Timing of Cortical Interneuron Migration Is Influenced by the Cortical Hem

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Cerebral cortical γ-aminobutyric acid (GABA)ergic interneurons originate from the basal forebrain and migrate into the cortex in 2 phases. First, interneurons cross the boundary between the developing striatum and the cortex to migrate tangentially through the cortical primordium. Second, interneurons migrate radially to their correct neocortical layer position. A previous study demonstrated that mice in which the cortical hem was genetically ablated displayed a massive reduction of Cajal-Retzius (C-R) cells in the neocortical marginal zone (MZ), thereby losing C-R cell-generated reelin in the MZ. Surprisingly, pyramidal cell migration and subsequent layering were almost normal. In contrast, we find that the timing of migration of cortical GABAergic interneurons is abnormal in hem-ablated mice. Migrating interneurons both advance precociously along their tangential path and switch prematurely from tangential to radial migration to invade the cortical plate (CP). We propose that the cortical hem is responsible for establishing cues that control the timing of interneuron migration. In particular, we suggest that loss of a repellant signal from medial neocortex, which is greatly decreased in size in hem-ablated mice, allows the early advance of interneurons and that reduction of another secreted molecule from C-R cells, the chemokine SDF-1/CXCL12, permits early radial migration into the CP.

Keywords: Cajal-Retzius cells, Cxcl12, Cxcr4, guidance cues

Introduction

γ-Aminobutyric acid (GABA)ergic interneurons regulate the balance of excitation and inhibition in the cerebral cortex and are therefore integral to the physiological processes of the forebrain (Gilbert and Wiesel 1985; Jones 1986; Freund 2003; Hensch 2005; Somogyi and Klausberger 2005; Woo and Lu 2006). As might be expected, altered GABAergic transmission has been reported in many human psychiatric and neurological disorders such as schizophrenia, epilepsy, autism, and Alzheimer’s disease (Young 1987; Fonseca et al. 1993; Keverne 1999; Cobos et al. 2005; Levitt and Gant et al. 2009). Understanding the control of interneuron development has therefore received considerable recent attention (Fishell 2007; Butt et al. 2008; Marsh et al. 2008; Potter et al. 2009).

Cortical interneurons in the mouse originate from progenitor cells in the telencephalic ganglionic eminences (Anderson et al. 1997, 1999) and adopt the same cortical layer as pyramidal neurons born at the same time in the cortical ventricular zone (VZ) (Hevner et al. 2004). Thus, coordinating the timing of different stages of interneuron migration may be key to determining the final position of the interneurons and their functional integration into cortical circuitry (Hevner et al. 2004; Li et al. 2008; Lopez-Bendito et al. 2008).

Several factors have been identified as regulators of cortical interneuron migration (Marin and Rubenstein 2003; Huang 2009). To reach the cerebral cortex, cortical interneurons are directed toward the corticostriate boundary from the ganglionic eminences. Neuregulin proteins are chemotactants in this early stage of migration. GABAergic cells born in the medial ganglionic eminence follow a corridor through the lateral ganglionic eminence (LGE) that expresses Neuregulin1 (Nrg1), which encodes the ligand of the tyrosine kinase receptor ErbB4 (Flames et al. 2004; Ghashghaei et al. 2006). Chemorepulsion additionally directs interneurons along this route, mediated by Neuropilin receptors (Nrp1, Nrp2), and activated by class-3 Semaphorins (Sema3a and Sema3f) in the striatal mantle zone (Marin et al. 2001).

Time-lapse videomicroscopy shows that the migratory behavior of interneurons in the cortical primordium is complex and dynamic (Ang et al. 2003; Li et al. 2008; Lopez-Bendito et al. 2008). We focus here, however, on a simplified model of interneuron movement: tangential migration in 2 streams through the marginal zone (MZ) and subventricular/intermediate zones (SVZ/IZ), followed by radial migration into the cortical plate (CP) (Huang 2009). Directly relevant to our study, invasion of the CP is delayed for both early- and late-born interneurons such that even early-born interneurons fail to enter the CP until after E15.5 (Lopez-Bendito et al. 2008).

