Formation of whisker-related barrels in primary somatosensory cortex (S1) requires communication between presynaptic thalamocortical afferents (TCAs) and postsynaptic cortical neurons. GAP-43 is crucially involved in targeting TCAs to postsynaptic S1 neurons but its influence on the interactions between these 2 elements has not been explored. Here, we tested the hypothesis that reduced early expression of presynaptic GAP-43 (GAP-43 heterozygous [HZ] mice) alters postsynaptic differentiation of barrel cells. We found a transient increase in cytochrome oxidase staining between P6 and P14 in HZ animals, indicative of increased metabolic activity in barrel cortex during this time. Golgi impregnation and microtubule-associated protein 2 immunohistochemistry showed anomalous dendritic patterning in GAP-43 HZ cortex at P5, with altered dendritic length and branching and abnormal retention of dendrites that extend into developing septa. This deficiency was no longer apparent at P7, suggesting partial recovery of dendritic pruning processes. Finally, we showed early defects in synaptogenesis from P4 to P5 with increased colocalization of NR1 and GluR1 staining in HZ mice. By P7, this colocalization had normalized to wild type levels. Taken together, our findings suggest abnormal postsynaptic differentiation in GAP-43 HZ cortex during early barrel development, followed by adaptive compensation and partial phenotypic rescue.

**Keywords:** cortical development, dendrite morphology, glutamate receptors, postsynaptic specialization

**Introduction**

The rodent whisker/barrel system is an important model of somatotopic map formation (Woolsey and Van der Loos 1970; Erzurumlu and Kind 2001; Lopez-Bendito and Molnar 2003). Clusters of neurons representing individual whiskers on the rodent snout form ordered arrays in the brain stem, thalamus, and somatosensory cortex. Development of cortical barrel patterns involves 2 phases (Molnar and Hannan 2000)—the convergence and ordering (somatotopy) of thalamocortical afferents (TCAs) to form a crude map, and the segregation of TCAs to form refined barrels (Maier et al. 1999; McIlvain et al. 2003).

GAP-43 is poised to affect both phases of cortical barrel formation. The first phase requires appropriate pathfinding by TCAs. GAP-43, an intracellular protein found in neural growth cones, is important for axonal pathfinding (Benowitz and Routtenberg 1997) in many cortical projections (Maier et al. 1999; Donovan et al. 2002). In mice that do not express GAP-43 (knockout [KO]), TCAs follow aberrant paths (Donovan and McCasland 2008), and barrel patterns fail to form (Maier et al. 1999). TCAs in GAP-43 heterozygotes (HZ), with reduced GAP-43 expression show more subtle pathfinding errors through the internal capsule and deep cortical layers (McIlvain et al. 2003). The second phase of barrel development, whereby barrel patterns are defined, relies on transfer of information between the pre- and postsynaptic cell, triggering synaptogenesis and postsynaptic differentiation in nascent barrels. The mechanisms by which TCAs communicate with cortical neurons are not well understood. However, defects in TCA segregation and barrel refinement are found in mouse mutants with both presynaptic (i.e., Adyl KO and MAO-A KO) and postsynaptic (i.e., NR1, PLCB1, and GluR5) signaling defects (Molnar and Hannan 2000; Erzurumlu and Kind 2001). The variable degree of pattern loss among these mutants suggests that multiple factors and compensatory mechanisms may contribute to map refinement. GAP-43 could be involved in this phase through its effects on both neurotransmission (Dekker et al. 1989; Haruta et al. 1997; Neve et al. 1998) and synaptic plasticity (Gianotti et al. 1992; Benowitz and Routtenberg 1997). GAP-43 HZ TCA arbors show aberrant widespread but sparse branching (McIlvain et al. 2003) during development suggesting errors in segregation within the cortex. At this time, GAP-43 HZ cortex has larger than normal barrels (McIlvain et al. 2003) suggesting a postsynaptic deficit that has not yet been explored.

In the present study, we investigated the consequence of GAP-43 deficiency on postsynaptic differentiation in an identified barrel (C3). We used a combination of dual immunofluorescence and confocal microscopy, cytochrome oxidase (CO) reactivity, microtubule-associated protein (MAP)-2 immunostaining, and Golgi impregnation to assay early postsynaptic development in GAP-43 HZ mice. We found early deficits in HZ barrels for dendritic pruning and patterning and glutamate receptor trafficking. Each of these early deficits was followed by an apparent compensatory response and partial phenotypic recovery during a period of increased metabolic activity. Our findings are consistent with homeostatic cortical responses to abnormal innervation by TCAs (Turrigiano and Nelson 2004; Perez-Otano and Ehlers 2005). They may reflect novel mechanisms for recovery of cortical function following abnormal development.

**Materials and Methods**

**Animals**

GAP-43 mice were generated as previously described (for procedures, see Maier et al. 1999). Mice were a C57BL/6 and A129/SV mixed background. GAP-43 wild type (WT) and HZ genotypes were determined following tail snip and DNA extraction and analyzed using multiplex polymerase chain reaction procedures. All GAP-43 animals were housed in the Department of Laboratory Animal Resources at

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**Postsynaptic Deregulation in GAP-43 Heterozygous Mouse Barrel Cortex**

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SUNY Upstate Medical University in Syracuse, New York. The treatment of all animals was in strict accordance with both Institutional Animal Care and Use Committee guidelines and those advocated by the National Institutes of Health. Pups were left with mothers until P21 (day of birth = P0) and then weaned and same sex housed as adults. Autoclaved lab chow and water were provided ad libitum. The facility was maintained on a 12 h light/dark schedule. All animals used in the following experiments were carefully matched for handling and age whenever possible.

**CO Histochemistry, Image Acquisition, and Image Analysis**

To study the effects of reducing GAP-43 on metabolic demand in the core barrel field of mouse somatosensory cortex, serial sections from P4-P21 cortices (for animal numbers, see Table 1) were processed for quantitative analysis of CO histochemistry (Wong-Riley and Kageyama 1986) as previously described (Wong-Riley and Welt 1980). Briefly, WT and HZ mice were sacrificed and perfused transcardially with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) to eliminate erythrocytes that may react with 3,3'-diaminobenzidine (DAB). Brains were then submerged in increasing concentrations of sucrose (15-50%) in 0.1 M phosphate buffer (PB), pH 7.4 overnight at 4 °C. Each hemisphere was flattened tangential to the pial surface overlying the barrel cortex on a freezing microtome and sectioned serially at 40 µm. HZ and littermate control sections were incubated together (see below), free floating at 37 °C in the dark for 3-7 h in a solution containing 0.05 g. DAB, 0.022 g. cytochrome C (Sigma, St Louis, MO), and 4.0 g. sucrose in 90 mL 0.1 M PB, pH 7.4.

