Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan

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INTRODUCTION

Functional maturation of visual cortex is linked with dynamic changes in synaptic expression of GABAergic mechanisms. These include setting the excitation–inhibition balance required for experience-dependent plasticity, as well as, intracortical inhibition underlying development and aging of receptive field properties. Animal studies have shown that there is developmental regulation of GABAergic mechanisms in visual cortex. In this study, we show for the first time how these mechanisms develop in the human visual cortex across the lifespan. We used Western blot analysis of postmortem tissue from human primary visual cortex (n = 30, range: 20 days to 80 years) to quantify expression of eight pre- and post-synaptic GABAergic markers. We found a complex pattern of different developmental trajectories, many of which were prolonged and continued well into the teen, young adult, and even older adult years. These included a monotonic increase or decrease (GABA_α1, GABA_α2), a biphasic increase then decrease (GAD65, Gephyrin), or multiple increases and decreases (VGAT, CB1) across the lifespan. Comparing the balances between the pre- and post-synaptic markers we found three main transition stages (early childhood, early teen years, aging) when there were rapid switches in the composition of the GABAergic signaling system, indicating that functioning of the GABAergic system must change as the visual cortex develops and ages. Furthermore, these results provide key information for translating therapies developed in animal models into effective treatments for amblyopia in humans.

Keywords: GABA, development, human, aging, visual cortex, inhibition, plasticity

Using pharmacological manipulations of GABA signaling, such as fluoxetine, to reinstate ocular dominance plasticity in adult visual cortex and facilitate recovery from amblyopia (Maya Vetencourt et al., 2008; Harauzov et al., 2010). Taken together, these studies are providing a better understanding for the role of GABAergic signaling in the maturation and function of the visual cortex, and have opened the door for the development of new treatments for neurodevelopmental disorders such as amblyopia.

The GABAergic synapse is a complex structure with a large set of pre- and post-synaptic proteins, many of which have been linked with experience-dependent plasticity in the cortex. On the pre-synaptic side these include: the two isoforms of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD), GAD65 and GAD67; the CB1 receptor that modulates GABA release; and the vesicular transporter VGAT that is responsible for loading GABA into synaptic vesicles (McIntire et al., 1997; Sagne et al., 1997). GAD65 is localized in the axon terminals and synthesizes the on-demand pool of GABA, while GAD67 is located in the cell body and synthesizes the basal pool of GABA (Feldblum et al., 1993, 1995). Knocking out GAD65 leads to a loss of critical period ocular dominance plasticity, however, it can be rescued by infusion of diazepam (Iwai et al., 2003). Activation of CB1 receptors is involved in regulating activity-dependent synaptic plasticity (Sjöström et al., 2003; Jiang et al., 2003).
et al., 2010) and blocking CB1 receptors during the critical period alters activity patterns (Bernard et al., 2005). Finally, VGAT contributes to an efficient up- and down-regulation of vesicular GABA content that affects post-synaptic currents and the fine-tuning of inhibitory strength (Engel et al., 2001).

On the post-synaptic side many components of the ionotropic GABA<sub>A</sub> receptor complex contribute to experience-dependent plasticity. Gephyrin is the GABA<sub>A</sub> receptor anchoring protein. Gephyrin clusters GABA<sub>A</sub> receptors (Essrich et al., 1998; Kneussel et al., 1999), and it seems to fulfill a modulator role for changes in synaptic activity and structure. Furthermore, expression levels of Gephyrin give an indication of the total amount of GABA<sub>A</sub> receptors at a given time. The GABA<sub>A</sub> receptor is a pentameric structure and is functionally diverse, with 20 known subunits. Three subunits are of particular interest, α1, α2, and α3, because they are developmentally regulated (Hendrickson et al., 1994; Chen et al., 2001; Bosman et al., 2002), they affect receptor kinetics (Laurie et al., 1992; Gingrich et al., 1995), and the α subunit is the interface for binding GABA (Smith and Olsen, 1995) and benzodiazepine (Sigel, 2002). Both α1 and α2 subunits play key functional roles, with α2 involved in regulating cell firing, and α1 necessary for critical period plasticity (Fagiolini et al., 2004). The α1 subunit also has high-affinity for binding GABA and benzodiazepine receptor ligands (Pritchett et al., 1989).