Factors identified as regulators of tangential and radial interneuronal migration include the chemokine CXCL12, previously called SDF-1 (stromal cell derived factor-1), which is generated by meningeal cells overlying the cortex, Cajal-Retzius (C-R) cells in the MZ, and pyramidal cell precursors along the SVZ/IZ migratory route (Daniel et al. 2005; Tiveron et al. 2006). CXCL12 in the MZ and SVZ/IZ provides interneurons with 2 "most permissive" routes through the cortical primordium (Borrell and Marin 2006; Lopez-Bendito et al. 2008). In mice deficient in the chemokine CXCL12, or its receptor CXCR4, interneurons alter their tangential migratory routes. When CXCL12 is disrupted in the SVZ/IZ, the cells switch their migration to the MZ route. If CXCL12/CXCR4 signaling is abolished, interneurons migrate broadly throughout the thickness of the cortical primordium and enter the CP prematurely (Stumm et al. 2003; Tiveron et al. 2006; Lopez-Bendito et al. 2008; Zhao et al. 2008). Thus, CXCL12/CXCR4 signaling not only directs interneurons along their 2 major tangential migration routes but also controls the timing of their exit from these routes into the CP (Li et al. 2008).

Other factors responsible for timing interneuron migration into the cortex have been demonstrated but not yet identified at the molecular level (Britto et al. 2006). Cell behavior in cortical slice cultures reveals that explants of the medial cortical primordium inhibit tangential migration of interneurons at early stages of corticogenesis. Subsequently, medial cortex becomes a permissive substrate for interneurons. Medially located cues therefore regulate the advance of interneurons from lateral to medial in the cortical primordium (Britto et al. 2006).
In a previous study, mice were engineered to lack a cortical signaling center, the cortical hem, by introducing the diphtheria toxin subunit A (dt-a) into the hem by Cre-lox recombination (Yoshida et al. 2006). Hem-ablated mice showed a massive loss of reelin-expressing C-R cells, demonstrating that the hem is the source of a major population of neocortical C-R cells (Yoshida et al. 2006). Given the production of reelin by C-R cells (D’Arcangelo et al. 1995; Ogawa et al. 1995; Meyer et al. 1999; Derer et al. 2001), and previous reports of neocortical layering defects in the reeler mutant mouse, which lacks reelin, and other mutants with defects in reelin signaling (Caviness 1982a; Caviness 1982b; Howell et al. 1997; Sheldon et al. 1997; Trommsdorff et al. 1999; Hammond et al. 2006), a perturbed neocortical layer distribution was expected in hem-ablated neocortex. Contrary to expectation, the laminar pattern was near normal. The sheet of C-R cells that covers the neocortical primordium therefore appears not to be required to direct the radial migration and laminar distribution of neocortical pyramidal neurons (Yoshida et al. 2006).

In the present study, we investigated the effect of cortical hem loss on interneuron migration, taking advantage of the previously generated mouse line (Yoshida et al. 2006). In contrast to the lack of major effects on pyramidal cells, we found prominent defects in the timing of interneuron migration in hem-ablated mice. Both the tangential advance of interneurons into the cortical primordium and the subsequent shift to a radial mode of migration were premature in hem-ablated mice compared with control mice. We therefore investigated the progression of interneuron migration in hem-ablated mice to determine which stages, and likely guidance mechanisms, were affected by loss of the hem.

