rb boutons with GAD67-IR boutons of PVch neurons, sections were quadruple-labeled for GAD67, GAD65, GAT1, and CB1r (Fig. 6). In 2 representative images (Fig. 6), CB1rb boutons that were GAD65+ and GAD67+/GAT1- could be seen around a soma (solid arrowheads; Fig. 6A–F) and presumably targeting dendrites (open arrowheads; Fig. 6G–I). In addition, a GAT1+/GAD67+ and GAD65-/CB1r- cartridge can be seen (arrows; Fig. 6G–I).

The GAT1 mean fluorescence intensity per GAD67-IR bouton within cartridges (1168.9 [SD 113.2]) was significantly greater than that in CB1rb boutons (94.0 [SD 37.1]; t [2] = 23.3, P = 0.002). The same bouton-labeling differences were seen with other GAD and GAT1 antibodies (Table 1). We next compared the mean GAT1 expression levels within cartridge and CB1rb boutons with that of GAD65+/GAD67+ boutons within the same image stacks. The GAT1 mean fluorescence intensity per bouton was different between the 3 groups studied (F [2.6] = 79.47, P < 0.001). Although the GAT1 mean fluorescence intensity per GAD65+/GAD67+ bouton (799.0 [SD 139.9]) was significantly larger (P < 0.001) than that of CB1rb boutons (94.0 [SD 37.1]), it was significantly smaller (P = 0.012) than that of cartridge boutons (1168.9 [SD 113.2]).

**Discussion**

GABAergic interneurons are a structurally and functionally diverse group of cells that together regulate the activity of neuronal networks giving rise to the brain oscillations necessary for information processing (Somogyi and Klausberger 2005; Skaggs et al. 2007). Recently, the Petilla Interneuron Nomenclature Group summarized morphological, electrophysiological, and neurochemical features that can be used to describe the phenotypes of GABAergic neurons (Ascoli et al. 2008). The resulting terminology was suggested to be a stepping stone toward future classification, with the assumption that as further information is gathered about these features and the role(s), each phenotype plays in shaping cortical function group membership would undoubtedly change.

The results of the present studies, which revealed significant differences in the bouton levels of GAD67, GAD65, and GAT1 across different subclasses of perisomatic-innervating interneurons, suggest that interneuron classification may be advanced by assessing differences in how cells handle GABA synthesis and reuptake. This information provides potentially important insights into the mechanisms used by interneurons to influence postsynaptic pyramidal cell activity. For example, tonic inhibition is thought to depend on GAT1-mediated release of GAD67-synthesized GABA (Richerson and Wu 2003). However, the findings that CB1r neurons have a high GAD65/GAD67 bouton ratio (Fig. 4) and express very low levels of GAT1 (Figs. 5 and 6) support the idea that CB1rb boutons use vesicular release (Alle and Geiger 2007), as opposed to nonvesicular GABA release (Soghomonian and Martin 1998), to mediate tonic inhibition. Furthermore, the finding that CB1rb boutons express very low levels of GAT1 may also underlie the observation that CB1rb vesicular GABA release can spillover to affect PV synapses in close proximity (Karson et al. 2009).

Our findings also provide new insights into the functional roles of GAD65 and GAD67. Prior studies suggest that the major role of GAD65 is activity-dependent GABA synthesis (vesicular) (Tian et al. 1999; Choi et al. 2002), whereas GAD67 synthesizes the cytosolic pool of GABA that can be released through reversible membrane transporters (Soghomonian and Martin 1998). However, across species and cortical regions PVch and PVr have very similar short-term depression (Maccalferrì et al. 2000; Gonzalez-Burgos et al. 2005; Gulyas et al. 2010), despite the apparent differences in GAD65 and GAD67 expression in synaptic boutons (Fig. 4). This would suggest that boutons expressing only GAD67 are capable of meeting the GABA release demands of a fast-spiking neuron, presumably via vesicular release.

The differential expression of GAD65, GAD67, and GAT1 in the boutons of the perisomatic-targeting interneurons studied here also has important implications for diseases, such as schizophrenia, in which expression of these proteins may be reduced in a cell type-specific manner (Woo et al. 1997; Volk et al. 2000; 2001; Hashimoto et al. 2003). Altered expression of these proteins in disease will not have a unitary effect on inhibition but will produce GABA neuron subtype-specific deviations from normal function. Importantly, the same approach used in the current investigation to determine relative expression of these proteins within defined subsets of GABA neuron boutons can be applied within human postmortem tissue (Sweet et al. 2010) to identify the specific subpopulations affected in disease.