Although there have been many animal studies of developmental and aging changes in expression of GABAergic signaling components in visual cortex (e.g., Shaw et al., 1991; Hendrickson et al., 1994; Hornung and Fritschy, 1996; Guo et al., 1997; Leventhal et al., 2003; Minelli et al., 2003), there have been relatively few studies of human visual cortex (Murphy et al., 2005). This poses a challenge when considering how to translate GABAergic drug treatments for amblyopia from animal models to human trials. To fill this gap, we have carried out a comprehensive study of changes in the expression of a collection of pre- and post-synaptic GABAergic signaling mechanisms in human visual cortex across the lifespan. Using Western blot analysis we quantified the developmental trajectories for four pre- and four post-synaptic components of GABA signaling. These results show a complex pattern of different developmental trajectories among the GABA signaling mechanisms in human visual cortex. Many of the changes are prolonged, highlighting a long time course for the development of GABA signaling in human visual cortex, much longer than would be predicted from animal studies. Furthermore, there are three transition stages when there are rapid switches in the relative amounts of the different components, indicating that functioning of the GABAergic system must change as the visual cortex develops and ages. A portion of these data has been presented previously (Pinto et al., 2008).

**MATERIALS AND METHODS**

**SAMPLES AND TISSUE**

Tissue samples were obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland (Baltimore, MD, USA). The samples were from the posterior pole of the left hemisphere of human visual cortex, including both superior and inferior portions of the calcarine fissure where the central visual field is represented in primary visual cortex (V1) according to the gyral and sulcal landmarks. The samples were from 28 individuals ranging in age from 20 days to 80 years (Table 1). All samples were obtained within 23 h postmortem, and at the Brain and Tissue Bank were fresh frozen after being sectioned coronally in 1-cm intervals, rinsed with water, blotted dry, placed in a quick-freeze bath (dry ice and isopentane), and stored frozen (−70°C). The individuals had no history of neurological or mental health disorders.

| Age group | Age   | Postmortem interval (hours) | Sex | Cause of death       |
|-----------|-------|----------------------------|-----|----------------------|
| Neonates  | 20 days | 14                         | F   | Pneumonia            |
| Neonates  | 86 days | 23                         | F   | Not known            |
| Neonates  | 96 days | 12                         | M   | Bronchopneumonia     |
| Neonates  | 98 days | 16                         | M   | Cardiovascular disorder |
| Neonates  | 119 days | 22                        | M   | Bronchopneumonia     |
| Neonates  | 120 days | 23                        | M   | Pneumonia            |
| Neonates  | 133 days | 16                        | M   | Accidental           |
| Neonates  | 136 days | 11                        | F   | Pneumonia            |
| Neonates  | 273 days | 10                        | M   | Sudden infant death syndrome |
| Infants   | 1.34 years | 21                       | M   | Dehydration          |
| Infants   | 2.16 years | 21                       | F   | Cardiovascular disorder |
| Infants   | 2.21 years | 11                       | F   | Accidental           |
| Young children | 3.34 years | 11                     | F   | Drowning             |
| Young children | 4.56 years | 15                     | M   | Accidental           |
| Young children | 4.71 years | 17                     | M   | Drowning             |
| Older children | 5.40 years | 17                     | M   | Accidental           |
| Older children | 8.14 years | 20                     | F   | Accidental           |
| Older children | 8.59 years | 20                     | F   | Cardiovascular disorder |
| Teens      | 12.45 years | 22                    | M   | Cardiovascular disorder |
| Teens      | 13.27 years | 5                      | M   | Asphyxia             |
| Teens      | 15.22 years | 16                     | M   | Multiple injuries    |
| Young adults | 22.98 years | 4                       | M   | Multiple injuries    |
| Young adults | 32.61 years | 13                      | M   | Cardiovascular disorder |
| Young adults | 50.43 years | 8                       | M   | Cardiovascular disorder |
| Young adults | 53.90 years | 5                       | F   | Cardiovascular disorder |
| Older adults | 69.30 years | 12                      | M   | Cardiovascular disorder |
| Older adults | 71.91 years | 9                       | F   | Multiple medical disorders |
| Older adults | 79.50 years | 14                      | F   | Drug overdose        |
Tissue samples (50–100 mg) were cut from the frozen block of V1 and suspended in cold homogenization buffer (1 ml buffer:50 mg tissue, 0.5 mM DTT, 1 mM EDTA, 2 mM EGTA, 10 mM HEPES, 10 mg/L leupeptin, 100 nM microcin, 0.1 mM PMSE, 50 mg/L soybean trypsin inhibitor). The tissue samples were homogenized in a glass-glass Dounce homogenizer (Contes, Vineland, NJ, USA). A subcellular fractionation procedure (synaptoneurosomes) (Hollingsworth et al., 1985; Titulaer and Ghijsen, 1997; Quinlan et al., 1999) was performed to obtain protein samples that were enriched for synaptic proteins. The synaptoneurosome was obtained by passing the homogenized sample through a coarse (100 μm) pore nylon-filter mesh followed by a fine (5 μm) pore hydrophilic mesh filter (Millipore, Bedford, MA, USA), then centrifuged at 1000 × g for 10 min to obtain the synaptic fraction of the membrane. The synaptic pellet was resuspended in boiling 1% sodium-dodecyl-sulfate (SDS) and stored at −80 °C. Protein concentrations were determined using the bichonchonic acid (BCA) assay guidelines (Pierce, Rockford, IL, USA). A control sample was made by combining a small amount of the prepared tissue sample from each of the cases.

