Heterotopia Formation in Rat but Not Mouse Neocortex after RNA Interference Knockdown of DCX

Subcortical band heterotopia (SBH) or double cortex is associated with significant impairments in neocortical function including mental retardation and epilepsy. Mutant alleles of DCX in humans typically cause SBH in females and lissencephaly in males, whereas Dcx null mutations in mice neither disrupt neocortical neuronal migration nor cause SBH formation. In utero RNA interference (RNAi) of Dcx in rats, in contrast, creates an animal model of SBH. Possible explanations for the discrepancies in results following loss of Dcx function include species differences and/or differences between RNAi knockdown and genetic deletion. We have carried out a series of in utero RNAi experiments to investigate possible species differences between rat and mouse to determine the molecular specificity of RNAi against Dcx and to identify the cellular constituents of SBH in the rat model. In utero RNAi in the rat consistently leads to both the formation of SBH and laminar displacement of transfected cells in normotopic cortex, whereas the same treatment in mouse fails to induce SBH but does create laminar displacement. Induction of SBH and impaired radial migration following RNAi against Dcx is rescued by overexpression of Dcx. Thus, both disruptions induced by RNAi are specific to interference of Dcx. SBHs contain transfected pyramidal cells as well as nontransfected cell types, including neocortical interneurons and glia. Together these results indicate that there is a species difference between rat and mouse with respect to RNAi-induced SBH formation and that SBH formation involves the recruitment of several unaltered cell types.

Keywords: doublecortin, epilepsy, lamination, neocortical malformations

Introduction

DCX is critical to the normal development and function of neocortex in humans (des Portes and others 1998; Gleeson and others 1998). Mutant alleles of DCX typically cause 2 distinct types of neocortical malformation depending on whether they are hemizygous in males or heterozygous in females (Gleeson 2000; Leventer and others 2000; Kato and Dobyns 2003). Heterozygosity for DCX mutations causes formation of prominent subcortical band heterotopia (SBH): a band of gray matter embedded in neocortical white matter underlying normal 6-layered cortex. SBH in females is thought to arise because random X inactivation creates a mixture of cells, some expressing a normal allele of DCX and others expressing a mutant allele. This hypothesis has not been directly tested, however, because DCX expression is transiently expressed in developing neocortex (des Portes and others 1998; Gleeson and others 1998; Meyer and others 2002) and because there is no current genetic animal model for X-linked SBH. In hemizygous males, the primary effect of DCX mutation is classic type 1 lissencephaly that, in addition to a dramatic loss of sulci and gyri, is characterized by a thickened 4-layered cortex. Further understanding of how DCX loss of function results in these 2 types of cortical disruption will require studies in animal models.

Targeted mutation of Dcx in mice does not result in neocortical radial migration deficits, although there are lamination deficits in hippocampus (Corbo and others 2002). In an effort to create an animal model of double cortex syndrome, we used RNA interference (RNAi) and in utero electroporation to knockdown Dcx protein expression in radial migrating neocortical neurons generated at the embryonic pallial ventricular zone (VZ) surface in rats (Bai and others 2003). Our reasoning was that there could be species-specific differences in Dcx function and that knockout could result in complications not present in RNAi knockdown. In utero RNAi of Dcx in rats caused a failure in radial migration and formation of SBH with striking similarity to human double cortex (Bai and others 2003). In the initial study we also performed in utero RNAi in several mice and found that Dcx RNAi impaired migration in the embryonic mouse brain as well. In that study, however, we did not examine postnatal time points following in utero RNAi and therefore did not test whether SBHs also form in the mouse after in utero RNAi against Dcx.

In the present study we tested for species-specific responses to RNAi by analyzing phenotypes in the postnatal brains of rats and mice. RNAi-treated mouse brains contained late-born neurons displaced to deep layers similar to RNAi-treated rat brains. In contrast, although RNAi resulted in SBH formation in every rat brain examined, none of the RNAi-treated mouse brains contained SBHs. We used rescue experiments to test whether Dcx RNAi phenotypes are dependent on the interference of Dcx protein expression. Coelectroporation with Dcx overexpression plasmids completely rescued the 2 distinct deficits in migration: formation of SBHs and laminar mistargeting of transfected cells. We used the rat model to characterize the cellular constituents within SBHs. Cellular phenotype analysis revealed the presence of inhibitory interneurons as well as glia within SBHs. Moreover, while inhibitory interneurons and glia within SBHs were never transfected and thus were generated from outside of the regions of transfection, the presence of multiple types of interneurons in SBHs suggests that migrating neocortical interneurons are attracted to aggregates of pyramidal neurons. In addition, the results indicate that heterotopia formation can involve direct impairment of migration and then recruitment of other cell types into the malformation.