**Materials and Methods**

**Mouse Lines**

The Institutional Animal Care and Use Committee (IACUC) of the University of Chicago approved all protocols, and mice were used according to National Institutes of Health (NIH) guidelines. Generation of hem-ablated mice has been previously described (Yoshida et al. 2006). Briefly, a cassette carrying an internal ribosome entry site (IRES), followed by stop codons flanked by loxP sites, followed by a cDNA encoding the diphtheria toxin subunit A (dt-a) (Lee et al. 2000), was inserted into the 3′-end of the Wnt3a locus, using standard embryonic stem cell technology (Yoshida et al. 2006). In mice carrying this mutant Wnt3a allele, activation of the toxin was prevented by the stop codons preceding dt-a (Lee et al. 2000). Dt-a was activated in the hem by crossing mice carrying the Wnt3a-IRESneo-dt-a-allele with an Emx1-IRES-Cre mouse line (Gorski et al. 2002). In cells that coexpressed Emx1 and Wnt3a dt-a was activated, killing the cells. In the telencephalon, such cells were confined to the cortical hem. Mouse-typed controls have the Wnt3a-IREScneo-dt-a-; Emx1-IRES-Cre- genotype and show an entirely normal hem. Mice with the Wnt3a-IREScneo-dt-a-; Emx1-IRES-Cre- genotype are referred as hem-ablated mice and die shortly after birth, apparently due to a jaw defect that prevents normal feeding. Hem-ablated mice were compared with littermate controls.

**Tissue Processing**

Brains were collected from embryos aged E12.5–E17.5 and fixed by immersion in 4% paraformaldehyde. Fixed brains were embedded in 30% sucrose, 10% gelatin in phosphate-buffered saline, and sectioned coronally or sagittally with a Leica SM2000R microtome. Sections were processed for in situ hybridization (ISH) with digoxigenin (Dig)-labeled riboprobes (Yoshida et al. 2006). Sections were first permeabilized by treatment with proteinase K (1 μg/mL) for 20 min. After posthybridization washes, bound Dig was detected with anti-Dig antibody (Roche). Fluorescent in situ hybridization (FISH) was performed on coronal and sagittal brain sections with fluorescein isothiocyanate (FITC)-labeled riboprobes. After posthybridization washes, bound FITC was detected with a peroxidase-conjugated anti-FITC antibody (Perkin Elmer) and incubation with the peroxidase substrate FITC-tetramide (Roche). For double FISH, following inactivation at 70°C, the second probe was localized with a peroxidase-conjugated anti-Dig antibody and Cy5-tetramide (Roche). At least 3 mutant and 3 control embryos were analyzed for expression of each gene at each age described in Results.

**Quantification**

At least 6 sections from 3 brains from each genotype were used for quantitative analysis of tangential migration of Lhx6-positive interneurons. Lhx6 is expressed in the vast majority of cortical interneurons (Cobos et al. 2006). Sections were imaged using an Axioscope with an Axiocam camera and software (Zeiss); contrast and brightness were adjusted with Adobe Photoshop CS4. The tangential distance traveled by interneurons from their entry point into the cortex at the corticostriate boundary to their migration front at a given age was measured using ImageJ software (series 1.4, NIH, public domain). The mean distance traveled through the cortical primordium was compared between control and hem-ablated brains at E12.5 and E13.5. Statistical significance was determined with the t-test (Microsoft Excel).

To compare the number of Lhx6-positive interneurons occupying the CP and the upper intermediate zone (uIZ) at E15.5 in hem-ablated and control brains, sagittal sections were processed with ISH for Lhx6. Four sections, evenly spaced through one hemisphere, were analyzed from each of 3 control and 3 hem-ablated brains, and a field of the cortical primordium, 1 mm wide, was selected from each section at the same dorsalventral level for cell counting. In each imaged field, upper and lower boundaries were drawn for the CP (Adobe Photoshop, CS4), which was visible at E15.5 as a densely packed cell layer, and for a band of equal width to the CP, immediately beneath, containing the uIZ. Cells were counted separately in the CP and uIZ bands. Total cell counts for the CP and uIZ in all sections from each brain were compared between control and hem-ablated cortices. The difference in cell number in the CP and uIZ between the 2 groups was evaluated with a one-tailed t-test (Microsoft Excel).

**Results**

**Genetic Ablation of the Cortical Hem Results in Aberrant Tangential Migration of GABAergic Neurons**

To test for differences in the migration of interneurons between hem-ablated and control brains, interneurons were identified by expression of Lhx6 and Erbb4 (Yau et al. 2003; Alifragis et al. 2004; Zhao et al. 2008), and tangential migration was assessed at 2 embryonic stages. In both hem-ablated mice and controls, interneurons migrated along a superficial stream in the MZ and a deeper and more populated stream in the SVZ/IZ (Ang et al. 2003; Tanaka et al. 2006; Huang 2009). At E12.5, there was no significant difference in the extent of tangential migration between hem-ablated mice and controls (Fig. 1A,B). At E13.5, however, in both migratory streams, Lhx6- and Erbb4-expressing interneurons extended further dorsally in the mutants than in controls (Fig. 1C–H).