IMMUNOBLOTTING

The samples (20 μg) were separated on sodium-dodecyl-sulfate polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF-FL) membranes (Millipore, Billerica, MA, USA). The blots were washed with PBS containing 0.05% Tween (PBS-T, Sigma, St. Louis, MO, USA) for 1 h (LI-COR Biosciences; Lincoln, NE, USA), then incubated in primary antibody overnight at 4°C using the following concentrations: GAD65, 1:500 (Chemicon International, Temecula, CA, USA); GAD67, 1:1000 (Chemicon International, Temecula, CA, USA); VGAT, 1:1000 (Synaptic Systems, Göttingen, Germany); CB1, 1:1000 (Cayman, Ann Arbor, MI, USA); Gephyrin, 1:500 (Chemicon International, Temecula, CA, USA); GABA α1, 1:500 (Imgenex, San Diego, CA, USA); GABA α2, 1:1000 (Imgenex, San Diego, CA, USA); GABA α3, 1:1000 (Imgenex, San Diego, CA, USA). The blots were washed with PBS containing 0.05% Tween (PBS-T, Sigma, St. Louis, MO, USA) (3 × 10 min), incubated (1 h, room temperature) with the appropriate IRDye labeled secondary antibody, (Anti-Mouse, 1:8000, Anti-Rabbit, 1:10,000) (LI-COR Biosciences; Lincoln, NE, USA), and washed in PBS-T (3 × 10 min). The blots were visualized using the Odyssey scanner (LI-COR Biosciences; Lincoln, NE). The blots were stripped and prepared to be re-probed with additional antibodies (Blot Restore Membrane Rejuvenation kit, Chemicon International, Temecula, CA, USA).

To visualize changes in expression of the GABAergic signaling mechanisms, the results were plotted in two ways. This was done to facilitate analysis of changes between developmental stages, to describe the pattern of changes, and to be able to quantify the time course of changes. First, the samples were grouped into developmental ages (<1 year neonates, 1–2 years infants, 3–4 years young children, 5–11 years older children, 12–20 years teens, 21–55 years young adults, and >55 years older adults) with three or more cases in each group and following the age groups used by Law et al. (2003) and Duncan et al. (2010). Group mean values and standard errors were calculated for each antibody and normalized to the mean level of expression for the youngest group. Second, we plotted scattergrams for each antibody that included both the average expression level (black symbols) for each case and every point from all runs (gray symbols). To help describe the pattern of change in expression across the lifespan, a weighted average curve was fit to each scatter plots using the locally weighted least squares method at 50% (dotted lines). In addition, an exponential decay function (solid lines) was fit to the scattergrams when there was a clear monotonic increase or decrease in the expression across the lifespan. The goodness of fit was determined (R) and the time constant (τ) for the rise or fall of expression level was calculated from the exponential decay function. The age when adult levels were reached was defined as 3τ. This provides an objective measure, representing the age at which expression had reached 87.5% of their asymptotic level.