Materials and Methods

Electroporation and In Utero RNAi

Short interfering RNA (siRNA) targeting the Dcx 3' untranslated region (DCX 3'UTR) was used in this study as described previously (Bai and
others (2003). A BLAST search for the sequence used (5′-GCUCAGUGAGACCAACAGGCUGAAGGCAGACAGCGUUGGUCUUGUGCAUCUGAGG-3′) returned matches to only 2 mRNA sequences in the database: rat and mouse Dcx. Previously we showed that this construct produced knockdown of Dcx protein expression in vivo and resulted in robust migration disruptions of neocortical neurons (Bai and others 2003). To fluorescently label transfected cells, the plasmid pLZRS-CA-gapEGFP (Okada and others 1999) was coelectroporated with RNAi plasmids. Previously we observed >90% cotransfection in vivo following cotransfection by electroporation.

Cells were transfected in vivo by utero electroporation as described previously (Bai and others 2003). Briefly, multiparous rats or mice (Wisstar or CD-1; Charles River, Wilmington, MA, USA) at 15 and 14 days gestation, were anesthetized with ketamine/xylazine (100/10 mixture, 0.1 mg/g, intraperitoneally); the uterine horns were exposed; and plasmids (1–3 μL) with Fast Green (2 mg/mL; Sigma, St. Louis, MO, USA) were microinjected by pressure (General Valve Picospritzer, Fairfield, New Jersey) through the uterus into the lateral ventricles of embryos by pulled glass micropipettes (0.5 μg/μL for pLZRS-CA-gapEGFP and pCAGGS-DSRed, 1.5 μg/μL for siRNA constructs). Electroporation was accomplished by discharging a 500-μF capacitor charged to 50–100 V with a sequencing power supply. The voltage pulse was discharged across a pair of copper alloy oval plates (1 cm × 0.5 cm) placed on either side of the head of each embryo through the uterus. All animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Immunocytochemistry for Light and Confocal Microscopy

Postnatal rats and mice were deeply anesthetized with halothane followed by ketamine/xylazine and perfused through the heart first with 0.9% saline followed by a phosphate-buffered (0.1 M) fixative containing 4% paraformaldehyde. Brains were removed from the skull and fixed with 4% paraformaldehyde overnight at 4°C, blocked in 2% agar, and sectioned in the coronal or horizontal plane on a vibratome (Leica VT1000S). Free-floating sections (50 μm) were then collected into different wells for immunocytochemistry. Sections were first washed with phosphate-buffered saline (PBS) and then blocked in 5% NGS and 0.2% Triton X-100 for 1 h. Sections were incubated with primary antibodies (BRDU 1:200, Accurate, Westbury New York; calbindin 1:1000, Chemicon, Temecula, California; calretinin (CR) 1:1000 Chemicon; gamma-aminobutyric acid (GABA) 1:100, Sigma, St. Louis, Missouri; GFAP 1:1000, Sigma; GFP 1:200, Chemicon; MBP 1:200 Sternebn Monolonsals, Lutherville, Maryland; NEUN 1:1000 Chemicon; NG2 1:2, generous gift of Akiko Nishiyama; parvalbumin 1:1000, Chemicon; TUJ1 1:1000 Babco, Richmond, California; rat brain pyramidal cells 1:1000, Swant, Bellinzona, Switzerland) in 0.1% Triton X-100, 2.5% NGS, and PBS at 4°C overnight. For use with light microscopy, sections were rinsed several times with 2.5% NGS in PBS and then incubated in biotinylated secondary antibodies (biotinylated goat anti-mouse, goat anti-rabbit; 1:200, Vector Labs, Burlingame, California) for 2 h at room temperature. Sections were rinsed several times with PBS and incubated for 1 h in an avidin-horseradish peroxidase mixture. Sections were rinsed in PBS and then reacted with 0.05% diaminobenzidine in the presence of 0.0015% H2O2. Sections were collected onto gelatin-coated slides, dried for several hours, and coverslipped with Cytoseal. Alternatively, secondary antibodies conjugated for various fluorochromes (Alexa Fluoroespheres (Alexa 488, 568, 635); goat anti-mouse, goat anti-rabbit; all 1:200, Molecular Probes, Eugene, Oregon) were used for confocal microscopy (Leica TCSSP2; laser lines at 488, 543, 633). In some tissue, nuclei were labeled with TO-PRO-3 (Molecular Probes) or counterstained with Neurotrace Nissl 530/615 (Molecular Probes).

