

# Patterning of the Dorsal Telencephalon and Cerebral Cortex by a Roof Plate-Lhx2 Pathway

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## Summary

The organizing centers and molecules that pattern the cerebral cortex have been elusive. Here we show that cortical patterning involves regulation of the *Lhx2* homeobox gene by the roof plate. Roof plate ablation results in reduced cortical size and *Lhx2* expression defects that implicate roof plate signals in the bimodal regulation of *Lhx2* in vivo. Bimodal *Lhx2* regulation can be recapitulated in explants using two roof plate-derived signaling molecules, *Bmp4* and *Bmp2*. Loss of *Lhx2* function results in profound losses of cortical progenitors and neurons, but *Lhx2* mutants continue to generate cortical neurons from dorsal sources that may include the roof plate region itself. These findings provide evidence for the roof plate as an organizing center of the developing cortex and for a roof plate-*Lhx2* pathway in cortical patterning.

## Introduction

In the course of dorsoventral patterning of the telencephalon, the roof plate undergoes some of the most dramatic morphological transformations in the developing nervous system. Unlike the spinal cord, which retains a tubular profile throughout life, the dorsal midline of the telencephalon becomes buried between the two cerebral hemispheres (Figure 1A). This relative invagination of the dorsal midline region is accompanied by a simplification of the roof plate into a monolayer epithelium and the formation of three distinct epithelial types in the pallium (dorsal telencephalon). The most dorsal type is choroid plexus epithelium, which is a monolayered neurosecretory epithelium that produces cerebrospinal fluid (CSF) and is contiguous with the midline roof. The most lateral type is cortical neuroepithelium, which represents a pseudostratified neurogenic epithelium that gives rise to neurons and glia of the cerebral

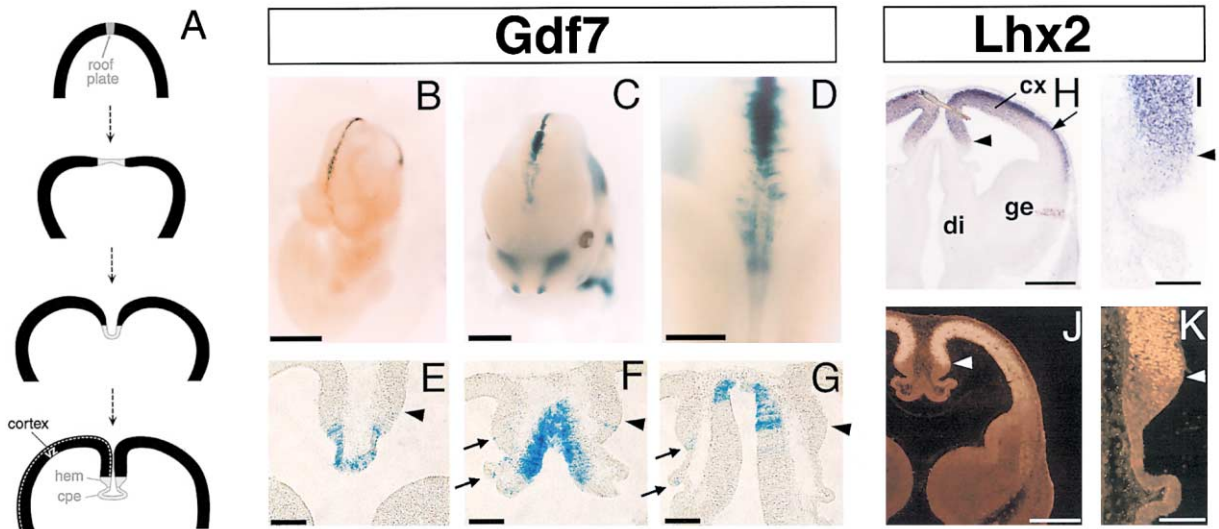
cortex. Finally, a small intermediate epithelium known as the cortical hem exists at the junction between choroid plexus and cortical neuroepithelium (Grove et al., 1998) (Figure 1A).

The organizing centers that transform and pattern the dorsal telencephalon are poorly understood, but the roof plate is a likely candidate. In the spinal region, the roof plate can induce dorsal cell types in explant cultures (Liem et al., 1997) and is selectively required for generating particular dorsal progenitors and neurons in vivo (Lee et al., 2000a). In the hindbrain, lack of a roof plate is also associated with a loss of dorsal cell types (Milonig et al., 2000), suggesting a conserved patterning function for the roof plate throughout the nervous system. The roof plate produces several secreted signaling molecules, among which the bone morphogenetic proteins (Bmps) are of central importance to roof plate-mediated patterning in the spinal cord (Liem et al., 1995, 1997; Lee et al., 1998; Lee and Jessell, 1999). While evidence for roof plate-derived Bmps in dorsal telencephalic development has been somewhat limited, multiple *Bmp* genes are expressed at high levels in the forebrain roof plate, including the choroid plexus and cortical hem (Furuta et al., 1997; Dou et al., 1999) (Figure 1A). Bmps have also been shown to regulate neural apoptosis and proliferation (Graham et al., 1994; Furuta et al., 1997; Mabie et al., 1999; Solloway and Robertson, 1999; Trousse et al., 2001), as well as gene expression (Furuta et al., 1997) in a fashion consistent with a primary role for Bmps in telencephalic dorsal midline development. Furthermore, exogenous Bmps can induce forebrain patterning defects (Golden et al., 1999), and mice lacking both *Bmp5* and *Bmp7* (Solloway and Robertson, 1999) or the *Bmp* antagonists *Chordin* and *Noggin* (Bachiller et al., 2000) have small and abnormal forebrains, although these abnormal phenotypes are complicated by the expression of *Bmp7* and other Bmps in the ventral midline (Solloway and Robertson, 1999; Lee et al., 2000a) and other regions outside the roof plate (Furuta et al., 1997).

In addition to the persistent questions regarding roof plate and *Bmp* function in dorsal telencephalic development, little is known about the intrinsic factors that represent the downstream targets of roof plate and *Bmp* signals in the dorsal telencephalon. Explant studies have implicated *Bmp4* and *Bmp2* in the activation of *Msx1* and suppression of *Foxg1* (*Bf1*) from the dorsal midline region (Furuta et al., 1997). Another potential target is the LIM homeodomain (LIM-HD) transcription factor *Lhx2*, which is downstream of roof plate-derived *Bmp* signals in the spinal cord (Liem et al., 1997) and has been previously implicated in dorsal telencephalic patterning. *Lhx2* is expressed at high levels by cortical VZ progenitor cells (Xu et al., 1993; Bertuzzi et al., 1999; Donoghue and Rakic, 1999; Nakagawa et al., 1999; Retaux et al., 1999; Rincon-Limas et al., 1999; Bulchand et al., 2001), and previous studies on *Lhx2* knockout mice have demonstrated an essential role for *Lhx2* in cortical development (Porter et al., 1997; Bulchand et al., 2001). Initial histologic and BrdU studies on *Lhx2*<sup>-/-</sup> embryos sug-

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**Figure 1. Anatomy of the Dorsal Telencephalon and Expression Patterns of *Gdf7* and *Lhx2***

(A) Coronal schematics (based on Bayer and Altman, 1991; not drawn to scale) of the mouse dorsal telencephalon from E9.5 (top) to E12.5 (bottom), with the *Bmp*-rich roof plate region shown in gray. The roof plate invaginates relative to the two hemispheres, and three epithelial types become identifiable—choroid plexus epithelium (cpe) and cortical hem (hem) in the dorsal midline region, and cortical neuroepithelium (cortex). Cortical neuroepithelial progenitor cells eventually occupy a deep layer known as the ventricular zone (VZ), while postmitotic cells populate more superficial layers (separated from VZ by dashed line).

(B–G) Roof plate-restricted expression of *Gdf7*. X-gal-stained embryos from *Gdf7*-Cre X conditional lacZ reporter matings.

(B–D) *Gdf7* activation is restricted to the midline roof plate and extends into the anterior forebrain at E10.5 (B) and E12.5 (C), higher power in (D)]. Anterior diencephalic staining appears split (D) due to its thin attenuated roof (G).

(E–G) *Gdf7* expression is activated in the telencephalic roof and adjacent cortical hem (E), including the choroid plexus epithelium and cortical hem more posteriorly, but only in scattered cells (arrows in F–G designate some of these cells). *Gdf7* activation in the diencephalon is also strong in the roof and adjacent neuroepithelium (F–G).

(H–K) Cortex-restricted expression of *Lhx2*. *Lhx2* mRNA (H and I) and protein (J and K) are present in the E12.5 cortical neuroepithelium, but not in the dorsal midline region. In the cortical VZ, *Lhx2* expression is graded (high-medial, low-lateral) with highest levels adjacent to the *Lhx2*-negative dorsal midline (H and J). The enrichment of *Lhx2* mRNA in the cortical preplate (arrow in [H]) is not seen at the protein level (J). Note the nuclear localization of *Lhx2* protein, which is typical for a transcription factor (K). Although the immunostains shown here utilized an antibody that detects both *Lhx2* and *Lhx9* (Liem et al., 1997), an indistinguishable staining pattern was obtained with an *Lhx2*-specific antibody (Lee et al., 1998), and *Lhx9* expression in the E12.5 cortical VZ was not detected by ISH or immunostaining with an *Lhx9*-specific antibody (Lee et al., 1998), consistent with previous studies (Bertuzzi et al., 1999; Retaux et al., 1999).

Arrowheads denote the cortex-hem border.

Abbreviations: cpe, choroid plexus epithelium; cx, cortex; di, diencephalon; ge, ganglionic eminence; vz, ventricular zone.

Scale bars: (B), 1 mm; (C, D, H, and J), 0.4 mm; (E–G), 0.2 mm; (I and K), 0.1 mm.

gested a proliferation defect of cortical VZ progenitors in the hippocampus and neocortex primordia (Porter et al., 1997). More recently, Bulchand et al. (2001) demonstrated a dorsal telencephalic patterning defect that involved an enlarged cortical hem and a missing or dramatically reduced hippocampus and neocortex, although the precise fate of presumptive cortical VZ progenitors that normally express *Lhx2* remained unclear.