The length of the reaction was determined for each age analyzed and strictly controlled across HZ and WT littermate controls. All sections within each litter were processed in the same reaction condition using a honeycomb-staining tray, which allowed reactions and washes to be performed at the same time for all sections. Sections were mounted on gelatin-coated slides and allowed to air-dry. Once dry, slides were run through an alcohol dehydration series to xylene, overslipped with dibutyl phthalate xylene (DPX) mounting medium (Electron Microscopy Sciences, Ft. Washington, Hatfield, PA), and allowed to air dry.

CO-reacted tangential sections were photographed with an RT Slider SPOT camera (Diagnostic Instruments, Inc., Sterling Heights, MI) for optical density analysis and quantification. The method for CO optical densitometry and quantification has been described previously (Wong-Riley and Kageyama 1986; Wong-Riley et al. 1993). To enable comparisons between sections and across genotypes, camera light levels (Wong-Riley and Kageyama 1986; Wong-Riley et al. 1993) as previously described (Wong-Riley and Welt 1980). Briefly, brains were rapidly removed from animals deeply anesthetized with isoflurane and rinsed briefly in 0.1 M PBS, pH 7.4. Brains were then removed, postfixed in 4% paraformaldehyde, both in 0.1 M sodium PB, pH 7.4. The brain was removed, postfixed in 4% PFA for 3 h at 4 °C and washed in sodium PBS (0.9% NaCl in 50 mM PB, pH 7.4). Transverse sections of the brain (60-µm thick) were produced with a vibratome in ice-cold PBS, at rostrocaudal levels including the barrel fields of the somatosensory cortex. Sections were afterward processed for CO histochemistry as previously described. To further test the specificity of the reaction with EM, sections from the same animals were incubated in parallel without cytochrome C.

After CO histochemistry, sections were rinsed in PB, postfixed flat in 1% osmium tetroxide for 30 min, dehydrated in ethanol and impregnated with resin (Durcupan ACM; Electron Microscopy Sciences). They were mounted between ACLAER® embedding films (Electron Microscopy Sciences) and cured at 55 °C for 48 h. Areas of interest, that is, the barrel fields of the somatosensory cortex were excised from the slides, glued at the tip of resin blocks, and sectioned (70-nm thin) with an ultramicrotome (Reichert Ultracut E; Leica Microsystems, Wetzlar, Germany). The ultrathin sections were collected on bare square mesh copper grids, stained with lead citrate, examined at 80 kV with a Hitachi 7650 electron microscope, and photographed with a ES1000W Erlangshen CCD Camera (Gatan, Warrendale, PA). For quantitative analysis in 1 P5, 1 P7, and 1 P10 animal, 45 micrographs (covering ~1030 µm²) were taken in the neuropil at ×3 k000, near the border between tissue and resin where the staining was most intense. CO-positive and CO-negative mitochondria were counted in all pictures, and the cellular profiles enclosing them were identified as dendritic, axonal, or glial (Peters et al. 1991) but also (Tremblay et al. 2007, 2009). The results were expressed in numbers of dendritic, axonal, or glial CO-positive mitochondria per 10³ µm² of neuropil. Labeling indices were also calculated for dendritic, axonal, and glial elements in percentage of CO-stained mitochondria over the total number of mitochondria. Lastly, no CO-stained mitochondria were observed on the control sections incubated without cytochrome C, which confirms the specificity of the histochemical reaction (not shown).

**MAP-2 Immunohistochemistry**

To study the effects of reduced GAP-43 on dendritic profiles in barrel cortex, P5 brains (animal numbers are shown in Table 1) were sectioned at 40 µm, either coronal or tangential to the plane of the barrel field. Sections were blocked in 5% normal goat serum (NGS) in 0.2% Triton X-100/0.1 M PBS, pH 7.4 and then incubated overnight in rabbit anti-MAP-2 primary antibodies (Millipore/Chemicon International, Inc., Billerica, MA; 1:750) followed by biotinylated goat anti-rabbit secondary antibodies (Vector Laboratories, Inc., Burlingame, CA; 1:200). Specific activity was detected using ABC reagent (Vector Laboratories Inc., 1:80) and visualized with DAB.

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age</th>
<th>WT (n)</th>
<th>HZ (n)</th>
</tr>
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<tbody>
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<td>CO analysis</td>
<td>P4-P5</td>
<td>34</td>
<td>40</td>
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<tr>
<td></td>
<td>P6-P14</td>
<td>57</td>
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<td></td>
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<td>3</td>
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<tr>
<td>MAP-2 immunohistochemistry</td>
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<td>19</td>
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<tr>
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<td>3 (30 cells)</td>
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<td>10 (44 cells)</td>
<td>25 (42 cells)</td>
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<tr>
<td>Dendritic analysis—orientation</td>
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<td>28 (53 cells)</td>
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<td>GluR1 immunohistochemistry</td>
<td>P6 and P7</td>
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<tr>
<td>NR1 immunohistochemistry</td>
<td>P6</td>
<td>6</td>
<td>12</td>
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</tbody>
</table>

### Golgi Impregnation

Brains from P5 and P7 mice (for animal numbers, see Table 1) were processed for Golgi analysis using the FD Rapid GolgiStain Kit (MTR Scientific, LLC, Germantown, MD) per manufacturer's instructions. Briefly, brains were rapidly removed from animals deeply anesthetized with isoflurane and rinsed briefly in 0.1 M PBS, pH 7.4. Brains were then submerged in a solution containing potassium dichromate, mercuric chloride, and potassium chromate (Solutions A and B) protected from light for 2-3 weeks. Brains were then incubated in a cryoprotectant solution (Solution C) for one week. Each hemisphere was blocked in...
a solution containing 0.5 g gelatin, 30 g albumin, and 0.02 g sucrose and allowed to harden for 30 min at room temperature. Blocks were then cut with a vibratome at 150 μm. Solution C was placed in the vibratome well during cutting to prevent subsequent cracking of the Golgi-impregnated sections. Sections were mounted on gelatin-coated slides in Solution C, air-dried, Golgi stained (Solutions D and E), and counterstained with cresyl violet.