The maximum dorsal extension of Lhx6-expressing interneurons along the SVZ/IZ route was quantified by measuring the distance of the migration front from the corticostriate boundary at rostral, mid, and caudal levels of the cortex. At E13.5, Lhx6-positive interneurons had migrated significantly further into the cortical primordium in hem-ablated mice compared with controls at all 3 levels (Fig. 1F). These
observations indicate that interneurons advance prematurely through hem-ablated cortex.

Subcortical Cues Regulating Interneuron Migration Are Unaffected

To determine the possible causes of early interneuron invasion of the cortical primordium, we first tested whether loss of the hem affects selected guidance cues in the developing striatum, through which the interneurons migrate, or disrupts the corticostriate boundary, or "anti-hem" (Assimacopoulos et al. 2003), which interneurons cross to enter the cortical primordium. Because the cortical hem is a dorsal telencephalic organizer, its loss could affect development throughout the dorsoventral axis of the telencephalon, including the anti-hem and ventral telencephalon. We found no obvious defects, however, in the expression of major interneuron guidance molecules in the ventral telencephalon (Corbin et al. 2001; Tanaka et al. 2003). At E13.5, no differences were observed between control and hem-ablated mice in the expression patterns of genes encoding Semaphorin3a (Sema3a) and Semaphorin3f (Sema3f) (Fig. 2A–D), which create an inhibitory territory for migrating interneurons (Marin et al. 2001; Tamamaki et al. 2003). Similarly, no differences were seen in the expression of the gene encoding Nrg1 (Fig. 2E,F), which guides ErbB4-expressing interneurons through the LGE (Flames et al. 2004; Ghashghaei et al. 2006), or in the position of ErbB4-expressing interneurons, relative to domains of Nrg1 (Fig. 2E,F'). Furthermore, Sema3a, Sema3f, and Nrg1 were expressed similarly in the cortical primordium of mutant and control mice (Fig. 2A–F).

The corticostriate boundary, or "anti-hem" (Assimacopoulos et al. 2003), produces several secreted signaling molecules, including FGF7, sFrp2, and TGFα, and is marked by boundaries of Dlx2, Emx2, Ascl1, and Gsx2 expression (Assimacopoulos et al. 2003; Backman et al. 2005; Long et al. 2009). Defects in the position of the anti-hem, or its production of signaling molecules, could well affect interneuron migration across the region. Indeed, defective positioning of the corticostriate boundary in the small-eye mutant causes an apparently excessive migration of interneurons into the cortical primordium (Gopal and Golden 2008). We saw no differences between control and hem-ablated brains, however, in the pattern or density of expression of Sfrp2, Tgfα (Fig. 3A–D), Fgf7, Ascl1, and Gsx2 (data not shown), indicating that the anti-hem was normally positioned in hem-ablated mice and that anti-hem signaling
was unaffected. Defects at the anti-hem are therefore unlikely to be responsible for the premature advance of interneurons into the cortical primordium.

In the cortical primordium, the chemokine CXCL12, produced by pyramidal neuron progenitor cells, provides a permissive substrate in the SVZ/IZ along which interneurons migrate (Tiveron et al. 2006). Similarly, CXCL12 produced by the meninges directs tangential interneuron migration in the neighboring MZ (Tiveron et al. 2006). At E13.5, Cxcl12 was expressed at equivalent levels in the SVZ/IZ and meninges in hem-ablated mice (see Supplementary Fig. 1), indicating that a major chemoattractant along the 2 tangential migratory routes was unaffected, at this age, by loss of the hem.