Four indices were calculated to quantify relative changes in expression levels of the GABAergic signaling components: maturation of receptor composition \(\left[\frac{\text{GABA} \alpha_1}{\text{GABA} \alpha_2}\right]\); changes in pre-synaptic production versus trafficking, \(\left[\frac{\text{GABA} \alpha_1}{\text{VGAT}}\right]\); and changes in pre-synaptic versus post-synaptic GABAergic signaling, \(\left[\frac{\text{GABA} \alpha_1}{\text{GABA} \alpha_3}\right]\). Statistical comparisons of differences in expression levels between the age groups were calculated using Kruskal–Wallis non-parametric analysis of variance and planned pairwise comparisons using a Tukey’s HSD test (p < 0.05).

RESULTS

The tissue samples were collected over a range (4–23 h) of post-mortem intervals and the first step was to determine if that interval affected expression of any of the GABAergic proteins. There were no significant correlations (p values >0.1) between postmortem interval and the expression level for any of the antibodies (GAD65, R = 0.26; GAD67, R = 0.08; VGAT, R = 0.20; GABA α1, R = 0.04; GABA α2, R = 0.25; GABA α3, R = 0.17; Gephyrin, R = 0.17; CB1, R = 0.19).

CHANGES IN PRE-SYNAPTIC GABAergic COMPONENTS

Expression levels of the two GABA synthesizing enzymes (GAD65 and GAD67) were quantified in human V1 across the lifespan. GAD65 is the isofrom that localizes in the axon terminals and is responsible for supplying the “on-demand” pool of GABA. GAD67 is the isofrom that is predominant in the cell body and supplies the basal pool of GABA (Feldblum et al., 1993; Esclapez et al., 1994 ).
Expression levels of GAD67 did not change across the lifespan (Figures 1A,B). In contrast, developmental changes were found for GAD65 expression levels ($p < 0.0002$; Figures 1C,D). GAD65 showed a progressive 60% increase in expression levels from early in life (<4 years) to the teenage and adult years ($p < 0.05$), with a slight decline into older adults (>55 years). The difference in the developmental trajectories of these two enzymes indicates that the basal pool of GABA is maintained across the lifespan, whereas the axonal pool has a time window of heightened production capacity.

To further examine how the pre-synaptic components of the GABAergic system change across the lifespan in human V1, we quantified the expression of two key inhibitory proteins, CB1 and VGAT (Figure 2). CB1 is a pre-synaptic receptor involved in modulating GABA release (Hajos and Freund, 2002); over the lifespan there were significant changes in the expression of CB1 in human V1 ($p < 0.0002$). In infants (<1 year) and pre-teens (5–11 years), CB1 expression was high, but in young children (1–2 years), adults (21–55 years), and older adults (>55 years) it was about 40% less ($p < 0.05$; Figures 2A,B). The GABA vesicular transporter VGAT showed a similar pattern of expression levels across the lifespan ($p < 0.002$). VGAT expression was highest in infants (<1 year) and pre-teens (5–11 years), and had about 32% less expression in adults (21–55 years; $p < 0.02$; Figures 2C,D).

To quantify changes in the balance between the mechanisms that produce and traffic GABA in the pre-synaptic terminal, we calculated an index of GAD65:VGAT expression. There were significant changes in the balance between GAD65:VGAT across the lifespan ($p < 0.0005$). There was more VGAT expression in both young children (<11 years) and older adults (>55 years; Figure 3). In contrast, there was an abrupt switch to much more GAD65 during the teenage ($p < 0.02$) and young adult years ($p < 0.001$). These shifts in the balance between GAD65 and VGAT indicate that the rate limiting component regulating GABA transmission changes across the lifespan (Figure 3).

**POST-SYNAPTIC CHANGES IN GABA<sub>α</sub> RECEPTORS**
Expression levels for three subunits of the ionotropic GABA<sub>α</sub> receptor were quantified (GABA<sub>α1</sub>, GABA<sub>α2</sub>, GABA<sub>α3</sub>). Each subunit exhibited a unique developmental trajectory across the lifespan (Figure 4). Quantification of GABA<sub>α1</sub> expression in human V1...
Figure 2 | Quantification of changes in expression levels of CB1 (A,B) and VGAT (C,D) in human visual cortex across the lifespan. The graphs follow the same conventions as described in Figure 1. CB1 levels are high in infants (<1 year) then fall into young children (1–2 years) before rising again in pre-teens (5–11 years) and falling again in teens (12–20 years) and leveling off (Kruskal–Wallis, \( p < 0.0001 \)) (A,B). For VGAT 2 bands were quantified (arrows at 50 and 57 kDa). VGAT levels are highest in infants (<1 year) then gradually fall into children (3–4 years) before rising again in pre-teens (5–11 years) and falling into adults (21–55 years) before finally rising again in older adults (>55 years) (Kruskal–Wallis, \( p < 0.001 \)) (C,D). The lines above the histograms identify the groups that were significantly different (Tukey’s post hoc HSD, \(* p < 0.05, ** p < 0.02, *** p < 0.001\)) (• average expression for each case; • all runs; dotted line is the weighted average).