**Golgi Analysis**

Golgi-impregnated cells were analyzed for several morphometric parameters using Bioquant v2.5 software (Bioquant Image Analysis Corp., Nashville, TN). Layer IV barrel cortex cells were located at low magnification (×10) using cresyl violet counterstaining and neighboring morphological landmarks when necessary. Once the appropriate location was found, the magnification was increased to ×40. Cells located closest to the ocular crosshairs were considered for analysis. Criteria for acceptance in this study were based on previous qualitative descriptions of layer IV stellate cells (Pasternak and Woolsey 1975). Branch lengths were carefully measured by tracing the primary branches (originating directly from the soma), while bifurcations were located closest to the ocular crosshairs were considered for analysis. Those cells that maintained septal-directed dendrites (regardless of also displaying hollow-oriented dendrites) were classified as "cells with septal-oriented branches." Cells that lacked septal-oriented dendrites (i.e., displaying hollow-oriented branches only) were classified as "cells without septa-oriented branches." In some cases, dendritic branches coursed out of the focal plane, making it difficult to discern an accurate orientation (see Fig. 4). In these cases, the branches in question were excluded, and a classification was based on the primary branch orientation only. Cells with multiple out of focus branches were often too difficult to classify properly and were excluded from the study. Dendritic protrusions were analyzed at P7 from 3 WT and HZ animals under ×100 magnification. Only cells lying on the barrel wall and showing orientation bias were considered for analysis. Those cells that maintained septal-directed dendrites (regardless of also displaying hollow-oriented dendrites) were classified as "cells with septal-oriented branches." Cells that lacked septal-oriented dendrites (i.e., displaying hollow-oriented branches only) were classified as "cells without septal-oriented branches." In some cases, dendritic branches coursed out of the focal plane, making it difficult to discern an accurate orientation (see Fig. 4). In these cases, the branches in question were excluded, and a classification was based on the primary branch orientation only. Cells with multiple out of focus branches were often too difficult to classify properly and were excluded from the study. Dendritic protrusions were analyzed at P7 from 3 WT and HZ animals under ×100 magnification. Two to three dendrites from each of 10 cells per animal were analyzed. Protrusions were identified as thin structures of up to 10 μm that emanated from dendrites. Protrusions were counted while manually changing the focus in order to identify all protrusions on a particular stretch of dendrite. No attempt to account for protrusions that were undetected due to their location atop or below the dendrite was undertaken with the understanding that this should introduce equal errors to the analysis of both groups (Rusakov and Stewart 1995). Protrusion density is defined as the density of all protrusions counted per animal divided by the total length of dendrite.

**NR1/GluR1 Immunohistochemistry**

To determine glutamate receptor distribution, P4–7 mouse brains were cut at 40-μm thickness, tangential to the plane of the barrel field. Tissue sections were processed for NR1 and GluR1 immunohistochemistry using a protocol adapted from Wong et al. (1997). Briefly, tissue sections were processed free floating at room temperature for 2 h in 10% NGS (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) or 10% normal horse serum (NHS; JRH Biosciences, Lenexa, KS) in 0.1 M PBS containing 0.1% Triton X-100 (PBS-TX). Sections were incubated at room temperature for 16–20 h in 10% goat or 10% horse block containing one of the following primary antibodies: rabbit anti-NR1 (1:500, Millipore) recognizing the N-terminus of the N-methyl-D-aspartic acid (NMDA) receptor or goat anti-GluR1 (1:500, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) to the N-terminus of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionyl (AMPA) receptor or biotinylated goat anti-rabbit or donkey anti-goat secondary antibodies (1:200, Vector Laboratories, Inc.). Specific reactivity was detected using ABC reagent (1:100, Vector Laboratories, Inc., Santa Cruz, CA) and visualized with DAB. The silver/gold enhancement protocol per Kitt et al. (1985) involved incubation in silver nitrate solution at 56 °C, followed by dipping in gold chloride solution and sodium thiosulfate. Dried slides were then run through an alcohol dehydration series to xylene, coveredslipped with DPX mounting medium (Electron Microscopy Sciences) and allowed to completely dry before analysis. Tissue was analyzed using both bright- and dark-field microscopy, and images were photographed using an RT slider SPOT camera (Diagnostic Instruments, Inc.).

**Immunofluorescence Microscopy**

To study colocalization profiles of NR1 and GluR1, P3–P7 brains (for animal numbers, see Table 1) were sectioned at 40-μm thickness, tangential to the plane of the barrel field. They were incubated at room temperature for 2 h in 5% normal donkey serum and 5% NHS in PBS-TX to block nonspecific binding. Sections were incubated for 16–20 h in 5% donkey/5% horse block containing the following primary antibodies: rabbit anti-NR1 (1:250, Millipore) and goat anti-GluR1 (1:250, Santa Cruz Biotechnology, Inc.). They were then processed sequentially with biotinylated donkey anti-rabbit (Amersham Pharmacia Biotechnology, Piscataway, NJ, 1:150) and Texas Red-conjugated streptavidin (Vector Laboratories, Inc., 1:250), followed by biotinylated horse anti-goat (Vector Laboratories, Inc., 1:250) and fluorescein-conjugated streptavidin (Vector Laboratories, Inc., 1:150). Sections were washed once between steps with PBS-TX, then mounted on subbed slides with Prolong Gold antifade (Molecular Probes, Carlsbad, CA), and allowed to dry.

**Fluorescent Image Acquisition and Unbiased Stereology**

Images with 0.2-μm pixel resolution were obtained for each hemisphere in 512 × 512 pixel matrices on a BioRad MRC 1024ES confocal microscope (Hercules, CA) mounted with a Nikon Eclipse E600 microscope. Sections were first viewed at ×10 magnification to locate barrel fields. When clear fields were evident, sections were centered on the C3 barrel and then examined at ×60 magnification at which the C3 barrel filled the field of view. A 1 × 1 mm 100 unit grid eyepiece reticle (Scope Instruments Company, Vestal, NY, #KR-100) for the Nikon E600E ocular (CFIW, 10 × 25) was placed in one eyepiece of the microscope. At ×60 magnification, random numbers between 1 and 100 (Microsoft Excel) were used in sequence to select grid squares, which were then centered and viewed at ×100 magnification. All images collected and analyzed fell within the C3 barrel hollow. Images were acquired using a Lasersharp 2001 program (BioRad, version 4.0) and scanned with simultaneous red (HeNe, λ=543 nm) and green (argon, λ=488 nm) channels. At each randomly selected location within the C3 barrel, the microscope was focused to a 3-μm depth, and 3 images were obtained by cutting Z displacement using a 1-μm step. The microscope condenser setting was constant for all sections. The gain and offset were set using a WT control as baseline for each age and maintained across genotype.