Here we provide evidence for a roof plate-*Lhx2* pathway that is involved in cortical patterning. The roof plate-specific *Gdf7* locus is used to ablate the roof plate and demonstrate that roof signals regulate *Lhx2* expression and cortical size. Roof plate regulation of *Lhx2* expression, as suggested by the ablation studies, can be mimicked *in vitro* using *Bmp4* or *Bmp2*, implicating *Bmp* signals as mediators of the roof plate-*Lhx2* pathway. We then describe three additional aspects of the *Lhx2*<sup>-/-</sup> patterning defect. First, we demonstrate that the patterning defect involves a massive excess of choroid plexus. Second, we identify the presumptive cortical VZ progenitors by their expression of the mutant *Lhx2* allele and provide evidence that *Lhx2* is required to “select”

a cortical VZ progenitor fate. Third, we show that the cortical VZ defect results in a lack of cortical plate neurons, but preplate neurons continue to be generated and may include the descendants of roof plate cells.

## Results

### Complementary Expression of *Gdf7* and *Lhx2* in the Roof Plate and Cortex

In order to begin addressing roof plate function in the telencephalon, we used a mouse line (Lee et al., 2000a) to demonstrate the expression of *Gdf7*, a *Bmp*-related signaling molecule (Lee et al., 1998), in the telencephalic roof plate. This mouse line expresses Cre recombinase from the *Gdf7* gene locus, and X-gal-stained embryos from matings between these *Gdf7*-Cre mice and a conditional lacZ reporter strain (Jackson Laboratories) (Soriano, 1999) demonstrated continuous lacZ expression in the roof plate at all levels of the nervous system, including the telencephalon (Figures 1B–1D). In the telencephalon, X-gal staining was detected at embryonic day 10.5 (E10.5) (Figure 1B) in the midline roof (lamina terminalis/

choroid plaque region) and adjacent cortical hem (data not shown), a pattern seen more clearly at E12.5 (Figures 1C–1G). LacZ expression was detected throughout the entire dorsal midline region that included the choroid plexus epithelium and cortical hem, but only in a small fraction of cells (Figures 1E–1G). Outside of the dorsal midline region, low-level lacZ expression was detected in dorsal surface ectoderm overlying the forebrain at E12.5 (data not shown), but other forebrain sites of expression were not identified. These studies indicated that, by E12.5, activation of Gdf7 expression in the telencephalon was largely restricted to roof plate cells.

In contrast to Gdf7, Lhx2 mRNA was expressed throughout the cortex, but not in the dorsal midline region at E12.5 (Figures 1H and 1I). The distribution of Lhx2 at the mRNA level was also seen at the protein level (Figures 1J and 1K). Within the cortical VZ, Lhx2 expression was graded, with highest levels in the hippocampal anlage dorsomedially and progressively lower levels in the neocortical primordium more laterally (Figures 1H and 1J). Low but detectable Lhx2 expression was seen in the far-lateral cortical VZ and in the ganglionic eminence VZ (Figures 1H and 1J). Lhx2 transcript and protein levels corresponded well in VZ progenitor fields, but the accentuation of Lhx2 mRNA in the cortical preplate was not seen at the protein level (compare Figures 1H and 1J). Although the polyclonal antiserum used in Figure 1 detects both Lhx2 (LH2A) and Lhx9 (LH2B) (Liem et al., 1997), an Lhx2-specific antipeptide antibody (Lee et al., 1998) yielded an indistinguishable staining pattern (data not shown), and Lhx9 mRNA and protein were not detected in the E12.5 cortex (data not shown; see Figure 1 legend). Lhx2 therefore appears to be the major LIM-HD transcription factor expressed by early cortical VZ progenitors, but it is not expressed in the Gdf7-expressing dorsal midline region.

#### Lhx2 Expression Defects following Gdf7-Mediated Roof Plate Ablation

The roof plate-restricted expression of Gdf7 allowed us to investigate the consequences of selective roof plate ablation, which resulted in Lhx2 expression abnormalities in the dorsal telencephalon. We used a mouse line that conditionally expresses a cellular toxin, the diphtheria toxin A chain (DTA) (Palmiter et al., 1987; Messing et al., 1992; Grieshammer et al., 1998), from the identical position within the *Gdf7* gene locus as the Cre recombinase (Lee et al., 1998). Embryos carrying both the conditional *Gdf7-DTA* allele and a ubiquitously expressed *Cre* allele (Lewandoski et al., 1997) (provided by Bruce Morgan) displayed a severe reduction in forebrain size by E10.5 (data not shown). At E12.5, mutant embryos exhibited an open neural tube (neural tube defect) that extended into anterior forebrain regions, which was associated with a markedly reduced telencephalon (Figures 2A and 2D). Sections showed that the ventrolaterally displaced telencephalon did contain cortical neuroepithelium, based on histology and Lhx2 expression pattern (Figure 2G). However, Lhx2 expression levels in the cortical neuroepithelium were significantly reduced in roof plate-ablated embryos (Figures 2A) and the normal graded pattern of Lhx2 expression was no longer apparent (Figures 2A and 2G), while Lhx2 expression in the

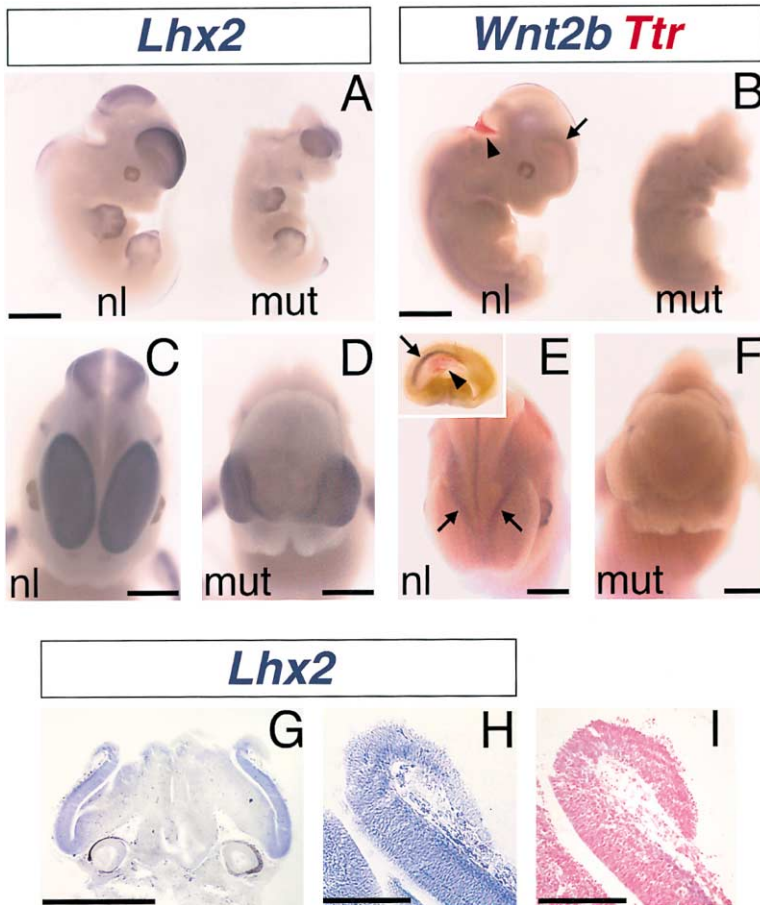
limbs appeared unaffected (Figure 2A). Thus, ablation of Gdf7-expressing cells resulted in reduced and less-graded Lhx2 expression, which was associated with a reduced cortical size.

In addition to its effects on Lhx2 expression in the cortex, roof plate ablation resulted in a reduction of the Lhx2-negative domain at the dorsal midline. Although Gdf7 activation was limited to only a fraction of cells in the choroid plexus and cortical hem (Figures 1E–1G), expression of Lhx2 extended close to the free ends of residual telencephalic neuroepithelium following Gdf7-mediated ablation (Figures 2G and 2H), indicating a significant reduction in the amount of Lhx2-negative tissue at the dorsal midline. This reduction was accompanied by a lack of monolayered choroid plexus epithelium histologically (Figure 2I), and little to no expression of the choroid plexus marker transthyretin (Ttr) or the cortical hem marker *Wnt2b* (Figures 2B and 2F), suggesting near-total losses of choroid plexus and cortical hem epithelia in roof plate-ablated embryos. These studies suggested that the roof plate regulates Lhx2 expression in both the developing cortex and the telencephalic dorsal midline. Since Gdf7 expression is largely restricted to roof plate cells, the ablation studies suggested that the roof plate acts in a non-cell-autonomous fashion to regulate Lhx2 expression in the developing cortex.

#### Bimodal Regulation of Lhx2 by Bmp4 and Bmp2 in Forebrain Explants

Since roof plate-derived Bmp signals mediate patterning of the spinal cord, we used explant cultures to investigate whether Bmps might mediate the roof plate effects on Lhx2 expression in the dorsal telencephalon. E10.5 or E11.5 dorsal forebrain explants grown for 36–48 hr without Bmp4 showed appropriate regional expression of Lhx2 (Figure 3A) and dorsal midline markers (Figures 3B and 3C), suggesting that dorsal telencephalic pattern was established and/or maintained in these cultures. When E10.5 forebrain or E11.5 telencephalic explants were exposed to 10  $\mu$ g/ml Bmp4 via bath application, Lhx2 expression was abolished (Figures 3D and 3F). In contrast, explants cultured in 2  $\mu$ g/ml or less Bmp4 maintained Lhx2 expression (Figures 3E and 3F, and data not shown).