Figure 3 | The index of GAD65:VGAT (A,B) gives insight into the relative balance between production and trafficking of GABA. The balance is in favor of VGAT early (<11 years) and late (>55 years) in development, but there is much more GAD65 during the teenage to adult years (12–55 years) (Kruskal–Wallis, \( p < 0.0002 \)) (A,B). The lines above the histograms identify the groups that were significantly different (Tukey’s post hoc HSD, \(* p < 0.05, ** p < 0.02, *** p < 0.001\)) (• average expression for each case; • all runs; dotted line is the weighted average).
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To determine the magnitude and time course of receptor maturation we calculated two indices (GABA_A_α1:GABA_A_α2, GABA_A_α1:GABA_A_α3). The GABA_A_α1:GABA_A_α2 balance was initially in favor of GABA_A_α2, then progressively shifted toward more GABA_A_α1 (Figure 5). We fit a τ (tau) function to the index to quantify the time course of the switch in receptor composition. The mature balance was reached at 4.5 years of age (3τ, τ = 1.50 years, R = 0.84; p < 0.0001; Figure 5B). The GABA_A_α1:GABA_A_α3 switch was smaller in magnitude and much more abrupt. There was initially more GABA_A_α3, but that switched within the first year to slightly more GABA_A_α1, and reached mature levels at about 8 months of age (3τ, τ = 0.23 years, R = 0.65; p < 0.0001; Figure 5D).

The three GABA_A receptor subunits that we quantified are classified into two categories, immature (GABA_A_α2, GABA_A_α3) and mature (GABA_A_α1), with the mature subunit conferring faster receptor decay times and great affinity for GABA compared with the immature subunits. To determine the magnitude and time course of receptor maturation we calculated two indices (GABA_A_α1:GABA_A_α2, GABA_A_α1:GABA_A_α3). The GABA_A_α1:GABA_A_α2 balance was initially in favor of GABA_A_α2, then progressively shifted toward more GABA_A_α1 (Figure 5). We fit a τ (tau) function to the index to quantify the time course of the switch in receptor composition. The mature balance was reached at 4.5 years of age (3τ, τ = 1.50 years, R = 0.84; p < 0.0001; Figure 5B). The GABA_A_α1:GABA_A_α3 switch was smaller in magnitude and much more abrupt. There was initially more GABA_A_α3, but that switched within the first year to slightly more GABA_A_α1, and reached mature levels at about 8 months of age (3τ, τ = 0.23 years, R = 0.65; p < 0.0001; Figure 5D).
switched to more Gephyrin during childhood (1–11 years of age), then a balance during the teenage and adult years, followed by an abrupt switch to relatively more GAD65 in older adults ($p < 0.05$; Figures 6C,D). These changes represent a shift in GABAergic signaling mechanisms from more pre-synaptic, to more post-synaptic, to a balance, to substantially more pre-synaptic. This index captures an important aspect of the changing nature of the pre- and post-synaptic GABAergic signaling mechanisms in human V1 across the lifespan.

There is not a single balance that is maintained across the lifespan.

**Discussion**

Our study is the first to characterize changes in pre- and post-synaptic components of the GABAergic signaling system across the lifespan in human primary visual cortex. We found a complex pattern of different developmental trajectories among these GABAergic signaling mechanisms. Many of the changes were prolonged and continued well into the teen, young adult, and even older adult years. For example, the peak of GAD65 expression was not reached until the teen to young adult years before switched to more Gephyrin during childhood (1–11 years of age), then a balance during the teenage and adult years, followed by an abrupt switch to relatively more GAD65 in older adults ($p < 0.05$; Figures 6C,D). These changes represent a shift in GABAergic signaling mechanisms from more pre-synaptic, to more post-synaptic, to a balance, to substantially more pre-synaptic. This index captures an important aspect of the changing nature of the pre- and post-synaptic GABAergic signaling mechanisms in human V1 across the lifespan.