Quantification of Lamination Disruption

To quantify the distribution of enhanced GFP (eGFP)-labeled cells in the neocortex, the position of cells was identified relative to the pial surface and white matter (relative units; pixels). The percent distance away from the white matter was computed (0–100%, where 0% = at the white matter and 100% = at the pial surface). The percentage of the total cells counted at each percent distance from the pial surface was plotted. Repeated-measures analyses of variance (ANOVA) were used to compare the position of eGFP+ cells across different conditions (SPSS, Chicago, Illinois).

Laminar Displacement following RNAi of Dcx

In utero electroporation resulted in golgi-like staining of transfected neurons in the postnatal neocortex. Intense cellular labeling was seen in all transfected brains, and there were no apparent decrease in the number, intensity, or quality of cellular labeling from P14 to P105. The intensity and persistence of the labeling late into postnatal development indicates that the transfection of VZ progenitors in utero results in a stable neuronal transfection. Transfection at E15 of eGFP expression plasmids without RNAi labeled pyramidal neurons solely in upper layers (Figs. 1A and 2A). In contrast, in all RNAi-treated brains examined (n = 20), transfected cells were both aggregated within heterotopia and were dispersed throughout normotopic cortex overlying SBHs. The scattered eGFP+ neurons in normotopic cortex had pyramidal morphologies with spiny dendrites consistent with their generation at the VZ surface. Quantification of the position of control and RNAi-transfected eGFP+ cells in normotopic cortex showed that control-transfected cells were confined to a peak position corresponding to layers 2/3, whereas RNAi-transfected neurons were distributed without a single peak throughout layers 2–6 (n = 11 sections from 6, P14 brains). Repeated-measures ANOVA revealed a significant difference between the positions of control and Dcx RNAi eGFP+ cells in normotopic neocortex (F = 6.49, df = 98, P < 0.001).

In 5 of 20 RNAi-transfected brains examined, displaced eGFP+ cells were arranged into conspicuous radial minicolumns that...
spanned nearly all lamina of normotopic cortex (Fig. 2). Similar columns were never found in any control-transfected brains. Representative images of the induced radial minicolumns are shown in Figure 2D–E1). Columns were 2–3 cells wide and contained chains of cells with intertwined apical dendrites. The minicolumns occurred throughout a range of postnatal time points: P14, P28, and P105. At present we do not know why the minicolumns are present in some transfections and not others but suspect that local differences in transfection location or transfection efficiency may play a role.

The presence of displaced neurons found in all lamina of neocortex raised the possibility that transected neurons may undergo laminar fate respecification. Thus, displaced neurons now found in deep lamina may extend axons to subcortical targets normally made by deep-layer neurons. To test this possibility, we performed retrograde labeling experiments by injections of rhodamine beads (Retrobeads; 530/590 nm, Lumafluor) into the pyramidal tract: an exclusive target of layer 5 neurons. In 3 brains tested (P60), we observed retrogradely labeled neurons confined to deep layers in close proximity to displaced eGFP+ neurons; however, no transected neurons were double labeled for both retrograde label and eGFP (Supplementary Figure online). Similarly, we did not find eGFP+ axons in subcortical targets for deep-layer cells (thalamus, tectum, brainstem) but did find labeled axons in the corpus callosum and ipsilateral cortical hemisphere. Together, these data indicate that RNAi of Dcx results in laminar mistargeting without a respecification of laminar fate.