**Fluorescent Image Analysis**

All images were analyzed while blind to the animal genotype. Collected image Z-series were analyzed using Laserpix software (BioRad, version 4.0.0.1.3 for Windows 95/NT/98). A 7 × 7 unsharp mask filter was applied to each image. A standard threshold was used for each channel (128; 0–256 scale) with all sections. Colocalization coefficient output files (see below) were exported into Microsoft Excel for each randomly selected location in C3 barrels from P3 to P7 mice in each genotype. Total red or green pixel counts, computed from collapsed Z-series images, were recorded and exported into Microsoft Excel for statistical analysis.

**Calculation of Colocalization Coefficient**

For each randomly selected region within the C3 barrel, immunolabeled puncta were classified as NR1 only, GluR1 only (rare in our samples), or colocalized NR1/GluR1. From these data, Laserpix calculated a "colocalization coefficient" or R/G ratio. The algorithms used are based on the Pearson’s correlation coefficient, a statistical measurement that analyzes the linear strength between 2 variables. The coefficient was calculated using the following equation: $R/G = \frac{2UV/UV + UD}{U^2/V + V^2/D}$, where $U = \sum_i U_i$, $V = \sum_j V_j$, and $D = \sum_k D_k$. The R/G ratio is the proportion of total NR1 puncta that are colocalized with GluR1 in our randomly selected samples. An R/G ratio equaling 1 means that all NR1 sites contain GluR1 (complete colocalization).
Results

Transient Increased Metabolic Activity in GAP-43 HZ Cortex during Development

CO reactivity is correlated with overall metabolic demand and functional activation in various nuclear groups under normal conditions (Wong-Riley and Kageyama 1986; Wong-Riley 1989). To determine if metabolic demand is impaired in GAP-43-deficient mice, we processed WT and HZ barrel cortex for CO reactivity from postnatal days "P4-P21" (Fig. 1). CO densities were compared in core barrel fields (B1-3, C1-3, D1-3; circled region in Fig. 1A) from sections processed simultaneously under identical conditions (see Materials and Methods, Wong-Riley et al. 1993). Since GAP-43 facilitates neurotransmission (Dekker et al. 1989) and total GAP-43 levels are reduced by 30–70% in HZ mice (Mcllvain et al. 2003), we expected to find decreased CO reactivity in HZ barrels. However, CO reactivity in core barrels was not significantly different during early development (P4–P5) in HZ than WT cortex (P < 0.05, Student’s t-test). Surprisingly, by P7, HZ core barrels were significantly more reactive than WT (P < 0.05, Student’s t-test), suggesting increased metabolic demand at this age. By late development (P21) CO reactivity in HZ barrels returned to WT levels.

In the adult mouse barrel cortex, CO-reactive mitochondria are predominantly localized in postsynaptic cortical dendrites (Wong-Riley and Welt 1980). To verify whether dendritic defects could underlie the transient increase in CO-reactivity observed in young HZ, we examined the subcellular localization of CO-positive mitochondria in the barrel cortex of P5, P7, and P10 WT mice using EM. At all examined ages, CO-positive mitochondria were frequently located in neuronal cell bodies but also throughout the neuropil (Fig. 2). Counts in number of sectional profiles per 107 μm2 of neuropil show that CO-positive mitochondria are much more numerous in dendritic elements (distal branches; 141–175 per 103 μm2), followed by axonal (axons and axon terminals; 29–51 per 103 μm2) and glial (astrocytic and microglial cells processes; 3–13 per 103 μm2) elements. Moreover, the proportion of CO-positive

Data Analysis

Statistical analysis was done using Microsoft Excel Data Analysis plugin software (Microsoft Office 2003). All data are reported as mean ± standard error. Sample size n (as shown in Table 1) refers to the number of animals processed for each experimental method described above. For statistical analysis, error bars represent standard error of the mean. Statistical analysis consisted of a standard Student’s t-test on raw data to determine the significance level between genotypes. Some data to determine the significance level between genotypes. Some

Figure 1. CO-reactivity differs in WT and HZ barrel cortex. Digital photomicrographs of CO-reacted barrel cortex were analyzed using National Institutes of Health image for average density measurements within the core barrel field (B1-3, C1-3, D1-3; outlined in A). Representative examples of CO-reacted barrel cortex at P5 and P7 in GAP-43 HZ and WT mice showing visible qualitative differences in CO-reactive intensity between HZ and WT cortices (A–D). Nissl-stained sections at P7 show no visible changes in barrel field cytoarchitecture in WT (E) compared with GAP-43 HZ mice (F). Quantitative analysis of mean core barrel field density among P4–P5, P6–P14, and P21 demonstrate that HZ core barrels become significantly more reactive between P6 and P14, returning to WT levels at P21 (G) (P < 0.05, Student’s t-test). Scale bar = 500 μm. Density scale bar = white (least reactive), black (most reactive). Data reported as mean ± standard error of the mean. *P < 0.05.

Figure 2. Subcellular localization of CO-stained mitochondria in the barrel cortex of WT mice. (A) Low-magnification electron micrograph showing the frequent localization of CO-stained mitochondria in neuronal cell bodies (colored in light yellow), as well as their relative distribution in the dendritic (light blue), axonal (light green), and glial (light pink) elements of the neuropil at P10. Scale bar = 1 μm. (B) A higher magnification of the field framed in A shows a dendritic branch that contains 5 CO-positive mitochondria (d–i) and a nearby dendrite with a CO-negative mitochondrion (white asterisk; d–i, dendritic and axonal elements without CO-positive mitochondria). Scale bar = 0.5 μm. (C) CO-stained mitochondria are located in 2 dendritic branches (d–i), one of which is making a synaptic contact with an axon terminal, also at P10. Scale bar = 0.5 μm. (D) Number of CO-stained mitochondria in dendritic, axonal, and glial elements per 107 μm2 of neuropil surface. (E) Dendritic, axonal, and glial labeling indices displayed as percentages of CO-positive mitochondria over total numbers of mitochondria in each type of structure.
Dendritic Barrel Patterns Are Disrupted in GAP-43 HZ Mice during Early Development