**Figure 2.** Distribution of major subpallial guidance cues for interneurons is similar in control and hem-ablated brains. (A-F) Coronal sections of E13.5 brains, processed with FISH for class III Semaphorins (Sema3a and Sema3f) (A-D) and Neuregulin1 (Nrg1) (E,F). Arrowheads in (A-F) mark the corticostriate boundary as a landmark for evaluating gene expression patterns. (A-F) Expression patterns of Sema3a, Sema3f, and Nrg1 appear highly similar between control and hem-ablated mice in the developing striatum (St) and neocortex (ncx). An exception is the cortical hem (ch) in which Nrg1 is strongly expressed in the control (E) but absent, as expected, in the hem-ablated mouse (F). Double FISH for Erb-B4 (green), marking interneurons, and Nrg1 (red) (E’,F’). Scale bar, 250 μm (A-F).

**Medial Cortex Controls Timing of Tangential Interneuron Migration and Is Reduced by Hem Loss**

Previous slice culture experiments demonstrated that overlaysing a cortical slice with a medial cortical explant inhibits interneuron migration at E12.5 (Britto et al. 2006) but that medial cortex becomes permissive for migration about a day later (Britto et al. 2006). The correspondence between the active period of this inhibitory cue and the timing of the premature advance of interneurons in hem-ablated cortex led us to examine whether medial neocortex is reduced in the hem-ablated brain.

Cre-mediated recombination directed by the Emx1-IRES-Cre mouse begins at E9.5 (Gorski et al. 2002). By E10, the cortical hem in Wnt3aIRESxneoxdt-a/+; Emx1IRESCre/+ mice was almost completely ablated, evident by the loss of characteristic hem expression of Wnt3a and Lmx1a as well as tissue loss (Yoshida et al. 2006). By E12.5, gene expression that identifies the hippocampal primordium was missing, and, at E18.5, gene expression markers of the hippocampal formation from the dentate gyrus to the subiculum were absent (Yoshida M, Grove EA, unpublished data). Loss of the hippocampus would be predicted given that loss of a single signaling molecule, Wnt3a, from the hem prevents hippocampal development (Lee et al. 2000).

In addition to loss of the hippocampus, we observed a large reduction in the primordium of medial neocortex in hem-ablated brains. At E13.5, the hippocampal and neocortex primordia express Emx1, an ortholog of Drosophila empty spiracles. The domain of Emx1 expression in hem-ablated cortex at E13.5 was much smaller than expected from the loss of the hippocampus alone (Fig. 4A,B), suggesting additional reduction of medial neocortex. In control mice at E13.5, Wnt7b is strongly expressed in the lateral neocortical primordium but relatively weakly expressed in the medial neocortical and hippocampal primordia (Fig. 4C). In hem-ablated mice, strong Wnt7b expression continued to the far medial edge of the

**Figure 3.** Corticostriate boundary is properly positioned in hem-ablated mice. (A-D) Coronal sections of E13.5 brains, processed with ISH for Slp2 and Tgfa. Arrowheads mark the position of the corticostriate boundary, or "anti-hem," which appears similarly positioned between the lateral ganglionic eminence and cortical primordium in control and hem-ablated brains. Anti-hem expression of Slp2 and Tgfa appears equivalently strong in both brains. Abbreviations: ncx, neocortex; LGE, lateral ganglionic eminence. Scale bar, 230 μm (A-D).
cortical primordium and the medial region of weak expression was virtually absent (Fig. 4D). The hippocampal primordium at E13.5 is further distinguished from the rest of the cortical primordium by comparatively weak expression of Bmpr1b encoding a type I bone morphogenetic protein receptor (Fig. 4E, arrowheads). As expected, this region was missing in the hem-ablated brain (Fig. 4F). The phenotype of a reduced medial neocortical primordium was observed in all mutant brains analyzed (n > 30). Future analysis will determine which areas are missing or reduced and the percentage of cortical tissue loss in hem-ablated brains.

Our observations support the hypothesis that inhibitory activity in medial neocortex transiently prevents tangential migration of interneurons (Britto et al. 2006), suggesting that when inhibitory medial cortex is reduced in hem-ablated mice, interneurons are free to advance prematurely toward the dorsomedial edge of the cortical primordium.