While bath application of high Bmp4 concentrations suppressed Lhx2, bead application of Bmp4 resulted in a dramatic bimodal effect on Lhx2 expression. Soaked beads were placed on the ventricular surfaces of E11.5 or E12.5 explants, then assayed for Lhx2 expression after 36–48 hr in culture. Whereas control beads soaked in BSA or EGF had no apparent effect on Lhx2 expression (Figures 3I and 3L), Bmp4-soaked beads altered Lhx2 levels in a “target” pattern (Figures 3G, 3H, and 3N). Lhx2 expression was suppressed immediately adjacent to the beads (Figures 3G, 3H, and 3N), which was consistent with the effects of high bath concentrations of Bmp4 on Lhx2 expression. Bmp4 beads also distorted the Lhx2 expression border at the cortex-hem boundary (Figure 3N), suggesting that exogenous Bmp4 can supplement endogenous signals to suppress Lhx2 expression at the dorsal midline. However, at some distance away from the Bmp4 bead and adjacent to the zone of suppression, there was a zone of enhanced Lhx2



**Figure 2. Lhx2 Expression Defects and Cortical Reduction following Gdf7-Mediated Roof Plate Ablation**

Whole-mount ISH (A–F) followed by coronal cryosectioning and histologic analyses (G–I) of E12.5 roof plate-ablated embryos and normal littermates from Gdf7-xneoX-DTA X  $\beta$ -actin-Cre matings. (A and B) Side-by-side lateral views; (C–F) superior views (anterior toward bottom); inset in (E), medial view of one telencephalic vesicle from the normal littermate. (A, C, and D) Lhx2 expression is reduced and less-graded following Gdf7-mediated roof plate ablation (A and D), while Lhx2 expression in the developing limbs appears normal (A). The Lhx2 expression defects are associated with a small cortex (A and D) and an open neural tube (D).

(B, E, and F) Expression of cortical hem-specific Wnt2b (blue, arrows) and choroid plexus-specific Ttr (red, arrowheads) are markedly reduced to absent following Gdf7-mediated ablation (B and F), despite the small fraction of cells in the choroid plexus and cortical hem that activate Gdf7 (Figure 1).

(G–I) Sections show a loss of graded Lhx2 expression in the cortex (G) and marked reduction to the Lhx2-negative dorsal midline domain (G and H). Residual cortical neuroepithelium is everted at its free edge and lacks the simple epithelium that characterizes choroid plexus at this age (I).

Abbreviations: mut, roof plate-ablated mutant; nl, normal littermate.

Scale bars: (A and B), 2 mm; (C–G), 1 mm; (H and I), 0.2 mm.

expression (Figures 3G, 3H, and 3N). Lhx2 expression then became indistinguishable from endogenous levels at still further distances from the bead. Beads soaked with Bmp2 also elicited the bimodal effect on Lhx2 expression (Figure 3J), whereas Bmp6 or coapplication of Bmp4 and its antagonist noggin (Zimmerman et al., 1996) had no apparent effect (Figures 3K and 3O), suggesting specificity to this effect. Immunostaining of sectioned explants revealed that Bmp4- and Bmp2-mediated suppression of Lhx2 was evident at the protein level within cortical VZ progenitor cells by 36–48 hr in culture (data not shown).

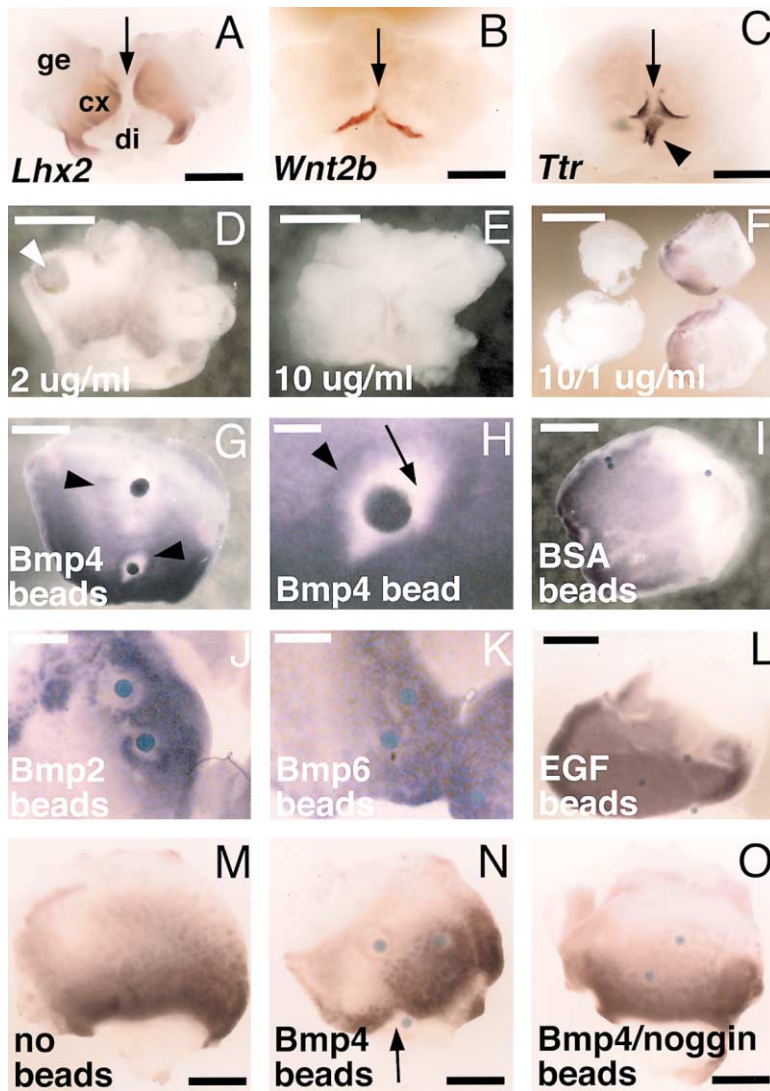
Importantly, the bimodal Lhx2 regulation by Bmp4 or Bmp2 corresponded well to the Lhx2 expression patterns relative to the roof plate in both normal and roof plate-ablated embryos in vivo. The zone of Lhx2 suppression that immediately surrounded Bmp4 or Bmp2 beads mimics the normal roof plate region, where Bmp4 and Bmp2 expression is high (Furuta et al., 1997) and Lhx2 expression is absent (Figures 1H–1K), and the Lhx2-negative dorsal midline domain was markedly reduced following roof plate ablation (Figures 2G–I). The adjacent zone of Lhx2 enhancement corresponds to the dorsomedial cortical neuroepithelium (hippocampal anlage) where the graded expression of Lhx2 is normally highest (Figures 1H and 1J), and roof plate ablation resulted in reduced and less-graded cortical Lhx2 expression (Figures 2A and 2G). Finally, the zone farthest from the Bmp4 or Bmp2 beads corresponds to the lateral

cortex (neocortical anlage), where lower levels of Lhx2 are normally expressed in vivo (Figures 1H and 1J).

#### Patterning Defects of the Dorsal Telencephalon in Lhx2 Knockout Mice

Since Lhx2 regulation by roof plate-derived Bmps provides a potential pathway for regulating dorsal telencephalic patterning, we studied the *Lhx2*<sup>-/-</sup> phenotype in further detail and discovered profound patterning defects of the dorsal telencephalon. Much of the presumptive neocortex and hippocampus in Lhx2 mutant embryos at E12.5 (Figure 4A) and E14.5 (approximately 1 day before mutant embryos begin to die) (Porter et al., 1997) possessed an abnormal monolayered and papillary appearance (Figures 4B and 4C) that was histologically indistinguishable from normal choroid plexus epithelium (Figures 4D–4F). This abnormal epithelium covered the *Lhx2*<sup>-/-</sup> dorsal forebrain throughout most of its rostrocaudal extent (Figures 4G–4I). The amount of thick neuroepithelium in the *Lhx2*<sup>-/-</sup> dorsal telencephalon was markedly reduced and present laterally. In addition to these telencephalic defects, the roof of the diencephalon, where choroid plexus of the third ventricle normally forms, was abnormally widened in *Lhx2*<sup>-/-</sup> embryos (Figures 4H and 4I). Other than a slight size reduction of the ventral telencephalon (Porter et al., 1997), this region appeared relatively unaffected by loss of Lhx2 (Figures 4G–4I).

The excessive simple and papillary epithelium in the



**Figure 3. Bimodal Regulation of Lhx2 Expression by Bmp4 or Bmp2 in Forebrain Explants**  
Whole-mount ISH analyses of dorsal forebrain or telencephalic explants cultured as flattened two-dimensional sheets for 36–48 hr. (A–C) The Lhx2 gradient (cortex), Wnt2b expression (cortical hem), and Ttr expression (choroid plexus) are maintained in an appropriate region-restricted fashion in E11.5 dorsal forebrain explants. Arrows indicate the dorsal midline, and the arrowhead in (C) designates Ttr-expressing choroid plexus epithelium in the diencephalon. (D–E) Lhx2 expression is suppressed in E10.5 dorsal forebrain explants cultured in 10  $\mu\text{g}/\text{ml}$  Bmp4, while Lhx2 expression in the cortex and eye (arrowhead in [D]) is maintained in 2  $\mu\text{g}/\text{ml}$  Bmp4. (F) Like the dorsal forebrain explants, 10  $\mu\text{g}/\text{ml}$  Bmp4 abolished Lhx2 expression in E11.5 telencephalic explants (left), while some Lhx2 expression is maintained at 1  $\mu\text{g}/\text{ml}$  (right). (G–O) Beads soaked in Bmp4 (G, H, and N) or Bmp2 (J) bimodally regulate Lhx2 expression in E11.5 or E12.5 explants—Lhx2 expression is suppressed next to the beads, but enhanced at a distance from the beads. Bmp4 beads also distort the normal cortex-hem expression border of Lhx2 (N). Beads soaked in Bmp6 (K), BSA (I), EGF (L), or Bmp4 together with an antagonist (noggin; [O]) do not affect Lhx2 expression. Arrows designate zones of suppression; arrowheads designate zones of enhancement. (H) Higher power view of (G). Abbreviations: cx, cortex; di, diencephalon; ge, ganglionic eminence. Scale bars: (A–F), 1 mm; (G and I–O), 0.5 mm; (H), 0.1 mm.

*Lhx2*<sup>-/-</sup> dorsal telencephalon was confirmed to be choroid plexus epithelium by marker analyses. All of the excessive simple epithelium showed strong expression of transthyretin (Figure 5A), a choroid plexus-specific marker (Figure 5E). In addition, this excessive tissue was intensely immunoreactive for Otx2 protein, which normally distinguishes choroid plexus epithelium from the adjacent cortex, cortical hem, and diencephalon at this age (Boncinelli et al., 1993) (data not shown). We then examined the status of the cortical hem and found a marked excess of cortical hem tissue in *Lhx2*<sup>-/-</sup> embryos, as previously reported (Bulchand et al., 2001), with Wnt2b- and Wnt5a-expressing cortical hem tissue representing most of the residual thick neuroepithelium that remained in the mutant dorsal telencephalon (Figure 5B and data not shown). Thus, the *Lhx2*<sup>-/-</sup> patterning defect involves an excess of both telencephalic epithelia that are associated with the dorsal midline region, the choroid plexus epithelium, and cortical hem.