GAP-43 HZ barrels are transiently larger than their WT counterparts (McIlvain et al. 2003), suggesting a possible dysregulation in postsynaptic development (Wong-Riley and Welt 1980). To determine whether dendritic cytoarchitecture was altered in developing GAP-43 HZ mice, we analyzed MAP-2 immunolabeling at P5 in WT and HZ mice. MAP-2 is a neuron-specific, cytoskeletal protein localized to cell bodies and dendrites (Matus et al. 1981), and anti-MAP-2 immunohistochemistry specifically labels layer IV dendrites in barrel cortex (White and Peters 1993; Majewska and Skangiel-Kramska 1996). We found consistent barrel patterning with MAP-2 immunohistochemistry at P5 in WT but not HZ cortex (Fig. 3). In coronal sections, MAP-2 clearly demarcated a positive barrel pattern in WT mice (Fig. 3A-C). By contrast, GAP-43 HZ cortex displayed a variable MAP-2 profile with largely reduced barrel patterning (Fig. 3F). Similarly, tangential sections showed a sharp barrel pattern with clear septal boundaries in WT mice (Fig. 3D) but no clear pattern in HZ mice (Fig. 3H), suggesting a deficit in the development of dendritic arborization in GAP-43 HZ mice.

Altered Dendritic Development in GAP-43 HZ Mice

Our MAP-2 staining suggests that early increases in barrel size in GAP-43 HZ mice result from altered dendritic patterning in these animals. Normal barrel size is restored in the adult (McIlvain and McCasland 2006), however, suggesting that postsynaptic cells can at least partially compensate for presynaptic GAP-43 deficiency. To assay whether increased metabolic activity during a specific developmental period reflects compensatory postsynaptic changes in dendritic arborization, we analyzed Golgi-impregnated neurons at P5 and P7 in WT (Fig. 4B,C) and GAP-43 HZ (Fig. 4D,E) barrels. At this stage of development, layer IV dendrites are still immature (Brown et al. 1995), and dendrite branch dynamics remain high allowing possible fast remodeling to compensate for altered presynaptic function.

Even as early as P5, both WT and GAP-43 HZ barrels showed oriented dendrites (cells labeled with white asterisks in Fig. 4B-E), providing the earliest known evidence that layer IV dendrites begin showing orientation preference during early postnatal development. Quantitative comparison of several dendritic parameters, including dendritic branch length, bifurcation number, and the percent of septal-oriented branches, indicated abnormal development in GAP-43 HZ barrels at P5. Primary branches were longer in WT barrels (Fig. 4F), and there was a significant increase in second-order branching in GAP-43 HZ mice at P5 compared with WT (Fig. 4L). A closer examination of dendrite orientation showed that significantly more oriented cells (65%) display primary branches oriented toward septa at P5 in GAP-43 HZ animals, compared with 23% in WT (Fig. 4M). The dendritic phenotype in GAP-43 HZ barrels likely reflects deficits in TCA targeting (McIlvain et al. 2003) in which TCA afferents display widespread poorly arborized branches in layer IV in GAP-43 HZ animals. This may lead to retention of septally oriented dendrites and an increase in secondary dendritic branches that contact the sparse TCAs.

All the dendritic deficits observed, however, were reversed in older animals. By P7, there was no significant difference in dendritic length or branching between GAP-43 HZ and WT animals (Fig. 4K,M) and 43% of HZ neurons versus 35% in WT displayed septal-oriented branches (Fig. 4N). The transient phenotype displayed by cortical dendrites might suggest an early activity-dependent failure to remodel cortical dendrites in response to reduced levels of GAP-43, and a delayed metabolically intensive compensatory process triggered in the postsynaptic cortical cell.

Altered Protrusion Density in GAP-43 HZ Mice at P7

We wondered whether altered dendritic development was indicative of aberrant synaptogenesis in layer 4 neurons in the GAP-43 HZ somatosensory cortex. Visual inspection showed that at P5 both WT and GAP-43 HZ dendrites bore thin, spindly, and infrequent protrusions suggesting a very immature synaptic phenotype (Fig. 5A,B). The density of these protrusions was highly variable between animals and even between cells and between different stretches of dendrite on the same cell, and therefore, we did not attempt quantification at this age. By P7, however, protrusion density had increased and became more stereotyped across different animals (Fig. 5C,D). Since we had observed a normalization of dendritic structure at P7, we wondered whether synaptic parameters were also similar in WT and GAP-43 HZ animals by this age. To determine...
whether synaptic parameters were comparable by P7, we quantified the density of protrusions of different genotypes at this age. Surprisingly, we found that protrusion density was significantly higher in GAP-43 HZ mice at P7 than in WT mice ($8.87 \pm 0.63$ protrusions/10 $\mu$m, $n = 5$ animals [30 cells; HZ]; $6.97 \pm 0.59$ protrusions/10 $\mu$m, $n = 3$ animals [30 cells; WT]; $P < 0.05$), suggesting that the compensatory dendritic remodeling observed at P7 may be the result of altered synaptic function.

**Glutamate Receptors Are Expressed in A Barrel Pattern during Early Development**

Early dendritic defects, their compensatory remodeling, and altered regulation of putative synaptic protrusions in GAP-43 HZ cortex are likely triggered through an interaction between the pre- and postsynaptic elements through glutamatergic signaling. To quantify the expression of glutamate receptors in developing barrels, we carried out immunostaining for glutamate receptors in WT and GAP-43 HZ cortex. In contrast to earlier studies which failed to find patterning of glutamate receptors in rat barrel cortex until the third postnatal week for NMDA receptors (Blue and Johnston 1995) and P10 for AMPA receptors (Brennan et al. 1997), we found robust patterning of both these glutamate receptors in early development (P4-P7) in the mouse (Fig. 6). AMPA and NMDA were located in complementary patterns, with GluR1 densest in barrel septa (Fig. 6A–C) and NR1 density highest in barrels (Fig. 6D-F). The imbalanced distribution of GluR1 staining in barrel septa might suggest a role for AMPA signaling in stabilization of afferents arising from the posterior medial (POm) thalamic nucleus around P3/4 (Olavarria et al. 1984; Gil et al. 2002) or callosal connections around P4/5 (Ivy and Killackey 1981). The localization of NR1 staining to barrels was further examined in GAP-43 mice at P5. Both WT and HZ mice show a positive barrel pattern (Fig. 6G,H). KO mice, in which barrels do not form, showed no NR1 barrel pattern, suggesting that NMDA receptor (NMDAR) distribution is highly linked to barrel development (Fig. 6I). While it is unclear whether the early barrel patterning of glutamate receptors observed in this study is due to the model system (mouse vs. rat) or experimental technique (immunolabeling vs. autoradiography), our results suggest that glutamate receptors are perfectly poised to play an important role in barrel development (Lee, Lo, et al. 2005).