**Laminar Dysplasia but No SBHs in the Mouse after RNAi of Dcx**

Species differences are one possible explanation for the differences between the Dcx knockout mouse and RNAi of Dcx in rats. We therefore performed RNAi experiments in mice and analyzed brains at P21. Similar to that observed in control-transfected rat brains, eGFP+ cells in control-transfected mouse brains at E14 were observed exclusively in upper layers of dorsolateral neocortex (Fig. 3). In contrast, Dcx RNAi in mouse resulted in laminar misplacement of transected neurons in all brains examined (n = 10). Similar to rat, eGFP+ cells were observed scattered throughout all lamina except layer 1. Figure 3B–B1 contains photomicrographs from a representative RNAi-transfected mouse brain where eGFP+ cell bodies can be seen in all layers. In layer 4 of barrel cortex of RNAi-transfected mouse brains, displaced eGFP+ cells were found primarily within septa (arrow in Fig. 3B1), although few cells were observed within barrel hollows; however, cell-dense septa and cell-sparse hollows appeared normal in Nissl-stained sections. Comparison of the laminar positions of transected cells in RNAi-treated mouse brains (P21, n = 10) with that of control-transfected brains revealed a significant shift in distribution of the position of transected neurons similar to that observed in the rat (F = 9.1, df = 98, P < 0.001). Transected cells in both control and RNAi conditions had callosal projecting axons characteristic of upper layer neurons (Fig. 3A, A1).

Although we found laminar displacement of neurons in 10 of 10 brains, we found no SBHs in RNAi-treated mice. Scattered
transfected eGFP+ cells could be observed in white matter, but these cells were few in number (<1% of all eGFP+ cells) and were not observed in aggregates. In addition, radial minicolumns were never observed in cortical lamina in the mouse following Dcx RNAi, although, nonradial clusters of transfected cells were observed in gray matter (Fig. 3C). Thus, there are species-specific differences in the development of SBHs following Dcx RNAi; however, there is also still a clear impairment in radial migration following RNAi in the mouse.

**Rescue of Laminar Dysplasia and SBH Formation by Dcx Overexpression**

RNAi is well known to create off-target effects (Scherer and Rossi 2003; Scherr and Eder 2004; Sledz and Williams 2004). This raised the specter that the RNAi-induced migration anomalies were due to off-target effects of Dcx RNAi vectors. The best test for specificity of RNAi is an “add back” or “rescue” experiment. In such experiments, RNAi plasmids are cotransfected along with overexpression plasmids. If the normal effect of the RNAi is attenuated by addition of only 1 target gene, then the argument is that the phenotype is due to knockdown of that target. We cotransfected the RNAi plasmid against the 3’ UTR of Dcx along with an expression plasmid missing the 3’ UTR and driven by a strong promoter (pCAGGS-DCX). Transfected brains (n = 9) were harvested at P60 and processed for eGFP and/or Dcx immunohistochemistry as well as Nissl staining. As shown in Figure 4, coelectroporation of Dcx RNAi with a Dcx overexpression plasmid resulted in both a reduction of SBHs and normal laminar position of transfected neurons (P14, n = 6 brains). In addition, SBHs were not detected in 3 rescue-treated brains, and small SBHs were detected in 3 others. RNAi-transfected brains contained SBHs that averaged 2.54 ± 0.29 mm along the medial/lateral axis and 0.64 ± 0.1 mm along the dorsal/ventral axis, whereas the 3 SBHs in the rescue experiments were 0.77 ± 0.15 mm (medial/lateral) and 0.18 ± 0.06 mm (dorsal/ventral) (F = 138.39, df = 1; P < 0.001 for medial/lateral; and, F = 73.28; df = 1; P < 0.001 for dorsal/ventral dimension). Analysis and comparison of the laminar positions of transfected cells in rescue-treated and control brains revealed overlapping peaks in the distribution of neurons corresponding to layers 2/3 that were not statistically different (F = 1.08, df = 98, P = 0.28). The rescue of both SBH formation and laminar displacement indicates that both types of malformations induced by RNAi are dependent upon Dcx expression.