#### Loss of Cerebral Cortex in Lhx2 Knockout Mice

The choroid plexus and cortical hem marker studies suggested a near-total loss of cortical VZ progenitor

cells, which was confirmed with additional markers. Only a small lateral region of the *Lhx2*<sup>-/-</sup> dorsal telencephalon lacked choroid plexus or cortical hem marker expression (Figures 5A and 5B), and this lateral remnant showed expression of the neomycin resistance gene (*Neo*) (Figure 5J) and exon 1 of the *Lhx2* gene (Figure 5K), both of which are present in the mutant *Lhx2* allele (Porter et al., 1997). Transcripts containing *Neo* or *Lhx2* exon 1 were not detected in the excessive cortical hem tissue (Figures 5J and 5K). These findings identified the lateral remnant as the location for the presumptive cortical VZ progenitor cells that normally express Lhx2.

Since presumptive cortical VZ progenitors in the *Lhx2*<sup>-/-</sup> lateral remnant do not express cortical VZ field markers (Bulchand et al., 2001), we examined if these cells had attained early telencephalic progenitor identity. The neural progenitor markers Nestin and RC2 (Misson et al., 1988; Lendahl et al., 1990; Noctor et al., 2001) were strongly expressed in the small lateral remnant, but only weakly expressed in the excessive cortical hem (Figures 5L and 5M). This pattern was consistent with normal Nestin and RC2 expression, which was diminished in the E12.5 cortical hem relative to the cortical

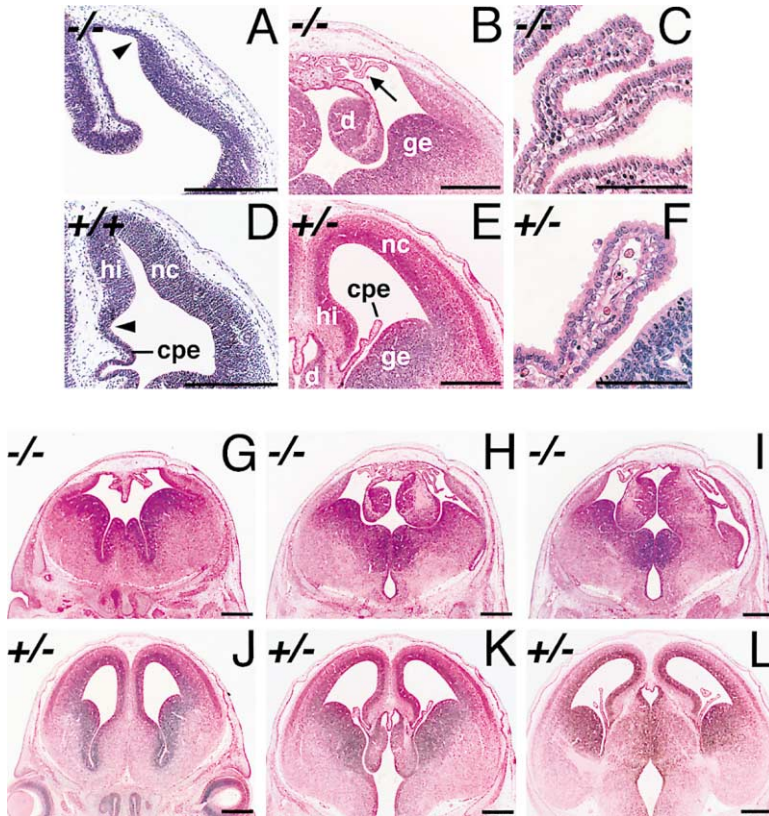


Figure 4. Excessive Choroid Plexus and Loss of the Cortex in the *Lhx2*<sup>-/-</sup> Dorsal Telencephalon

E12.5 cresyl-violet (A and D) or E14.5 H&E histologic analyses of *Lhx2* homozygous null (A–C and G–I) or normal littermates (D–F and J–L). Images within each vertical pair represent comparable rostrocaudal levels.

(A–F) Much of the presumptive cortical neuroepithelium in *Lhx2*<sup>-/-</sup> animals becomes a monolayered and papillary epithelium ([A and B], high-power view in [C]) that is histologically indistinguishable from normal choroid plexus epithelium ([D and E], high-power view in [F]). The normal choroid plexus-cortical hem junction at E12.5 (arrowhead in [D]) appears similar to the junction between simple and thick epithelia in *Lhx2*<sup>-/-</sup> embryos (arrowhead in [A]). Residual thick neuroepithelium in the mutant dorsal telencephalon is present laterally (A and B).

(G–L) Excessive simple and papillary epithelium occupied the *Lhx2*<sup>-/-</sup> dorsal forebrain throughout its rostrocaudal extent ([G–I], rostral to caudal), including anterior regions (G) normally devoid of such epithelium (J). Residual thick neuroepithelium is located laterally within the mutant dorsal telencephalon at all levels (G–I). The choroid plexus of the diencephalon is also abnormal, showing widening and loss of papillarity (H and I), while the ganglionic eminence appears relatively unaffected.

Abbreviations: cpe, choroid plexus epithelium; d, diencephalon; ge, ganglionic eminence; hi, hippocampus; nc, neocortex.

Scale bars: (A, B, D, E, and G–L), 0.4 mm; (C and F), 0.1 mm.

VZ (Figures 5N and 5O). In addition to Nestin and RC2, Foxg1 (Bf1) was expressed at low but detectable levels in the lateral remnant, but not in the excessive cortical hem (Figures 5C and 5I). Foxg1/Bf1 is normally expressed in the cortical VZ, but not in the dorsal midline region (Figure 5G) (Tao and Lai, 1992) and represents one of the earliest known markers of the telencephalon (Tao and Lai, 1992; Shimamura et al., 1995). These studies indicated that presumptive cortical VZ progenitors lacking *Lhx2* are able to attain a telencephalic progenitor identity.

As predicted by the drastic reduction of specified cortical VZ progenitor cells, definitive cortical plate neurons were not identified in the *Lhx2* mutant telencephalon. By E14.5, the cortical plate normally forms a distinct layer of closely packed bipolar neurons with radially oriented nuclei (Figures 6C and 6D). However, the superficial region of the *Lhx2*<sup>-/-</sup> telencephalon lacked a definitive cortical plate and had few, if any, radially oriented cells (Figures 6A and 6B). At both E12.5 and E14.5, the mutant superficial layer contained significant numbers of neurons based on immunoreactivity for TuJ1 (Figures 6E and 6H), including neurons with horizontally oriented processes (Figure 6H). These cells also expressed the preplate markers *Lhx2* (Figure 5K) and *Lhx9* (Bertuzzi et al., 1999) (data not shown), and many of the cells stained for Reelin, the product of Cajal-Retzius preplate neurons (D'Arcangelo et al., 1995; Ogawa et al., 1995), at both E12.5 (data not shown) and E14.5 (Figure 6I).

The *Lhx2*<sup>-/-</sup> dorsal telencephalon therefore appeared to contain preplate neurons, but few if any cortical plate neurons.

#### Evidence for a Dorsal Midline Source of Cortical Neurons

Although subcortical proliferative zones are sources of GABAergic interneurons (Anderson et al., 1997), Cajal-Retzius neurons (Lavdas et al., 1999), and potentially glutamatergic neurons (Tan et al., 1998) in the normal cortex, the neurons that persist in the *Lhx2*<sup>-/-</sup> dorsal telencephalon appear to be largely derived from dorsal proliferative zones. The mutant superficial layer does contain GABAergic (Figure 6G) as well as glutamatergic cells (Figure 6F), but significant numbers of subcortically derived cells are not observed in the normal dorsal cortex by E12.5 (Anderson et al., 1999; Parnavelas, 2000), when neurons are already numerous in *Lhx2* mutants (Figure 6E). In addition, little if any expression in the mutant superficial layer is seen at either E12.5 (Figure 5D) or E14.5 (Figure 6J) for *Dlx1*, a marker of subcortically derived neurons (Anderson et al., 1997). Thus, subcortical proliferative regions do not appear to contribute significantly to the neuronal population in the *Lhx2*<sup>-/-</sup> dorsal telencephalon by E14.5, which implicates dorsal proliferative zones in the genesis of these neurons.

The TuJ1 studies on *Lhx2*<sup>-/-</sup> embryos suggested that the dorsal midline region can generate neurons. TuJ1-positive cells were readily identified within the enlarged

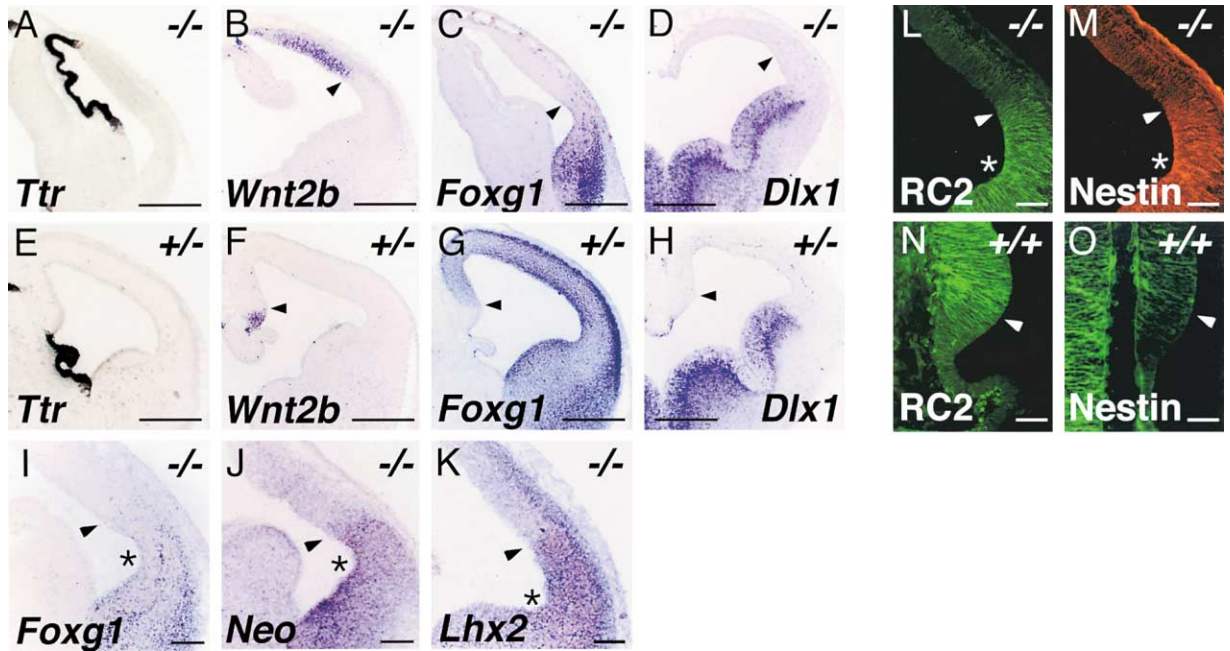


Figure 5. Excessive Dorsal Epithelia and Loss of the Cortical VZ in *Lhx2*<sup>-/-</sup> Embryos