**Loss Of Cxcl12-Expressing Cajal-Retzius Cells in Hem-Ablated Mice Is Associated With Early Interneuron Invasion of the CP**

To integrate into the developing CP, tangentially migrating GABAergic cells switch their mode of migration from tangential to radial, invading the CP from the MZ and SVZ/IZ migratory streams in a temporally regulated manner (Li et al. 2008; Lopez-Bendito et al. 2008; Zimmer et al. 2008). In wild-type mice, interneurons invade the CP after E15.5 (Li et al. 2008; Lopez-Bendito et al. 2008). In hem-ablated mice, however, interneurons entered the CP prematurely.

At E15.5, as expected, most Lhx6-expressing interneurons in control cortex were in the MZ and SVZ/IZ, with few labeled cells in the upper IZ and almost none in the CP (Fig. 5A). In hem-ablated cortex, by contrast, many more interneurons appeared in the CP and in the upper IZ. In total, the CP in hem-ablated cortex contained about 4 times as many Lhx6-expressing interneurons as the CP in control cortex (Fig. 5C). The greater number of interneurons invading the CP in the mutant is unlikely to result from a generally larger number of interneurons that have migrated into the cortical primordium. First, an active mechanism is believed to be required for interneurons to enter the CP (Li et al. 2008; Lopez-Bendito et al. 2008). Second, the total number of interneurons invading the entire cortical primordium is not 4 times greater in the hem-ablated mice than in controls (Figs 1, 5).

The effects of precocious invasion of the CP were also evident later in corticogenesis. At E17.5, Lhx6-expressing interneurons appeared in the upper IZ and CP of control brains (Fig. 6C) but were substantially more profuse in the CP of hem-ablated brains. Conversely, there were markedly fewer Lhx6-expressing cells in the lower IZ and SVZ of hem-ablated cortex compared with control brains, suggesting that cells had left the SVZ/IZ migratory stream in greater numbers in the mutants than in controls (Figs 6C, D).

Previous reports indicate that disruption or loss of CXCL12/CXCR4 chemokine signaling causes aberrant migration by interneurons, including a premature entry into the CP (Stumm et al. 2005; Tiveron et al. 2006; Li et al. 2008; Lopez-Bendito et al. 2008). In control mice at the earliest stages of corticogenesis, Cxcl12 expression near the MZ is confined to the meninges overlining the brain (Supplementary Fig. 1). C-R cells in the MZ begin to express Cxcl12 at about E14.5 (Daniel et al. 2005; Lopez-Bendito et al. 2008). At later embryonic stages, therefore, both the meninges and C-R cells produce CXCL12 near the cortical pial surface (Fig. 6A). Meanwhile, by E16.5, the SVZ/IZ source of CXCL12 declines in both control and mutant mice. Thus, at later stages of corticogenesis, C-R cells contribute substantially to available CXCL12. Consequently, as a result of the massive loss of C-R cells in hem-ablated brains, CXCL12 is considerably reduced (Fig. 6A,B). Furthermore, the timing of the reduction of CXCL12 correlates well with the timing of precocious interneuron migration into the CP in hem-ablated mice. Our findings therefore suggest that reduced CXCL12 accounts for the premature migration of interneurons into the CP of hem-ablated brains.

**Discussion**

A previous study indicated that secretion of reelin by a dense layer of C-R cells is not required for near-normal pyramidal cell lamination (Yoshida et al. 2006). A low level of reelin from residual C-R cells and from sources outside the cortical MZ is sufficient for migration and layer targeting (Yoshida et al. 2006). Whether interneurons respond to reelin from C-R cells remains unclear. In mice lacking all reelin, or the reelin receptor adaptor protein Disabled1 (Dab1), interneuron layering is perturbed (Hevner et al. 2004; Pla et al. 2006; Yabut et al.
2007), but the distribution of pyramidal neurons and inter-
neurons remains coupled, suggesting interneurons respond to
cues from pyramidal cells already in place or that reelin affects
interneurons and pyramidal neurons in a correlated manner
(Hevner et al. 2004; Pla et al. 2006; Yabut et al. 2007). Thus, we
might expect the reduction of C-R cell–produced reelin in
hem-ablated brains to have little effect on interneuron
migration.