**Anomalous Glutamate Receptor Expression in C3 Barrel Mouse**

In the barrel cortex, early synaptogenesis occurs through the production of NMDAR-dominated silent synapses that are then converted, in an activity-dependent manner, to mature...
Dendrites (* from GAP-43 HZ animals had increased protrusion density as compared with WT. Density had increased and appeared more uniform between dendrites on a single cell (C, D). Mature spines were visible along with thin, immature protrusions. Dendrites from GAP-43 HZ animals had increased protrusion density as compared with WT dendrites (**P < 0.05; E). Scale bar = 10 μm.

Figure 5. Dendritic protrusion density is increased on layer 4 neurons in GAP-43 HZ animals. Dendritic protrusion density was low and highly variable between dendrites at P5 in both WT and HZ animals (A, B). The majority of protrusions at these ages had immature morphologies with thin stalks and no delineated head. By P7, protrusion density had increased and appeared more uniform between dendrites on a single cell (C, D). Mature spines were visible along with thin, immature protrusions. Dendrites from GAP-43 HZ animals had increased protrusion density as compared with WT dendrites (**P < 0.05; E). Scale bar = 10 μm.

AMPA/NMDA-containing synapses (Petralia et al. 1999). In transgenic mice with altered barrel development, this conversion is often curtailed leaving the cortex in an immature state (Lu et al. 2003; Daw et al. 2007; Inan and Crair 2007). To test whether GAP-43 HZ mice had altered glutamate receptor trafficking, we used unbiased stereology to analyze the overall expression and degree of colocalization of NR1 and GluR1 receptor subunits in the C3 barrel of WT and GAP-43 HZ cortices. We were able to identify barrels based on the low-magnification images as shown in Figure 6. Figure 7 shows typical immunolabeling patterns at P5 in WT (Fig. 7A-C) and GAP-43 HZ (Fig. 7D-F) barrels. At P5, there are fewer NR1 and GluR1-reactive puncta in GAP-43 HZ cortex suggesting deficits in synaptogenesis at this age in GAP-43 HZ animals. Additionally, the degree of colocalization between these 2 markers is increased in GAP-43 HZ cortex suggesting an overabundance of mature, nonsilent synapses at this age. In comparison, at P7 both NR1 and GluR1 puncta were more numerous in GAP-43 HZ cortex than in WT, suggesting increased synaptogenesis to compensate for the early deficit and supporting the increase in protrusion density assayed in Golgi-stained cells (Fig. 5). As expected, in WT cortex, the colocalization of NR1 and GluR1 increased between P5 and P7 likely reflecting the normal conversion of silent synapses into mature ones (Isaac et al. 1997; Feldman et al. 1999). Interestingly, colocalization of the 2 markers declined in GAP-43 HZ animals to WT levels at P7, possibly reflecting the normalization of one aspect of synaptogenesis at this age in parallel with the reorganization of cortical dendrites.

Discussion

Our data indicate that presynaptic GAP-43 assists in the regulation of postsynaptic differentiation in the developing mouse barrel cortex. Using a variety of experimental approaches, we report an unexpected increase in cortical reorganization during a latent postnatal period reflected in increased CO reactivity, dendritic remodeling, and changes in glutamate receptor trafficking in GAP-43 HZ barrel cortex after P6. The shifts in GAP-43 HZ CO were inconsistent with reduced neurotransmission and postsynaptic activity, a predicted effect of GAP-43 deficiency (Dekker et al. 1989). Instead, our results suggest a transient deregulation of cortical postsynaptic differentiation with an intense developmental period of compensatory activity in the postsynaptic cell.

Altered Regulation of Oxidative Metabolism in GAP-43-Deficient Cortex

CO reactivity is traditionally used as a marker for gross barrel morphology. Indeed, the primary use for CO in the barrel cortex has been for this type of general analysis. This is especially true in the morphological survey of mice with barrel pattern deficits (for review, see Inan and Crair 2007). However, CO reactivity can also indicate changes in relative levels of neuronal activity (for review, see Wong-Riley 1989) and has been shown to predict changes in activity levels within postsynaptic dendritic compartments of rodent barrel cortex (Wong-Riley and Welt 1980). In this report, we demonstrated that CO reactivity was deregulated postnatally in barrel cortex of GAP-43 HZ animals with a period of increased metabolic activity spanning from P6 to P14 (Fig. 1). While many variables can contribute to altered CO immunoreactivity, this period coincided with a period of dendritic remodeling and synaptic...
reorganization suggesting increased metabolic activity within the cortex during this time may be linked to postsynaptic processes. Additionally, CO reactivity in GAP-43 HZ cortex returned to WT levels by P21, a time when barrel sizes are also indistinguishable in the 2 genotypes (McIlvain and McCasland 2006) and compensatory postsynaptic reorganization may be complete. Our data suggest that after an early period of altered postsynaptic development, layer IV cells undergo metabolically intensive changes that subside when postsynaptic development normalizes in older animals.

Altered Synaptic Development in GAP-43 HZ Cortex at P5

The fine tuning of somatotopic maps is thought to be dependent on synaptic signaling between TCAs and layer IV cortical neurons. Our data show altered synaptogenesis in layer IV of barrel cortex during the first postnatal week in GAP-43--deficient cortex. While we expect that our data largely represent developmental changes in TCA synapses, it is important to note that it is unlikely that we are looking at TCA synapses exclusively, and other afferent systems may contribute. While intracortical connectivity begins in the second to third postnatal week and continues into adulthood (Miller et al. 2001), afferents arising from subplate neurons form barrel-like clusters in layer IV between P4 and P6 (Pinon et al. 2009). Whether these subplate neurites form synapses in layer IV has not been explored.

Importantly, Pinon et al. (2009) also found that early selective whisker removal prevents subplate afferent reorganization from hollow to septa. This finding suggests that sensory periphery information, conveyed by TCAs, is important for subplate neurite patterning in layer IV, as has been shown for 5-HT afferents (Bennett-Clarke et al. 1994). It is likely that subplate neurites (Pinon et al. 2009) and 5-HT afferents (Bennett-Clarke et al. 1994) are both picking up barrel pattern cues from TCAs. In this regard, the Pinon et al. (2009) findings appear to further stress the importance of early TCA clustering and of the sensory periphery, in “driving” barrel formation (Killacky et al. 1995) and the patterning of other afferent systems.