ISH (A–K) and immunostaining (L–O) of E12.5 coronal cryosections from *Lhx2* homozygous null (top and bottom rows) and normal littermates (middle row). Images within a vertical pair represent comparable rostrocaudal levels. (I) is a higher power view of (C).

(A and E) The suspected excess of choroid plexus in *Lhx2*<sup>-/-</sup> embryos is confirmed (A) using the choroid plexus-specific marker transthyretin (Ttr) (E).

(B and F) The hem-specific marker *Wnt2b* (F) also reveals a dramatic excess of the cortical hem in *Lhx2*<sup>-/-</sup> embryos (B), which represents most of the residual thick neuroepithelium in the mutant dorsal telencephalon.

(C and G) The telencephalon-specific marker *Foxg1* (Bf1) (G) showed weak but detectable expression in the *Wnt2b*-negative lateral remnant of the mutant dorsal telencephalon. *Foxg1*/Bf1 expression in the ganglionic eminence appears unaffected by loss of *Lhx2* (C).

(D and H) Expression of the ganglionic eminence-specific marker *Dlx1* (H) appears unchanged in mutant animals (D).

(I–K) Cells of the lateral remnant express the neomycin resistance gene (*Neo*) (J) and *Lhx2* exon 1 (K), which are present in the mutant *Lhx2* allele (Porter et al., 1997), thus identifying these cells as the presumptive *Lhx2*-expressing cortical VZ progenitors. These cells also weakly express *Foxg1*/Bf1 (I), indicating their telencephalic identity.

(L–O) Cells of the lateral remnant also strongly express the neural progenitor markers RC2 (L) and Nestin (M), while the enlarged hem shows diminished expression of both markers. The cortical hem of normal littermates also shows diminished RC2 and Nestin expression compared to adjacent neuroepithelium (N and O). These studies defined a preserved cortex-hem border in *Lhx2* mutants, but one that had shifted to a position close to the ganglionic eminence, indicating near-total loss of the cortical VZ.

Arrowheads designate the cortex-hem border; asterisks designate the corticostriatal sulcus.

Scale bars: (A–H), 0.4 mm; (I–O), 0.1 mm.

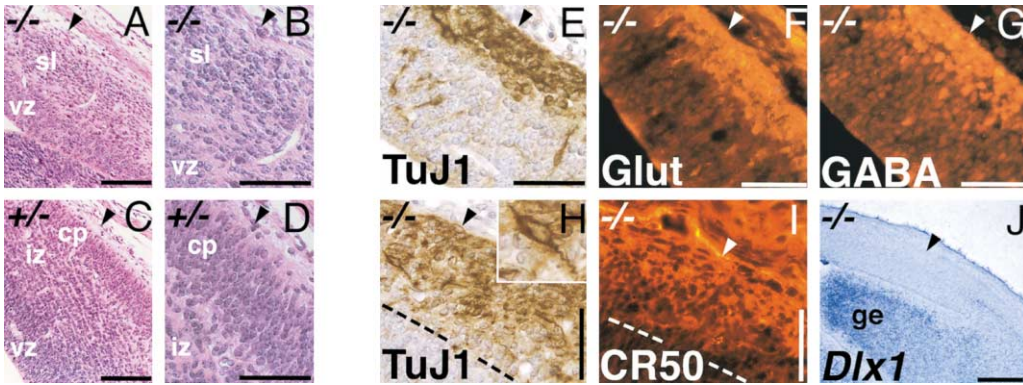
cortical hem VZ of *Lhx2* mutants at E12.5 (Figure 6E) and to a lesser extent at E14.5 (data not shown). Since the cortical VZ and subcortical progenitor zones in *Lhx2*<sup>-/-</sup> embryos are unlikely to be the source of these neurons, they are most likely generated directly by progenitor cells of the cortical hem.

Unexpectedly, fate mapping of the *Gdf7*-expressing cells also identified the dorsal midline region as a potential source of cortical neurons. At E12.5, *lacZ* expression within the VZ was tightly restricted to the dorsal midline region (Figures 7A and 7B), with no X-gal staining detected in the cortical VZ of multiple serially sectioned embryos (data not shown). In association with dorsal midline cells, however, *lacZ* expression was also detected in superficial cells of the cortical preplate and marginal zone (Figures 7A–7C). At E12.5, these superficial cells were most often found in the hippocampal region (Figures 7A and 7B) and occasionally in the nearby neocortex (Figure 7C), and were usually associated with heavily stained neuroepithelial cells in the dorsal midline region (Figures 7A and 7B). Almost all *lacZ*-

expressing cells in the superficial cortex were subpial in location and occasionally possessed horizontally oriented processes (Figure 7E). At E14.5, X-gal-stained superficial cells were more numerous (Figure 7D), and at least some showed coexpression of the neuronal marker TuJ1 (Figure 7F). Rare *lacZ*-expressing cells in the VZ were also detected outside the dorsal midline region at E14.5, including in the neocortical VZ (data not shown), which complicated the E14.5 analysis. Nonetheless, since *Gdf7* activation in neuroepithelial cells at E12.5 remained tightly restricted to the dorsal midline region, these findings suggest that some cortical marginal zone neurons are the descendants of *Gdf7*-expressing neuroepithelial cells of the dorsal midline region, which implicates the dorsal midline as a potential additional source of cortical neurons.

## Discussion

In this report, we provide evidence that roof plate signals regulate the expression of an essential cortical tran-



**Figure 6. Loss of Cortical Plate Neurons, but Persistence of Dorsally Derived Preplate Neurons in the *Lhx2*<sup>-/-</sup> Dorsal Telencephalon**

Histologic (A–D) and marker analyses (E–J) of the abnormal superficial layer in the *Lhx2*<sup>-/-</sup> dorsal telencephalon at E14.5 (A–D and H–J) and E12.5 (E–G). (E–H) Paraffin DAB immunoperoxidase. (F–G and I) Cryosection immunofluorescence. (J) ISH. Inset in (H), magnified view of field in (H).

(A–D) H&E-stained sections at a comparable rostrocaudal and dorsoventral position in *Lhx2* null and normal littermates. The mutant superficial layer (sl) lacks a defined cortical plate (A and B). In contrast to the radial orientation of normal cortical plate neurons (D), the nuclei of cells in the mutant superficial layer generally appeared horizontally oriented (B).

(E–J) At E12.5, numerous TuJ1-positive neurons are present in the mutant superficial layer (E), which includes both glutamatergic (F) and GABAergic cells (G). TuJ1-positive cells are readily detected in the ventricular zone of the enlarged cortical hem (E), implicating the cortical hem as a potential source of neurons.

(H–J) At E14.5, some of the TuJ1-positive neurons in the mutant superficial layer possess horizontally oriented processes (inset in [H]) and express Reelin, although Reelin immunoreactivity in the *Lhx2*<sup>-/-</sup> dorsal telencephalon is abnormally diffuse (I). *Dlx1* expression is not detected in the mutant superficial layer despite normal ganglionic eminence expression (J), suggesting few if any subcortically-derived neurons in the *Lhx2*<sup>-/-</sup> dorsal telencephalon by E14.5.

Arrowheads designate the pial surface; dashed lines designate the lower border of the mutant superficial layer.

Abbreviations: cp, cortical plate; ge, ganglionic eminence; iz, intermediate zone; sl, superficial layer in *Lhx2* mutants; vz, ventricular zone.

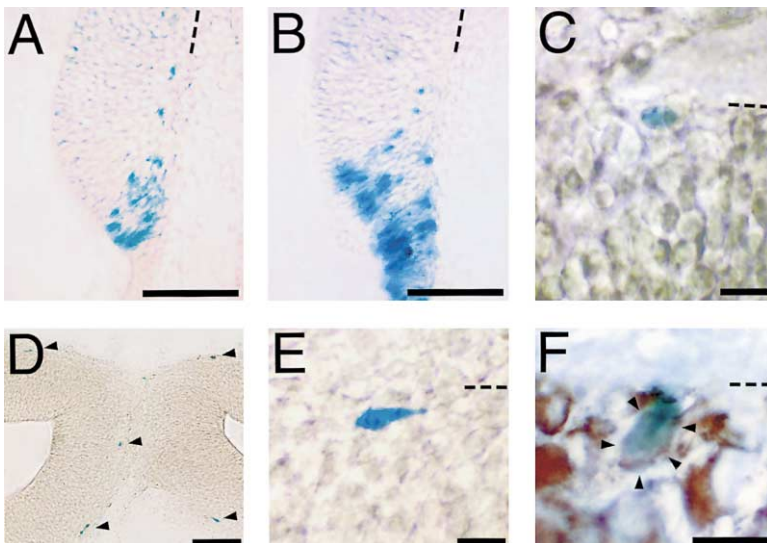
Scale bars: (A–C), 0.1 mm; (B, D, and E–I), 0.05 mm; (J), 0.2 mm.

scription factor, *Lhx2*. In the forebrain, *Gdf7* expression is largely restricted to roof plate cells, and ablation of these cells results in defective *Lhx2* expression within the telencephalon and a reduced cortical size. The ablation studies suggest that roof plate signals are necessary to both suppress and enhance *Lhx2* expression in different regions of the dorsal telencephalon, and both modes of *Lhx2* regulation can be recapitulated by *Bmp4* or *Bmp2* in explant cultures. Finally, we show that *Lhx2*

is crucial for normal telencephalic patterning and corticogenesis. These findings suggest that a roof plate-*Lhx2* pathway mediated by *Bmp* signals is involved in patterning of the dorsal telencephalon and cerebral cortex (Figure 8).