C-R cells produce another secreted ligand, CXCL12,
however, which has been demonstrated to regulate the
migration of interneurons in the cerebral cortex (Stumm
et al. 2003; Berger et al. 2007; Li et al. 2008; Lopez-Bendito et al.
2008). Only time-lapse video microscopy would allow us to identify
unequivocally whether one or both interneuron migratory
streams invade the CP prematurely in the absence of the hem.
Meanwhile, some observations suggest that interneurons
migrate into the CP precociously from both streams. At
E15.5, cells were seen apparently detaching from the MZ to
enter the CP. Moreover, a large excess of interneurons was
present in the upper IZ, presumably heading for the CP from
the SVZ/IZ (Fig. 5). By E17.5, both the IZ and SVZ were more
depleted of interneurons in the mutant cortex compared with
controls (Fig. 6), suggesting an early clearance of the SVZ/IZ
pathway in the mutant.

The mechanism of CXCL12 control of interneuron migration
is not yet known, but in 2 models, CXCL12 activity maintains
interneurons in tangential migration and must be suppressed to
allow the cell to escape and integrate into the CP. One proposal
is that CXCL12 is initially a chemoattractant for local
interneurons but that later in corticogenesis, interneurons lose
their responsiveness to CXCL12 and are free to migrate radially
into the CP (Lopez-Bendito et al. 2008), which contains a chemoattractant
for interneurons (Lopez-Bendito et al. 2008). In a second
proposal, CXCL12 influences the exploratory behavior of
interneurons as they migrate (Lysko and Golden 2009). In
the first model, a reduction of CXCL in the MZ, such as occurs
in hem-ablated mice, might be expected to affect primarily
local interneurons migrating through the MZ. In the second
model, loss of CXCL12 in the MZ could affect interneurons
anywhere in its range of diffusion, a model that is perhaps more
consistent with our findings.

The migration of interneurons in hem-ablated mice shows an
additional abnormality, not seen in mice deficient in Cxcl12/
Cxcr4 signaling or any other mutant mouse of which we are
aware. At E12.5, interneurons have crossed into the cortical
primordium in both mutants and controls, and the position of
the migratory front is not significantly different between the 2
groups. By contrast, at E13.5, the migratory front is significantly
more advanced dorsomedially in hem-ablated mice compared
with controls. This implies that the initial crossing of
interneurons into the cortical primordium is relatively normal in hem-ablated cortex but that subsequent progress is increased. A previous study supports the hypothesis that differential promotion and inhibition of interneuron migration in the lateral and medial cortical primordium plays a key role in the timing of tangential interneuron migration (Britto et al. 2006). Our findings provide further support for this idea. After genetic deletion of the hem, the lateral cortical primordium appears intact, and in keeping with previous observations that the lateral cortex at E12.5 is an attractive substrate for interneurons (Britto et al. 2006), we saw no difference between hem-ablated and control mice at E12.5 in interneuron migration into lateral cortex. Between E12.5 and E13.5, medial cortical produces a cue that is transiently inhibitory to interneurons in slice culture (Britto et al. 2006). A reduction of medial cortex, as is observed in hem-ablated brains, could therefore allow interneurons to invade further into the cortical primordium by E13.5. In conclusion, we identified premature tangential and radial interneuron migration in hem-ablated mice. Final layer position of the interneurons born at different ages could not be determined directly because the hem-ablated mice die at birth. However, the early entry of interneurons into the CP suggests, at least, that they were unlikely to arrive in the same layer at the same time as pyramidal cells born at the same age, potentially disrupting interneuron integration into cortical circuitry (Hevner et al. 2004; Li et al. 2008; Lopez-Bendito et al. 2008). Strikingly, the cortical hem, a structure that is distant from both the source and entry point of cortical interneurons, nonetheless regulates their migration. Specifically, the hem establishes cell types and tissues that in turn provide cues directing the timing of both tangential and radial interneuron migration.

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

Funding
National Institutes of Health (R37 H059962 to E.A.G.).

Notes
Conflict of Interest: None declared.

References