**Cellular Constituents of SBHs**

The abovementioned results indicate that in utero RNAi of Dcx in developing rat neocortex remains the only model of SBH dependent upon loss of Dcx function. We have used this model to further explore the formation of heterotopia by determining the identities of cells within SBHs. Heterotopia contained a mixture of both transfected (eGFP+) and untransfected (eGFP−)
cells. All transfected cells examined within SBHs were positive for the neuronal markers NEUN (100%, \( n = 3 \) brains; P14), TUJ1 (100%, \( n = 3 \) brains, P14; Fig. 5B), and PYR (100%, \( n = 1 \) brain; P70; Fig. 5B1). Transfected neurons within SBHs had atypical pyramidal morphologies with spiny dendrites and were atypically oriented; cells could be found at nearly all orientations including inverted. Axons from transfected neurons often exited the SBHs and coursed through the corpus callosum or entered the overlying neocortex. Many untransfected cells within the SBHs were also positive for the neuronal markers NEUN, TUJ1, and PYR (100%, \( n = 7 \) brains). Untransfected cells in the SBHs, however, which did not express neuronal markers, were positive for glia markers. As shown in Figure 5H–L, numerous untransfected cells within SBHs were MBP+, GFAP+, and NG2+ cells. Consistent with the observation that 100% of transfected cells in the SBHs colocalized with neuronal markers, none of the eGFP+ cells in SBHs were positive for the glial markers. Thus, transfected cells within the SBHs are exclusively neurons, whereas the total population of cells within the heterotopia is diverse.

**Interneurons in SBHs**

The abovementioned results suggested that untransfected neurons and glia are recruited into the forming heterotopia. We further hypothesized that neurons generated far from the region of transfection may also be recruited into the heterotopia. Because the electroporation method used here targets cells exclusively at the dorsal pallial VZ surface and because GABAergic interneurons in the rodent forebrain are generated in the ventral ganglionic eminence (GE), we determined whether GABAergic interneurons are present in SBHs. Transfected eGFP+ neurons within or outside of SBHs were never positive for any of the 4 GABAergic interneuron markers: GABA, GAD65, parvalbumin, or CR. A small population (4%) of transfected neurons was positive for calbindin; however, layer 2/3 pyramidal neurons can also express calbindin (Demeulemeester and others 1989; van Brederode and others 1991). Moreover, eGFP+ /calbindin+ neurons had pyramidal morphologies and spiny dendrites and so are unlikely to be GABAergic interneurons (Fig. 6). The remaining calbindin+ neurons (96%) in SBHs were eGFP+ with multipolar morphologies; these cell types are likely to be calbindin+/GABA+ neurons (Ang and others 2003).

Together these observations indicate that electropropagation of the VZ fails to transfect interneurons, and this supports accumulated evidence that interneurons in the rodent cortex are not generated from the pallial VZ surface.

Although interneurons were not transfected by in utero electroporation, we observed significant numbers of GABA+, GAD65+, parvalbumin+, CR+, and calbindin+ neurons in all SBHs examined (Fig. 6; \( n = 3 \) brains, P14; \( n = 1 \) brain, P28; \( n = 2 \) brains, P105). In addition, parvalbumin+ terminals were found surrounding eGFP+ neurons, suggesting the formation of "basket synapses" in heterotopia typical of interneuron circuitry in normotopic cortex (Fig. 6C2). Despite the presence of significant numbers of inhibitory neurons within SBHs, we observed no qualitative difference in the density or distribution of interneurons in normotopic cortex overlying SBHs (Fig. 6E–G). Although quantitative stereology will be required to determine if there is a significant change in interneuron number in normotopic cortex, our current qualitative results indicate that SBH formation involves recruitment of interneurons into areas where pyramidal neurons have stalled in their radial migration in the intermediate zone (IZ).
Discussion

RNAi of Dcx in rats consistently resulted in 2 types of migrational disruption, whereas RNAi of Dcx in mice resulted solely in laminar displacement. Both phenotypes are rescued following coelectroporation of RNAi and Dcx expression vectors in the rat. This data combined with previously reported data confirming a decrease of Dcx protein expression levels in vivo following RNAi (Bai and others 2003) provides strong evidence that the RNAi-induced disruptions in migration are due to Dcx loss of function.

Although the similarities in the development and structure of rodent neocortex are generally emphasized, there are significant species differences between the adult rat and mouse neocortex. In addition to the obvious differences in size, mouse neocortex is significantly thinner, has increased neuronal densities in all layers, and has a thinner layer 2/3 relative to other layers (DeFelipe and others 2002), suggesting differences in neuronal proliferation and migration. In each of these quantitative measures, the values for rat are intermediate to those for mouse and human (DeFelipe and others 2002). A related underlying difference in development shared by human and rat but not by mouse may account for the apparent differences seen with Dcx RNAi in this study between mouse and rat and in the effects of germ line Dcx mutations between mouse and human (Corbo and others 2002). Bayer and Altman (1991) provided quantitative data of many features of the developing rat VZ, IZ, and CP, such as changes in thickness, cell density, and nuclear morphology and orientation. Complimentary data in fetal mouse and human are unknown, making direct comparisons difficult. In the future, comparisons of the structural and morphological features of the developing VZ, IZ, and CP between these species might help identify differential requirements of Dcx.