#### The Roof Plate-*Lhx2* Pathway and *Bmp* Signals

Our studies suggest that roof plate signals regulate *Lhx2* expression in the dorsal telencephalon (Figure 2). Roof



**Figure 7. Evidence for a Dorsal Midline Source of Cortical Marginal Zone Neurons**

X-gal histochemistry (A–E) and combined X-gal histochemistry/TuJ1 immunoperoxidase (F) of E12.5 (A–C) and E14.5 (D–F) embryos from *Gdf7-Cre X* conditional *lacZ* reporter matings. (A–C) At E12.5, *lacZ*-expressing neuroepithelial cells of the dorsal midline region are associated with stained cells in the superficial hippocampus (A and B). Most of these cells are located in subpial positions within the hippocampus (A) and nearby neocortex (C).

(D–F) At E14.5, subpial *lacZ*-expressing cells are more numerous within the hippocampus and neocortex (arrowheads in [D]). Some of these cells have horizontally oriented processes relative to the pial surface (hippocampal cell in [E]) and are surrounded by a brown rim of TuJ1-immunoreactivity (arrowheads around neocortical cell in [F]), thus indicating a neuronal identity.

Dashed lines designate the pial surface.

Scale bars: (A, B, and D), 0.1 mm; (C, E, and F), 0.01 mm.

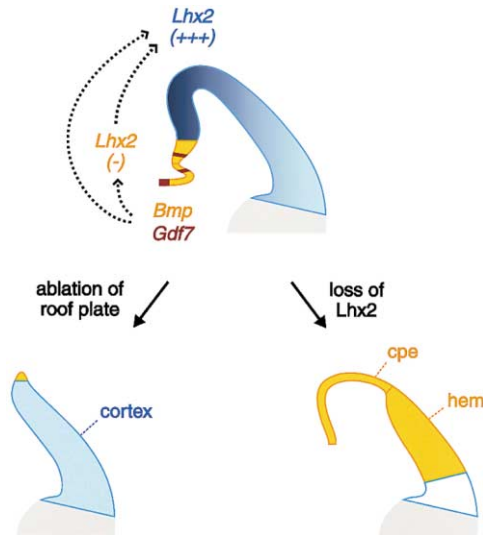


Figure 8. The Roof Plate-Lhx2 Pathway in Dorsal Telencephalic Patterning

Coronal schematics of the telencephalon in normal, roof plate-ablated, and Lhx2 knockout embryos at E12.5. Orange, Bmp-expressing roof plate region, including the choroid plexus and cortical hem; brown, Gdf7-expressing roof plate cells; blue, cortical neuroepithelium expressing the Lhx2 gradient; gray, the ganglionic eminence, partially depicted. Roof plate signals that include Bmp4 and Bmp2 suppress Lhx2 expression from the dorsal midline and enhance Lhx2 expression in the cortex. Lhx2 enhancement in the cortex may be a direct or indirect effect of roof plate signals. Ablation of Gdf7-expressing roof plate cells results in a reduced Lhx2 suppression domain at the dorsal midline and reduced less-graded Lhx2 expression in the cortex, which is accompanied by a reduction in cortical size. Loss of Lhx2 results in a massive excess of dorsal midline epithelia and a selective loss of cortical VZ progenitor cells and cortical plate neurons. Specification of cells in the *Lhx2*<sup>-/-</sup> lateral remnant (open blue box) suggest that Lhx2 “selects” a cortical VZ progenitor fate from already-specified dorsal telencephalic progenitor cells. Abbreviations: cpe, choroid plexus epithelium.

plate ablation results in cortical Lhx2 expression that is lower than normal and lacks its characteristic graded pattern (Figures 2A and 2G), suggesting that roof plate signals establish and/or maintain the cortical Lhx2 gradient. Roof plate signals may also regulate Lhx2 expression at the dorsal midline, where the normal Lhx2 suppression domain (Figures 1H–1K) is markedly reduced following roof plate ablation (Figures 2G and 2H). Loss of Lhx2 suppression is accompanied by losses of choroid plexus epithelium and cortical hem (Figures 2E, 2F, and 2I), whereas loss of Lhx2 function promotes a massive excess of these epithelia (Figures 4–5). This raises the possibility that roof plate-mediated suppression of Lhx2 may be involved in promoting choroid plexus and cortical hem fates.

The explant studies provide evidence that Bmp4 and Bmp2, but not Bmp6, represent two of the roof plate signals responsible for suppressing Lhx2 at the dorsal midline (Figure 3). These *in vitro* findings are consistent with the normal absence of Lhx2 expression (Figures 1H–1K) in the Bmp-rich dorsal midline region (Furuta et al., 1997; Grove et al., 1998; Dou et al., 1999) and with the loss of Lhx2 suppression following roof plate ablation (Figures 2G and 2H). In previous studies using similar

explants and Bmp concentrations, beads containing Bmp4 or Bmp2, but not Bmp7, were shown to elicit other gene expression (Foxg1/Bf1 suppression and *Msx1* upregulation) and cellular changes (increased apoptosis and decreased proliferation) that mimic the dorsal midline region (Furuta et al., 1997). Thus, Bmp4 and Bmp2 may be particularly important mediators of telencephalic dorsal midline development.

Bmp4 and Bmp2 may also mediate the roof plate enhancement effect on Lhx2 expression in the cortex, since beads with Bmp4 or Bmp2, but not Bmp6, were able to enhance Lhx2 expression at a distance (Figure 3). Like the Bmp-mediated suppression, Bmp-mediated enhancement of Lhx2 corresponds well to both the normal expression and roof plate ablation studies, since cortical Lhx2 levels are normally highest in the region immediately adjacent to the Bmp-rich dorsal midline (Figures 1H and 1J) and roof plate ablation leads to reduced levels of cortical Lhx2 (Figures 2A and 2G). Interestingly, previous explant studies suggested that Bmp4 or Bmp2 beads suppress Foxg1/Bf1 expression, but do not enhance its expression at a distance (Furuta et al., 1997). These effects are consistent with Foxg1/Bf1 expression *in vivo*, since Foxg1/Bf1 is absent from the dorsal midline region, but has a cortical expression gradient (high-ventral, low-dorsal; Figure 5G) (Tao and Lai, 1992) that is opposite to that of Lhx2 (Figure 1H and 1J). Bmp4 and Bmp2 may therefore be critical for regulating the gradients of Lhx2 and other cortically expressed genes in addition to regulating dorsal midline development. It is worth noting, however, that Bmp4 and Bmp2 represent only two of several Bmp (Furuta et al., 1997) and other signaling proteins (Grove et al., 1998) that are expressed in the dorsal midline region and could potentially be involved in regulating Lhx2.

Two different mechanisms could underlie the Bmp-mediated enhancement of Lhx2 expression at a distance. The first is a gradient mechanism involving differential short and long-range effects that depend on Bmp concentration, which is believed to be a common mechanism in the developing nervous system (Knecht and Harland, 1997; Liem et al., 1997; Marchant et al., 1998; Mabie et al., 1999). Although our studies are consistent with a gradient effect, such a mechanism would predict an intermediate bath concentration of Bmp4 or Bmp2 that uniformly enhances Lhx2 expression in explants, an effect that we have yet to observe (data not shown). Thus, the second possibility is a relay or cascade mechanism, in which Bmps from the dorsal midline act at short range to induce a second signaling center, such as the cortical hem (Grove et al., 1998), which then enhances Lhx2 expression in the neighboring cortex (Figure 8).

#### The Roof Plate as a Signaling Center for the Dorsal Telencephalon

Our studies suggest that roof plate signals regulate cortical size. The roof plate-restricted expression of Gdf7 leads to selective roof plate ablation, but loss of the roof plate also results in a reduced amount of cortical tissue (Figures 2A and 2D). This suggests that the cortical reduction is a non-cell-autonomous consequence of roof plate loss, thus implicating roof plate-derived

signals in the regulation of cortical size. This roof plate-dependent effect on cortical size could be mediated by *Lhx2* and/or *Wnt* signals, both of which are critical for normal cortical VZ proliferation (Porter et al., 1997; Lee et al., 2000b) and show reduced expression following roof plate ablation (Figures 2A and 2F).

*Gdf7*-expressing roof plate cells may also have a specific role in forming the telencephalic choroid plexus and cortical hem. These two epithelia are virtually undetectable following *Gdf7*-mediated ablation (Figures 2B, 2F, 2H, and 2I), despite evidence that the vast majority of cells in these epithelia never express *Gdf7* (Figures 1E–1G). *Gdf7*-positive cells may therefore be required to induce *Gdf7*-negative cortical hem and choroid plexus cells, although it is possible that some other nonspecific mechanism could account for the extensive loss of these epithelia in roof plate-ablated embryos.

A comparison of the roof plate-ablation phenotypes in the telencephalon and spinal cord suggests similar roof plate functions in both regions. In the spinal cord, roof plate ablation results in a loss of dorsalmost progenitors (*Math1* and *Neurogenin1*-positive), but only a reduction of the major and more distant dorsal progenitor pool (*Pax7*-positive) (Lee et al., 2000a). This is similar to the telencephalon, where the dorsal-most choroid plexus and cortical hem epithelia are lost, while cortical VZ progenitors are only reduced (Figure 2). Furthermore, the telencephalon and spinal cord defects are both accompanied by losses of pro-proliferative *Wnt* signals (Dickinson et al., 1994; Ikeya et al., 1997; Lee et al., 2000b) from dorsal domains (Figure 2F) (Lee et al., 2000a). These similarities suggest that after the onset of *Gdf7* expression, the roof plate may have conserved roles in the direct induction of nearby progenitors or epithelia and the indirect *Wnt*-dependent expansion of more distant dorsal progenitors.