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There is a heterogeneous array of GABAergic neurons and circuits in primate visual cortex (Jones, 1993). The diversity of anatomical and physiological characteristics points to 14–19 different types of GABAergic neurons in the cortex (Gupta et al., 2000). They are spread across all layers of the cortex and in primate visual cortex the largest concentration is in layers II–III and IVa (Beaulieu et al., 1992). The GABA$_A$ receptor is also very diverse. This pentameric receptor has at least 20 different subunits, including $\alpha$, $\beta$, $\gamma$, $\sigma$, $\epsilon$, $\theta$, and $\pi$, that combine to make a very wide range of different GABA$_A$ receptors (Sieghart, 1995; Cherubini and Conti, 2001; Rudolph et al., 2001). GABAergic interneurons make connections onto specific compartments of cortical pyramidal neurons. Synapses containing $\alpha$-subunits are differentially distributed across pyramidal neurons with all types of $\alpha$-subunits found at synapses on dendrites, $\alpha_1$ and $\alpha_2$ are also found on the soma, and only $\alpha_2$ is on the axon initial segment. This diversity of morphologies, receptor compositions, and connectivity patterns confers a wide range of specific

FIGURE 6 | Changes in expression of Gephyrin (A,B) and the index of Gephyrin:GAD65 (C,D) during postnatal development. Expression levels of Gephyrin are lowest in infants (<1 year) and older adults (>55 years) and higher for the rest of the lifespan (Kruskal–Wallis, $p < 0.005$) (A). The Gephyrin:GAD65 index shows a shift from more GAD65 in infants (<1 year) to more Gephyrin in children (1–11 years) to a balance from the teenage to adult years (12–55) then a switch to much more GAD65 in aging (>55 years) (Kruskal–Wallis, $p < 0.008$) (C). The lines above the histograms identify the groups that were significantly different (Tukey’s post hoc HSD, *$p < 0.05$, **$p < 0.02$, ***$p < 0.001$) • average expression for each case; • all runs; dotted line is the weighted average).
functions to the different GABAergic circuits (Hensch, 2005). This heterogeneity poses a challenge for understanding the organization and function of GABAergic signaling.

The diversity in the GABAergic system introduces a number of sources of variability. We found substantial inter-individual variability for expression of the GABAergic proteins in human visual cortex and this probably reflects the heterogeneous nature of the GABAergic system. In addition, we also found substantial variability when measuring the expression levels of the GABAergic proteins from individuals. This measurement variability likely arises because the GABAergic system represents a smaller fraction of the total number of neurons and synapses in visual cortex compared to the excitatory system. This smaller sample size will contribute to increased measurement variability when quantifying GABAergic expression. For example, GABAergic signaling in primate visual cortex comprises about 20% of the total number of neurons (Beaulieu et al., 1992; Jones, 1993), and an even smaller portion (17%) of the total number of synapses (Beaulieu et al., 1992). Based on the difference in proportion of excitatory versus inhibitory synapses, statistics predicts that there should be about 4.8 times more measurement variability when quantifying expression of GABAergic synaptic proteins. This is very close to the larger measurement variability that we have found when quantifying the expression of inhibitory (gephyrin) versus excitatory (PSD-95) anchoring proteins in rat cortex (Pinto and Murphy, unpublished results). Additional variability in higher mammals will arise from inter-individual differences in the number of inhibitory synapses (Beaulieu and Colonnier, 1985), and the relatively smaller number of inhibitory synapses per neuron in primates compared with rats (Beaulieu et al., 1992). Taken together, the diversity and smaller proportion of GABAergic synapses predicts that there must be substantial variability when measuring expression of GABAergic synaptic proteins. Furthermore, previous studies suggest that there is greater synaptic specialization in non-human primates (Beaulieu et al., 1992) raising the possibility of even greater diversity in human cortex. In spite of these inherent sources of variability, our large data set makes it clear that there are significant changes in GABAergic expression in human visual cortex across the lifespan.