The biochemical and cellular determinants of Dcx function within cells is becoming increasingly well defined. Dcx stabilizes and associates with microtubules (Gleeson and others 1999; Yoshiura and others 2000; Bielas and Gleeson 2004), and this function is dynamically regulated by the phosphorylation state of Dcx. Several kinases can phosphorylate Dcx, and the capacity for Dcx to stabilize microtubules decreases upon phosphorylation of Dcx (Reiner and others 2004; Tanaka and others 2004). This dynamic microtubule-stabilizing function may be important for coordinating the outgrowth of processes with nucleokinetisn movements (Gdalyahu and others 2004; Graham and others 2004; LoTurco 2004). A current challenge is to link these biochemical functions to specified modes of migration or transitions between different modes that occur through neuronal migration in vivo (Nadarajah and others 2001; Kriegstein and Noctor 2004; LoTurco 2004). The 2 distinct phenotypes created by RNAi of Dcx may indicate that different cells may require Dcx
neurons with RNAi is consistent with a critical interaction between pyramidal neurons and interneurons during migration through the IZ. Similarly, Hammond and others (2004) described non-cell autonomous disruptions of interneuron migration in "supercortex" that form in p35−/− and p35+/+ chimeric mice (Hammond and others 2004). Similar conclusions have been made with other chimeric mutants (Gilmore and Herrup 2001). Non-cell autonomy between neurons in disorders of migration implies cooperative interactions between migrating neurons and suggests that relatively large malformations in neocortex may form if merely a subset of neurons is disrupted from their normal migration pattern. Correspondingly, as shown in our rescue experiments, if only the abnormal cells are molecularly rescued then the entire malformation is prevented.

**Supplementary Material**

Supplementary Figure can be found at (http://www.cercor.oxfordjournals.org/).

**Supplementary Figure.** No evidence of corticospinal projections made by displaced eGFP+ neurons in layer 5. Neurons transfected with Dcx RNAi (eGFP+) are displaced and can be found in layer 5 in close proximity to retrogradely labeled corticospinal neurons (red; A–A2). Higher magnification and three-dimensional rendering of eGFP+ cell in A–A2 reveals a lack of co-localization with the retrograde label (B–C). Arrowheads in B2 point to retrogradely labeled neuron (red arrowhead) and eGFP+ transfected neuron (green arrowhead). Scale bars: A–A2 = 100 μm; B–B3 = 15 μm; C = 30 μm.

**Notes**

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for different aspects of their migration. For example, cells that use Dcx for extension of processes may require Dcx to enter the cortical plate (CP), whereas those that use Dcx for nucleokinesis may require Dcx for appropriate laminar positioning. A prediction of this model is that radial migration in mouse requires Dcx primarily for nucleokinesis, whereas in human and rat a significant proportion of migrating neurons require Dcx for process growth and invasion into the CP. Data regarding differences in the proportion of cells employing translocation versus locomotion modes in mouse, rat, and human are unknown. Interestingly, Dcx localization appears to be similar in embryonic mouse and rat, with high levels of protein expression in the IZ, subplate, CP, and few Dcx+ cells in the VZ (Francis and others 1999; Gleeson and others 1999). Localization studies in humans, however, have revealed a distribution of Dcx different from mouse and rat. For example, radial columns of Dcx+ neuroblasts spanning the entire neuroepithelium (VZ-preplate), have been observed in 5.5- to 6.5-week-old human fetal neocortex (Meyer and others 2002).

Interneurons in the rodent neocortex migrate from the GE through both the marginal zone and the IZ (Anderson and others 1999, 2001, 2002; Xu and others 2003, 2004). The correspondence of the birth date and laminar position of interneurons and pyramidal neurons suggests an interaction between these cell types generated in different regions (Nadarajah and others 2002, 2003). Whether or not migrating interneurons interact with migrating cortical pyramidal neurons as they migrate through the IZ in order to secure a defined laminar position is not currently known. The presence of all subtypes of interneurons in SBHs following interruption of only pyramidal
SBHs and are never eGFP+ normotopic cortex are GABA A2 PV, or CR. Scale bars: 1330 µm.

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References