While our studies implicate roof plate signals in cortical expansion, it remains uncertain if roof plate signals are involved in the *initial* induction of the cortex. At least three possibilities, which are not mutually exclusive, could account for cortical induction in roof plate-ablated embryos. The first is that early roof plate signals may be present despite *Gdf7*-mediated ablation, due to early persistence of *Gdf7*-negative roof plate cells and/or onset of *Gdf7* ablation that is too late to prevent cortical induction. The second possibility is that alternative sources of inductive cues may compensate for roof plate loss. Both the cortical neuroepithelium itself and dorsal surface ectoderm are known to express multiple *Bmp* transcripts around the time of anterior neural tube closure (Furuta et al., 1997), including *Bmp4*, *Bmp2*, and *Bmp7* in the cortex (Furuta et al., 1997; Dou et al., 1999) and *Bmp2*, *Bmp5*, and *Bmp7* in the dorsal surface ectoderm (Furuta et al., 1997). Since dorsal surface ectoderm also fails to form properly following *Gdf7*-mediated ablation (Figure 2G), presumably due to direct expression of *Gdf7* in this tissue (data not shown), we cannot exclude the possibility that the dorsal surface ectoderm has important signaling properties during early development. The third possibility is that the roof plate may not be critical at all for inducing the cortex, but may only have a role in its expansion.

### **Lhx2 in Dorsal Telencephalic Patterning**

Our studies confirm previous observations of *Lhx2* mRNA (Xu et al., 1993; Bertuzzi et al., 1999; Rincon-Limas et al., 1999; Bulchand et al., 2001) and its graded distribution (Donoghue and Rakic, 1999; Nakagawa et al., 1999; Retaux et al., 1999) in the cortical VZ, and provide evidence for a similar distribution of *Lhx2* protein (Figure 1J), the absence of *Lhx2* from the dorsal midline region (Figures 1H–1K), and the dependence of *Lhx2* expression on roof plate function (Figure 2). Some features of the forebrain phenotype in *Lhx2*<sup>-/-</sup> embryos have also been previously reported (Porter et al., 1997; Bulchand et al., 2001). In particular, a recent study of these animals demonstrated a dorsal telencephalic patterning defect that involved an enlarged cortical hem (Bulchand et al., 2001). Interestingly, an excess of choroid plexus epithelium was not reported, although this tissue was noted to be difficult to assess (Bulchand et al., 2001).

Our studies confirm the excess of cortical hem tissue in *Lhx2*<sup>-/-</sup> embryos (Bulchand et al., 2001), but also demonstrate a marked excess of choroid plexus (Figures 4 and 5). The lack of mutant *Lhx2* allele expression in the excessive choroid plexus and cortical hem of *Lhx2*<sup>-/-</sup> embryos (Figures 5J and 5K) suggests that these dorsal telencephalic epithelia *enlarge* at the expense of cortical neuroepithelium. *Lhx2* expression in cortical neuroepithelium may therefore be required to restrict the growth of *Lhx2*-negative dorsal midline tissue in the normal forebrain.

The striking modularity to the dorsal telencephalic enlargement and cortical loss in *Lhx2*<sup>-/-</sup> embryos (Figures 4 and 5) is suggestive of a modular basis to forebrain development (Puelles and Rubenstein, 1993; Rubenstein and Shimamura, 1997). The *Lhx2*<sup>-/-</sup> patterning defect suggests that the dorsal midline (choroid plexus and cortical hem), cerebral cortex, and ganglionic eminence represent distinct modules along the telencephalic dorsoventral axis (Figure 8), with *Lhx2* selectively defining the cortical module in a fashion that is complementary in some ways to the *Dlx* genes in the ganglionic eminence. Modular telencephalic development may enable progenitor cells from dorsal midline, cortical, and subcortical regions to develop independently prior to mixing their progeny in the mature cerebral cortex (Paravelas, 2000; Wilson and Rubenstein, 2000).

### **Lhx2 as a “Selector” Gene for Cortical VZ Progenitor Fate**

In addition to regulating proliferation (Porter et al., 1997), *Lhx2* appears to have a fundamental role in the specification of cortical VZ progenitor cells. Partially specified cells are located in a small lateral remnant of the E12.5 *Lhx2*<sup>-/-</sup> dorsal telencephalon based on their expression of the mutant *Lhx2* allele (Figures 5J and 5K). Lateral remnant cells appear to be normally specified as telencephalic progenitor cells, since they express high levels of *Nestin* and *RC2* (Figures 5L and 5M) and low but detectable levels of *Foxg1/Bf1* (Figure 5I). They also seem to show normal expression of the dorsal marker *Ngn2*, but fail to express genes that identify more medial (*EphB1* and *Lmo2*) or more lateral (*Scip* and *Lmo3*) cortical VZ fields (Bulchand et al., 2001). *Lhx2*<sup>-/-</sup> lateral rem-

nant cells therefore appear to possess a normal dorsal telencephalic progenitor identity, but fail to become specified into bona fide cortical VZ progenitor cells.

Taken together, these studies suggest that Lhx2 is required for the establishment of a cortical VZ progenitor fate and that Lhx2 specifically functions to “select” this fate from already-specified dorsal telencephalic progenitors. The data provide evidence for a hierarchy of specification events in which neural (Nestin and RC2), telencephalic (Foxg1/Bf1) and dorsal (Ngn2) progenitor identity are established prior to or in parallel with the Lhx2-dependent selection of a generic cortical VZ progenitor fate, which precedes the specification of individual progenitor fields. The putative selector function for Lhx2 in cortical VZ progenitors may complement a similar role for Lhx5 in dorsal midline development (Zhao et al., 1999). The Lhx2 selector function may also be evolutionarily conserved, since *apterous*, the *Drosophila* ortholog of *Lhx2*, is a classic “selector” gene that acts in the dorsal fly wing (Diaz-Benjumea and Cohen, 1993; Williams et al., 1993; Blair et al., 1994).

#### Lhx2 and the Dorsal Midline Region in the Generation of Cortical Neurons

Lack of a specified cortical VZ in *Lhx2*<sup>-/-</sup> embryos leads to a corresponding lack of cortical plate neurons (Figure 6). The apparent absence of a defined cortical plate from all cortical regions suggests that the entire cortex depends on Lhx2 for its genesis. Interestingly, the *Lhx2*<sup>-/-</sup> dorsal telencephalon does contain neurons, but most appear to be preplate neurons that do not have a subcortical origin. These neurons lack expression of the subcortical neuronal marker Dlx1 (Anderson et al., 1997) (Figure 6J) and are present in sizeable numbers by E12.5 (Figure 6E), when significant numbers of subcortically derived neurons have yet to arrive in the normal cortex (Anderson et al., 1999; Parnavelas, 2000). Dorsal progenitor zones therefore represent the likely source for most, if not all, of the *Lhx2*<sup>-/-</sup> dorsal telencephalic neurons, but the major dorsal source normally is the cortical VZ, which is virtually absent in E12.5 *Lhx2*<sup>-/-</sup> embryos (Figure 5).

Two lines of evidence indicate that the dorsal midline region may be a source of cortical neurons in normal and *Lhx2*<sup>-/-</sup> embryos. First, TuJ1-positive neurons are readily detectable within the enlarged cortical hem VZ of *Lhx2*<sup>-/-</sup> embryos (Figure 6E), suggesting that the cortical hem produces neurons. The possibility that these TuJ1-positive cells migrated into the hem VZ from another site seems unlikely, since the Lhx2 mutant cortex lacks a cortical VZ as well as subcortically derived neurons. Second, the Gdf7-lacZ fate mapping studies suggest that Gdf7-expressing neuroepithelial cells in the dorsal midline region give rise to marginal zone neurons (Figure 7). LacZ-expressing marginal zone neurons are more numerous toward the dorsal midline region where Gdf7 activation is initiated, and importantly, these marginal zone cells are detectable by E12.5, when lacZ-expressing neuroepithelial cells appear to remain exclusively restricted to the dorsal midline region. This strongly suggests that the lacZ-expressing marginal zone cells are the descendants of Gdf7-expressing neuroepithelial cells of the dorsal midline region. The relative paucity

of lacZ-expressing marginal zone cells in these studies is consistent with the small fraction of dorsal midline cells that activate Gdf7 expression (Figures 1E–1G). Correspondingly, this paucity almost certainly underestimates the number of cortical cells that may be derived from the dorsal midline region.

Although a dorsal midline source of neurons has not been previously described for the cortex, potential dorsal midline origins for neurons in the midbrain, hindbrain, and spinal cord have been previously described (Dymecki and Tomasiewicz, 1998; Lee et al., 2000a). This suggests that, in addition to its signaling function, the roof plate region may have a conserved role in the generation of neurons throughout the developing nervous system. For the cerebral cortex, the roof plate region would then join the now well-recognized subcortical sites (Anderson et al., 1997; Parnavelas, 2000) as sources of cortical neurons outside the cortical VZ.

#### Experimental Procedures

##### Animal Matings and Genotyping

Embryos from *Lhx2*<sup>+/-</sup> matings were PCR typed using primers for *neo* and/or *Lhx2* exon 3. The morning of the vaginal plug was designated day 0.5, and gestational ages were confirmed by crown-rump measurements (Kaufman, 1992). Although *Lhx2* null animals die in utero by E15–E16 (Porter et al., 1997), E14.5 *Lhx2*<sup>-/-</sup> embryos were obtained in numbers that approximated the expected Mendelian ratio and were grossly and histologically indistinguishable from normal littermates. Gdf7-Cre mice (Lee et al., 2000a) were mated with a conditional lacZ reporter strain (B6;129S-*Gtrosa26*<sup>tm1Sor</sup>; Jackson Lab) (Soriano, 1999). The Gdf7-xneox-DTA mice (Lee et al., 2000a) were mated with a  $\beta$ -actin Cre line (Lewandoski et al., 1997) (provided by Bruce Morgan). C57BL/6J mice (Jackson Lab) were used for the normal expression studies and explant cultures.