The findings of the present study raise several important questions. First, how do differences in GAD expression levels in monkey PFC compare with what is known in rodents? Although previous studies have analyzed axon terminal levels of GAD65 and GAD67 in rodents (e.g., see Fukuda et al. 1998), they have not performed quantitative cell type-specific comparisons. However, one study did perform a comparison of possible markers for chandelier cartridges in the rat medial PFC and hippocampus (Hardwick et al. 2005). The authors concluded that in the medial PFC neither GAD65 nor GAD67 could be used to identify cartridges, while in the hippocampus,
these structures were identifiable with GAD65 but not GAD67. In support of the idea that PV-IR boutons in the rat hippocampus express GAD65, GAD65 immunoreactivity at the AIS was shown in primary rat hippocampal cultures (Burkarth et al. 2007). Although neither of these 2 studies performed a quantitative analysis of GAD65 and GAD67 expression in cartridges,
Figure 6. Examples of quadruple-labeling used to assess relative levels of GAT1 fluorescence intensity in cartridge and CB1r, boutons. Monkey PFC cryostat sections (40 μm) were quadruple-labeling for GAD65, GAD67, CB1r, and GAT1. Single channel (A,B; D,E; G,H; J,K) and merged (C,F,J,L) projection images of deconvolved image stacks (5 z-planes 0.25 μm apart). Since 4 labels cannot be displayed together in a single image, they have been separated into 2 RGB images that contain GAD65 (red), GAD67 (green), and CB1r (blue; C and I) or GAT1 (blue; F, L). Solid arrowheads (A-F) point to CB1r+/GAD65+/GAD67+/GAT1—boutons surrounding a soma, arrows (G-L) point to a GAT1+/GAD67+ and GAD65−/CB1r—cartridge, and open arrowheads (G-L) point to CB1r+/GAD65+ and GAD67−/GAT1—boutons presumably targeting dendrites. Bars = 10 μm. (M) A bar graph of the number of CB1r, GAD65+/GAD67+, and cartridge boutons (100 each; selected randomly from the entire data set) that fall within 3 different GAT1 mean fluorescence intensity bins.
together their findings suggest that some differences might exist between rodent and primate. Thus, given the potential utility of rodent models of altered GAD expression for understanding human cortical function and/or dysfunction future, comparative studies should be performed that directly assess interneuron subtype-specific bouton expression of GAD65 and GAD67.

Our findings also raise the question of whether the reported levels of GAD in PVch and CB1rb, boutons are static or change over development. To our knowledge, interneuron subtype-specific GAD65/GAD67 bouton ratios have not been examined over development in primates. There are limited data in human and rodent about GAD mRNA and protein expression at the tissue level. For example, in the human primary visual cortex, GAD67 protein expression is unchanged from <1 year to adult, while GAD65 expression increases through adolescence and then begins to decline after early adulthood (Pinto et al. 2010). In the rat, at both the message and protein level, GAD65 and GAD67 expression is spatiotemporally regulated over development (Greif et al. 1992; Ma et al. 1994; Kiser et al. 1998; Dkhissi et al. 2001; Popp et al. 2009). Future experiments that assess if and/or how the cell type-specific bouton expression pattern of GAD65 and GAD67 changes over development will provide valuable information for defining the roles these different isoforms.

Finally, can the expression levels of one GAD change to compensate for disease-associated reductions in the other isoform? Data from knockout animal studies suggest that such compensation does not occur (Asada et al. 1996, 1997). Specifically, GAD67 mRNA and protein levels are not upregulated in mice lacking GAD65 (Asada et al. 1996). Likewise, GAD65 mRNA and protein levels are not altered in GAD67 knockout mice (Asada et al. 1997). However, it is important to mention that in the latter study, the tissue assessed was from newborn animals because the mice die shortly after birth as a result of having a cleft palate. Taken together, these studies suggest that GAD expression levels of one isoform are not directly tied to those of the other. It remains possible, however, that decreases in expression of one GAD isoform later in development, and/or selectively within interneuron subtypes, may be associated with compensatory upregulation of the other GAD protein’s activity. Indeed, some evidence suggests that GAD67 could perform some of GAD65’s role in GABA transduction (Greif et al. 1992; Ma et al. 1994; Kiser et al. 1998; Dkhissi et al. 2001; Popp et al. 2009). Future experiments that assess if and/or how the cell type-specific bouton expression pattern of GAD65 and GAD67 changes over development will provide valuable information for defining the roles these different isoforms.

In conclusion, the findings presented here represent a first step in using information about how the axon terminals of interneurons handle GABA synthesis and reuptake to expand the criteria for interneuron classification, using measures that are clearly functionally significant. The next step is to assess how other “classic” populations of interneurons that target dendrites (e.g., Martinotti cells, neurogliaform cells) compare on these measures.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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