During early synaptogenesis, a significant portion of synapses do not contain functional AMPA receptors rendering them silent (Liao et al. 1999; Petralia et al. 1999). Appropriate silent synapses are then converted into mature functional synapses by long-term potentiation (LTP)-like mechanisms. By P6–P7, the proportion of silent synapses decreases and by P9 they disappear altogether, suggesting that the developmental period of P6–P9 may be particularly important for thalamocortical synaptic rearrangement in layer IV of rodent barrel cortex (Feldman et al. 1999). Many of the transgenic models of altered barrel development appear to have deficits in the shift from silent to functional synapses and show immature AMPA to NMDA signaling in mature animals (Daw et al. 2007). Surprisingly, GAP-43 HZ mice appear to show deficits in early development with a reduced number of glutamate puncta and increased colocalization of AMPA and NMDA as compared with WT at P5. While we cannot be sure that all the analyzed glutamate staining is synaptic, previous studies have demonstrated that colocalization of AMPA and NMDA staining is largely limited to synapses (Liao et al. 1999) making this more probable. Additionally, we observed increased colocalization as development progressed in WT animals (Fig. 7) showing that our staining can detect the established conversion of silent to functional synapses during this developmental period. Our data suggest that early synaptogenesis is abnormal in GAP-43 HZ mice, with few synapses with mature signaling characteristics being generated instead of the normal large number of NMDA-only synapses (Fig. 8). This may be due to the TCA deficit whereby disturbed, sparse arbors lead to the generation of few contacts and synapses on cortical neurons (McIlvain et al. 2003) that in turn inhibits dendritic growth but may induce increased secondary branching around TCA contacts (Niell et al. 2004). These synapses may be inappropriately strengthened due to increased glutamate signaling in GAP-43--deficient mice (Albright et al. 2007) and lead to the retention of septally oriented dendrites.

Alternatively, HZ mice may display defects in long-term depression (LTD)/LTP which are known to regulate the removal and insertion of AMPA into the synapse (Isaac et al. 1995; Liao et al. 2001), thus regulating synapse maturation. Indeed, it has been shown that “barrelless” mutants (lacking adenylyl cyclase 1 activity) maintain synapses that are rarely silent but are “stuck” in an immature state containing few functional AMPA receptors (Lu et al. 2003). The authors conclude that it is possible that the NMDA-only “silent synapse” may not be the default immature state during development. Instead, cortical synapses may contain a mixture of NMDA and AMPA, consistent with the observation of increased colocalization between AMPA and NMDA receptors in GAP-43--deficient cortex (Pinon et al. 2009). The ratio of AMPA to NMDA receptors may be altered in GAP-43 HZ cortex, potentially affecting synaptic plasticity and the development of barrel structure.
Figure 8. Schematic representation of GAP-43 effects on postsynaptic differentiation. (4A) At P5 WT cortical neurons display dendrites oriented toward the barrel hollow and a large number of immature synapses that are functionally silent due to the presence of NMDA but not AMPA receptors. In contrast, GAP-43 HZ mice display cortical deficits in the development of dendritic and synaptic architecture at these ages. HZ cortical neurons display shorter average dendrites, increased secondary branches, and increased retention of septal-oriented dendrites possibly due to the sparser, less confined TCAs that are present at these ages (McIlvain et al. 2000). HZ cortical dendrites have fewer NR1 and GluR1 puncta and show higher colocalized NR1/GluR1 sites indicative of functional synapses. (B) Between P5 and P7, WT neurons undergo a switch in synaptic glutamate receptor composition in an activity-dependent manner, whereby silent synapses are converted into functional synapses through the insertion of AMPA receptors (Petralia et al. 1999). Interestingly, this does not occur in GAP-43 HZ animals. In fact, there is a rapid addition of synaptic sites such that synaptic density increases as compared with WT, and AMPA/NMDA colocalization is reduced as compared with HZ WT animals but is comparable to WT P7 animals. This suggests that homeostatic mechanisms may be employed to compensate for the deficits in early development in HZ cortex. These mechanisms increase synaptic number and restore the AMPA/NMDA balance that is crucial for developmental plasticity. Dendritic parameters also normalize at P7 in HZ cortex with reestablishment of dendritic length and arborization and pruning of septal-oriented dendritic branches.

AMPA. The removal or insertion of AMPA into the synapse is then regulated by LTD and LTP, respectively, during development. We show significant increases in AMPA in GAP-43 HZ mice (Fig. 7). According to the above stated model, this might suggest trafficking errors stemming from potential deregulation of LTD and LTP and a delay of development (discussed below).

Compensatory Mechanisms May Explain GAP-43 HZ Shifts from P5 to P7

We observed a normalization of dendritic parameters by P7 in GAP-43 HZ mice with normalization of the average dendritic length, the number of secondary branches, and the dendritic orientation toward barrel hollows. This was likely a result of the pruning of spurious secondary branches and inappropriately oriented dendrites, alongside increased growth of new and existing primary dendrites oriented toward the barrel hollow. The fact that dendritic morphology can normalize within a short period of 2 days is astounding although developmental changes in dendritic lengths of up to 1 μm/min have been reported (Mumm et al. 2006).