Monyer and Markram (2004) aptly describe studying GABAergic interneurons as a daunting task because of the diverse nature of this system. They also point out that combinatorial approaches are helping to clarify the diversity of GABAergic interneurons. In our study, we used a large number of samples from human visual cortex covering the lifespan to quantify the expression and developmental trajectories of eight components of the GABAergic system. The number of proteins quantified allowed us to capture a unique perspective on both pre- and post-synaptic development that would be missed by studying a smaller set of synaptic proteins. Furthermore, by quantifying the expression of GABAergic proteins in a preparation enriched for synaptic proteins (Hollingsworth et al., 1983; Titulaer and Ghijsen, 1997; Quinlan et al., 1999), we are able to tie the current findings closer to functional changes at the synapse. This is especially important for changes that affect experience-dependent plasticity (Quinlan et al., 1999). This contrasts with other approaches, such as measuring mRNA expression of GABAergic components (e.g., Duncan et al., 2010), because it is hard to make the link between mRNA expression and the level of protein expression at the synapse. Finally, the developmental trajectories that we describe reflect the average across the whole group of samples and this type of cross sectional design cannot address how the levels of these GABAergic markers change in an individual. The higher level of variability in expression that we found for older children through young adults suggests that the timing of rapid changes may vary across individuals.

The immunoblotting technique used for the current study does not allow for an analysis of developmental changes in the variety of different morphological types of GABAergic interneurons, specific intracortical inhibitory circuits, or locations of receptor subunits on different compartments of the post-synaptic neuron. It will be important for future studies to extend this work to include anatomical methods that describe and quantify laminar and morphological changes in GABAergic neurons, as well as tracing the specific locations of GABAergic synapses in cortical circuits. These will be challenging studies because of the difficulty obtaining postmortem tissue that is both appropriate for anatomical investigations and covers a wide range of ages. Despite these obstacles they should be a priority, especially since site-specific optimization of GABA synapses may trigger experience-dependent plasticity in visual cortex (Katagiri et al., 2007).

**TRANSITIONAL STAGES IN GABAergic SIGNALING**

The pattern of changes in expression of the GABAergic signaling mechanisms was complex and there was not one or two key components driving these changes. Instead, each pre- and post-synaptic mechanisms followed different developmental trajectory that combine to create a complex pattern of overall changes. The expression patterns for individual proteins can be characterized as either no change (GAD67, GABA$_{\alpha3}$), a monotonic increase or decrease (GABA$_{\alpha1}$, GABA$_{\alpha2}$), a biphasic increase then decrease (GAD65, Gephyrin), or multiple increases and decreases (VGAT, CB1) across the lifespan. These different developmental trajectories may be linked to functional changes and the different roles that these synaptic proteins play in GABAergic signaling. For example, the multiple increases and decreases found for CB1 may reflect the multiple roles that it has in synaptic development, plasticity, and regulating GABA release (Hajos and Freund, 2002). By calculating a series of indices to quantify the balance between the various pre- and post-synaptic GABAergic mechanisms, we found three transitional stages during early development, teen years, or aging when there were large switches in the relative amounts of the components of the GABAergic signaling system. The three distinct transitional stages in GABAergic signaling reflect important times during the lifespan when there must be significant changes in the function of GABAergic inhibition. Interestingly, these transition stages line up with time points when visual functions are changing and when the visual system is sensitive to experience-dependent change (Faubert, 2002; Lewis and Maurer, 2005; Maurer et al., 2007a,b). The first stage reflects an early developmental transition between neonates/infants (<2 years) and young children (3–5 years) when there was a switch in composition of the GABA$_{\alpha}$ receptor. The relative expression switched from more of the immature subunits (GABA$_{\alpha2}$ and GABA$_{\alpha3}$) to more of the mature (GABA$_{\alpha1}$) subunit. The GABA$_{\alpha1}$ subunit has a special role in development of the visual cortex driving experience-dependent plasticity whereas GABA$_{\alpha2}$ modulates neuronal firing (Fagiolini et al., 2004). The kinetics of the GABA$_{\alpha}$ receptor speed up threefold when the
GABA_α1 subunit dominates (Gingrich et al., 1995) and gamma bursts depend on circuits dominated by GABA_α1 (Cardin et al., 2009; Sohal et al., 2009). Furthermore, the switch has pharmacological implications as the α2 subunit exhibits the anxiolytic effects of benzodiazepines, whereas α1, α3, and α5 mediate the sedative and amnesic effects (Rudolph et al., 1999; Löw et al., 2000). This developmental switch in GABA_α1 receptor subunits also occurs in rats (Bosman et al., 2002), cats (Chen et al., 2001), and macaque monkeys (Hendrickson et al., 1994). For these species, however, the switch is correlated with different aspects of the critical period. In rats, the switch occurs before the start of the critical period (Heinen et al., 2004), but in cats and macaque monkeys the switch overlaps the critical period for ocular dominance plasticity (Hendrickson et al., 1994; Chen et al., 2001). The current results show that in human V1 this transitional switch in expression of GABA_α1 receptor subunits spans the critical period, when disrupting vision can cause long lasting changing in visual acuity (Lewis and Maurer, 2005). Perhaps this first stage defines the period of ocular dominance plasticity in human V1.

In the second stage, the teenage transition was a switch from a greater capacity for trafficking GABA (more VGAT) to a greater capacity to produce the on-demand pool of GABA (more GAD65). In addition, there was a high level of GABA_α1 expression during this stage. These suggest that in teens and young adults the pool of on-demand GABA can be readily replenished and binds with α1 containing receptors to maintain a high level of phasic inhibition. Perhaps this combination is needed for optimal coding of neural signals, especially during periods of sustained neural activity. Optimal orientation tuning of receptive fields (Leventhal et al., 2003) and perceptual performance on orientation discrimination (Edden et al., 2009) depend on the level of GABA concentration. It seems likely that the high levels of GAD65 and GABA_α1 at this stage are contributing to optimal visual performance on tasks such as orientation discrimination.

In the third stage, during the transition to older adults, there were two switches in the balance between components of the GABAergic signaling system: from greater capacity to produce GABA (more GAD65) to greater capacity to traffic GABA to the synapse (more VGAT); and from relatively more post-synaptic (more Gephyrin) to more pre-synaptic (more GAD65) expression. Both of these changes were large switches from those found for young adults and point to significant changes in functioning of GABAergic synapses in the aging visual cortex. Furthermore, the time course of the GABAergic changes in aging is similar to the decline of visual abilities. The changes could contribute to the loss of orientation tuning (Leventhal et al., 2003) and processing of complex visual stimuli that are particularly vulnerable during aging (Habak and Faubert, 2000; Faubert, 2002). For example, the perception of a moving stimulus in the context of a moving surround is particularly affected in aging (Betts et al., 2005) and a loss of GABA inhibition has been suggested as the mechanism underlying this perceptual loss. It seems likely that the changes in the balance of GABAergic components at aging synapses contribute to the visual losses that accompany aging.

**LINKS WITH EXPERIENCE-DEPENDENT PLASTICITY**

Previous studies have shown that a specific excitatory-inhibitory balance and expression of the GABA_α1 subunit are required for ocular dominance plasticity in the developing rodent visual cortex (Hensch et al., 1998; Fagiolini et al., 2004). Furthermore, inhibition is abnormal in visual cortex of monococularly deprived (Burchfiel and Duffy, 1981) and strabismic cats (Singer et al., 1980). Our findings suggest that the period of ocular dominance plasticity in the human visual cortex is prolonged. If ocular dominance plasticity follows the development of GABA_α1 then it would extend through childhood into teenage and possibly even to young adult years. This idea is consistent with the prolonged period of visual changes found in children with cataracts (Lewis and Maurer, 2005; Maurer et al., 2007a,b). Importantly, the current findings indicate that primary visual cortex continues to have the necessary balance of GABAergic signaling mechanisms to mediate experience-dependent plasticity well into the teenage years. In addition, because the development of GAD65 expression may extend into the young adult years it is possible that this affects plasticity in primary visual cortex and contributes to visual recovery promoted by perceptual learning treatment in adults with amblyopia (Levi and Li, 2009). Finally, the losses in aging may contribute to reduced synaptic plasticity and may be part of a more generalized change in the balance of synaptic proteins (Williams et al., 2010) in the aging cortex that could underlie age-related perceptual and cognitive declines.

The prolonged window of GABA changes in human visual cortex provides an opportunity to translate experimental treatments that target GABAergic signaling in the visual system into therapeutics that may even be useful in treating amblyopia in young adults. For example, new studies have shown that fluoxetine can reinstate ocular dominance plasticity in the adult cortex by reducing intracortical inhibition (Maya et al., 2008; Harauzov et al., 2010). The current study provides the necessary information about GABAergic signaling in human cortex to begin the process of translating the exciting therapies being developed in animal models into effective treatments for amblyopia in humans.

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