What could be the trigger for such compensatory remodeling? Electrophysiological studies suggest that neurons maintain an optimal level of synaptic input by globally increasing synaptic strength during periods of low activity and decreasing it during high activity (Turrigiano et al. 1998; Turrigiano and Nelson 2004; Perez-Otano and Ehlers 2005). While several homeostatic mechanisms have been identified, many cells implement compensatory synaptic changes through activity-dependent modulation of receptor composition of existing synapses (Craig 1998) or the creation of new synapses (Harris 1999). Such changes have been described following activity blockade in vitro (Rocha and Sur 1995; Wierenga et al. 2005) and during deprivation paradigms in vivo (Lund et al. 1991; Desai et al. 2002). Our findings suggest that both mechanisms can contribute to dendritic reorganization in developing GAP-43 HZ barrels (Fig. 8). We found an increased density of GluR1 and NR1 puncta at P7 in GAP-43 HZ animals compared with WT suggesting an increase in the number of postsynaptic sites. We also found an increase in the number of protrusions on GAP-43 HZ dendrites at P7, and while we cannot be certain that all of these are functional synapses strictly from Golgi analysis, leaving the possibility that increased protrusion number in GAP-43 HZ mice does not reflect an increase in synaptic contacts, the data do indirectly support the notion that synaptogenesis is upregulated in P7 GAP-43 HZ animals. This increased synaptogenesis agrees with the idea that dendritic normalization occurs as a result of homeostatic mechanisms that increase postsynaptic sensitivity when early synaptogenesis is disrupted by presynaptic GAP-43 insufficiency. Interestingly, the amount of colocalization between these 2 stains was decreased compared with P5 and not statistically different than in P7 WT animals, suggesting that the increase in synaptogenesis may serve to add new functional and NMDA-only silent synapses and bring the synapse population into the correct balance for this developmental stage. The increase in postsynaptic sensitivity may reinstate plasticity mechanisms that allow the pruning of inappropriately oriented or branched dendrites and direct the growth of dendrites oriented toward the barrel hollow. Taken together, our findings represent in vitro evidence of phenotypic rescue in GAP-43 HZ cortex. These compensatory mechanisms may contribute to restoration of normal barrel size by P26 (McIlvain and McCasland 2006).

Pre- and Postsynaptic Interactions in Barrel Cortex

The proper development of the barrel cortex is dependent on the interaction of TCAs and postsynaptic cortical neurons. For instance, cortical inactivation of NMDAR1 results in disruption of both pre- and postsynaptic modules of developing barrels (Iwasato et al. 2000; Lee, Iwasato, et al. 2005; Lee, Lo, et al. 2005). Therefore, it is not surprising that presynaptic GAP-43 deficiency in TCAs results in altered development of layer 4 neurons. The interaction between presynaptic GAP-43 and postsynaptic plasticity has been demonstrated, and GAP-43 phosphorylation by protein kinase C can rescue LTP from NMDAR blockade (Routtenberg 1999; Routtenberg et al. 2000; Kleschevikov and Routtenberg 2001). While complete deletion of GAP-43 results in a severely disrupted cortical map (Maier et al. 1999), partial disruption of GAP-43 (HZ) yields a phenotype somewhat similar to the cortical disruption of NMDAR1. Cortex-specific NR1 KO mice (Ctx-NR1-KO) display a loss of postsynaptic differentiation (i.e., cortical neurons no longer segregate to form barrels, and there is a loss of dendrite orientation bias [Iwasato et al. 2000]). GAP-43 HZ barrels show near normal placement of cortical neurons (Fig. 1F); however, we have demonstrated that GAP-43 HZ dendrites display a transient deficit in dendritic development within barrels (Fig. 4). Within the presynaptic barrel module, Ctx-NR1-KO TCAs are distended.
from P3 to P7 resulting in abnormal patterning (Lee, Iwasato, et al. 2005; Lee, Lo, et al. 2005) suggesting that abnormal postsynaptic activation of NMDAR results in disruption of presynaptic patterning, most likely due to retrograde signaling (Lee, Iwasato, et al. 2005). GAP-43 HZ mice display poorly arborized and distended TCAs at these ages (McIlvain et al. 2003). These findings suggest that a “dialogue” between the pre- and postsynaptic membrane is important for pattern formation in the neocortex and that GAP-43 is an important component of this signaling cascade. The similarities between GAP-43 HZ mice and Ctx-NR1-KO mice further suggest a link between NMDAR activation and GAP-43 activation.

Despite the reciprocal signaling between TCAs and cortical neurons, our data suggest that cortical neurons are able to compensate for presynaptic deficits and reestablish some aspects of their developmental program even when TCA signaling is disrupted. While early synaptogenesis and dendritogenesis appear to be disrupted in GAP-43 HZ, homeostatic mechanisms may be implemented to alter synaptic development and reestablish normal dendritic and synaptic patterns. This may be a general feature of synaptic development in the somatosensory system, as near normal patterns of TCAs have been described in mature mice with cortical deficits (Datwani et al. 2002; Inan et al. 2006; Watson et al. 2006). Thus, while synaptic activity may be an important determinant of developmental patterns in somatosensory cortex, TCAs and cortical neurons may retain the ability to compensate for deficits in their synaptic partners.

**Developmental Delay**

Our findings could also be explained by a delay in barrel development in GAP-43 HZ mice. Published reports show a 24–36 h delay in barrel segregation between WT and GAP-43 HZ (McIlvain and McCasland 2006) as well as a delay in radial glia maturation. A developmental delay stemming from TCA pathfinding errors (McIlvain et al. 2003), inefficient terminal arborization (McIlvain et al. 2003), and/or reduced 5-HT innervation (Donovan et al. 2002) may help to explain these effects. Interestingly, no differences were observed in the critical period for whisker ablation induced plasticity, a mechanism possibly governed by neurotrophins (Domenici et al. 1994) that appear unaffected in GAP-43 HZ mice. A delay of development explanation would also agree with the scenario that early TCA synapses are not silent but are later rendered silent by LTD-like mechanisms (Lu et al. 2003). Thus, our observation of fewer synapses with stronger colocalization of NMDA and AMPA components at P5 in GAP-43 HZ mice could be indicative of an earlier phase of development before LTD has depressed the early synaptic contacts.

**GAP-43 Dosage Regulates Circuit Development in Mouse Barrel Cortex**

It is interesting to note that several developmental disorders, including autism, schizophrenia, DiGeorge syndrome, and Down syndrome, are known gene dosage disorders (Cook and Scherer 2008). GAP-43 HZ animals have reduced levels of GAP-43 (25--50%) but equivalent levels by P26 (McIlvain and McCasland 2006). This reduction in GAP-43 levels (HZ) has a profound effect on barrel development; that is, distinct from the GAP-43 KO phenotype, including TCA misrouting and insufficient terminal arborization (McIlvain et al. 2003), delays in barrel segregation and radial glia maturation (McIlvain and McCasland 2006) and the deregulation of several postsynaptic components shown in this study. This illustrates how dosage of a key regulator of neuronal growth is capable of multiple phenotypes that can profoundly alter circuit development. These effects are consistent with the emerging concept that genetic copy number variation may play a key role in the etiology of neuropsychiatric disorders, including autism (Cook and Scherer 2008).

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**References**


Dekker LV, De Graan PN, Versteeg DH, Oestreicher AB, Gispen WH. 1989. Phosphorylation of B-50 (GAP-43) is correlated with...