##### Histology and In Situ Hybridization

Standard H&E or cresyl violet staining was performed on 4  $\mu$ m sections of Carnoy's-fixed paraffin-embedded tissue. Nuclear fast red was performed according to manufacturer protocol (Vector Labs) on 25  $\mu$ m cryostat air-dried sections following whole-mount in situ hybridization.

Whole-mount embryo in situ hybridization (ISH) was performed as described (Wilkinson and Nieto, 1993), using digoxigenin-labeled riboprobes, an alkaline phosphatase (AP)-conjugated anti-digoxigenin Fab fragment (Boehringer-Mannheim), and NBT/BCIP substrates. The following modifications were applied for explants: 2 hr fixation, 1  $\mu$ g/ml Proteinase K for 3 min, and the addition of 1% SDS to wash solutions to prevent sticking. Explants were flattened with cover slips on glass slides as needed for photography. Double whole-mount ISH was performed as previously described (Hauptmann and Gerster, 1994), using digoxigenin-labeled Wnt2b and fluorescein-labeled Ttr riboprobes, an AP-conjugated anti-fluorescein Fab fragment (Boehringer-Mannheim), and Vector Red substrate (Vector Labs).

Section ISH was performed as described (Wilkinson and Nieto, 1993), using 14–30  $\mu$ m cryostat sections of 4% paraformaldehyde-fixed, 30% sucrose/PBS-infused tissue frozen in OCT. Acetylation steps following Proteinase K digestion were omitted. 10% high-molecular weight polyvinyl alcohol (70–100 kDa; Sigma) was included in some substrate reactions (De Block and Debrouwer, 1993).

Templates for probes: mouse *Lhx2* cDNA (Roberson et al., 1994) (gift of R. Maurer), and mouse ESTs for transthyretin (IMAGE clone 1078224), Wnt2b (IMAGE clone 353765), Wnt5a (IMAGE clone 775462), Foxg1/Bf1 (IMAGE clone 388688), and Dlx1 (IMAGE clone 348426). In addition to available sequence information, all EST clones were verified by insert size, diagnostic restriction digests, and replication of previously described expression patterns. The *neo* template was generated by PCR with SP6 and T7 promoter-containing primers.

### Immunohistochemistry and X-Gal Histochemistry

For fluorescent immunostaining, 14–20  $\mu\text{m}$  cryosections were blocked with 5% serum/PBST, then incubated overnight at 4°C with primary antibodies diluted in block. Secondary antibodies diluted in block were applied for 1 hr at room temperature, stained for 1–2 min in 2  $\mu\text{g}/\text{ml}$  Hoechst 33342 (Molecular Probes) for nuclear counterstaining, and mounted in Citifluor (Ted Pella). For TuJ1 DAB immunoperoxidase, 4  $\mu\text{m}$  paraffin sections of Carnoy's-fixed tissue were deparaffined in the microwave and processed according to manufacturer protocol for ABC amplification and DAB reaction (Vector Labs), with the following steps: 3%  $\text{H}_2\text{O}_2$  treatment for 30 min, 2% serum block, primary antibody dilutions in PBS only, biotinylated secondary antibody diluted in block, brief (three dips) counterstaining in Gill's hematoxylin and blueing in 0.1 M Tris (pH 8.4).

X-gal whole-mount staining was performed as previously described (Mercer et al., 1991), followed by cryoprotection in 30% sucrose, freezing in OCT, serial cryosectioning, and permanent mounting. For X-gal staining of sections, embryos were fixed in 4% paraformaldehyde for 4 hr, cryoprotected in 30% sucrose, then frozen in OCT. 30–60  $\mu\text{m}$  sections were air-dried for 2 hr to overnight, then treated with solutions containing 0.1% Triton X-100 in place of deoxycholate and NP40. For combined X-gal/TuJ1 staining, overnight X-gal staining was followed by 2% serum block, then TuJ1 immunostaining as described above.

Primary antibodies: Lhx2/Lhx9 (rabbit polyclonal, Gabi Tremml and Tom Jessell), 1:250; Lhx2 (guinea pig polyclonal, Kevin Lee and Tom Jessell), 1:1000; Lhx9 (guinea pig polyclonal, Kevin Lee and Tom Jessell), 1:500; RC2 (ascites, Miyuki Yamamoto, Developmental Hybridoma Studies Bank), 1:200; Nestin (mouse monoclonal Rat-401 supernatant, Ron McKay), 1:4; TuJ1 (rabbit polyclonal, Research Diagnostics), 1:4000–12000; CR50 (mouse monoclonal, Masaharu Ogawa), 1:10; Otx2 (rabbit polyclonal, E. Boncinelli), 1:500. Cy3 or FITC-conjugated goat secondary antibodies (Jackson ImmunoResearch) were applied at 1:100–200. Biotinylated goat anti-rabbit secondary antibody (Vector Labs) was applied at 1:250–500.

### Explant Cultures

The protocols were based on previous descriptions (Burrows et al., 1997; Furuta et al., 1997). Briefly, overlying skin (E10.5) or ectomesodermal tissue (E11.5 and older) was removed by forceps in HBSS. Explanted tissues were pinched off with forceps, placed in fresh HBSS, unfurled and trimmed with forceps or microscissors, oriented by meninges, then transferred by bent microspatula and forceps onto polycarbonate membranes (Costar, 0.2  $\mu\text{m}$  pore size), shiny side up, floating on media in tissue culture plates. Explants were placed into 15–20  $\mu\text{l}$  drops of media (1:1 DMEM:F12, 5% FCS, 25  $\mu\text{g}/\text{ml}$  insulin) and cultured for 36–48 hr at 37°C in a humidified 5%  $\text{CO}_2$  incubator.

For bath application experiments, explants were oriented VZ down onto 13 mm membranes in 24-well plates preblocked with 0.2  $\mu\text{m}$ -filtered serum-containing media. Media both below (at least 200  $\mu\text{l}$ /well) and above the membranes was removed by pipette before changing to media with recombinant human Bmp4 (Genetics Institute, Cambridge, MA).

For bead experiments, explants were oriented VZ up on 25 mm membranes in six-well plates and incubated for 1 hr to allow explant flattening and slight adherence. Bead preparation was performed as described previously (Furuta et al., 1997) using siliconized microcentrifuge tubes with 10  $\mu\text{l}$  aliquots of the following growth factors at 10  $\mu\text{g}/\text{ml}$ : recombinant human Bmp4, Bmp2, and Bmp6 (Genetics Institute, Cambridge, MA), recombinant human EGF (Fisher/Collaborative Biomedical), 0.1% cell culture-grade BSA (Gibco/BRL) in PBS. Beads were rinsed briefly in PBS prior to placement with forceps, pipetteman, or pulled microcapillary pipettes and mouth aspirator. Conditioned media from a noggin-expressing cell line (from Richard Harland) was mixed 1:1 with 10  $\mu\text{g}/\text{ml}$  Bmp4 prior to bead soaking.

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### References

- Anderson, S.A., Eisenstat, D.D., Shi, L., and Rubenstein, J.L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. *Science* 278, 474–476.
- Anderson, S., Mione, M., Yun, K., and Rubenstein, J.L. (1999). Differential origins of neocortical projection and local circuit neurons: role of *Dlx* genes in neocortical interneuronogenesis. *Cereb. Cortex* 9, 646–654.
- Bachiller, D., Klingensmith, J., Kemp, C., Belo, J.A., Anderson, R.M., May, S.R., McMahon, J.A., McMahon, A.P., Harland, R.M., Rossant, J., and De Robertis, E.M. (2000). The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature* 403, 658–661.
- Bayer, S.A., and Altman, J. (1991). Neocortical development (New York: Raven Press).
- Bertuzzi, S., Porter, F.D., Pitts, A., Kumar, M., Agulnick, A., Wassif, C., and Westphal, H. (1999). Characterization of Lhx9, a novel LIM/homeobox gene expressed by the pioneer neurons in the mouse cerebral cortex. *Mech. Dev.* 81, 193–198.
- Blair, S.S., Brower, D.L., Thomas, J.B., and Zavortink, M. (1994). The role of *apterous* in the control of dorsoventral compartmentalization and *PS* integrin gene expression in the developing wing of *Drosophila*. *Development* 120, 1805–1815.
- Boncinelli, E., Gulisano, M., and Broccoli, V. (1993). *Emx* and *Otx* homeobox genes in the developing mouse brain. *J. Neurobiol.* 24, 1356–1366.
- Bulchand, S., Grove, E.A., Porter, F.D., and Tole, S. (2001). LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. *Mech. Dev.* 100, 165–175.
- Burrows, R.C., Wancio, D., Levitt, P., and Lillien, L. (1997). Response diversity and the timing of progenitor cell maturation are regulated by developmental changes in EGFR expression in the cortex. *Neuron* 19, 251–267.
- D'Arcangelo, G., Miao, G.G., Chen, S.C., Soares, H.D., Morgan, J.I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374, 719–723.
- De Block, M., and Debrouwer, D. (1993). RNA-RNA in situ hybridization using digoxigenin-labeled probes: the use of high-molecular-weight polyvinyl alcohol in the alkaline phosphatase indoxyl-nitroblue tetrazolium reaction. *Anal. Biochem.* 215, 86–89.
- Diaz-Benjumea, F.J., and Cohen, S.M. (1993). Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* 75, 741–752.
- Dickinson, M.E., Krumlauf, R., and McMahon, A.P. (1994). Evidence for a mitogenic effect of Wnt-1 in the developing mammalian central nervous system. *Development* 120, 1453–1471.
- Donoghue, M.J., and Rakic, P. (1999). Molecular gradients and compartments in the embryonic primate cerebral cortex. *Cereb. Cortex* 9, 586–600.
- Dou, C.L., Li, S., and Lai, E. (1999). Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cereb. Cortex* 9, 543–550.
- Dymecki, S.M., and Tomaszewicz, H. (1998). Using Flp-recombinase

- to characterize expansion of Wnt1-expressing neural progenitors in the mouse. *Dev. Biol.* 201, 57–65.
- Furuta, Y., Piston, D.W., and Hogan, B.L. (1997). Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development* 124, 2203–2212.
- Golden, J.A., Bracilovic, A., McFadden, K.A., Beesley, J.S., Rubenstein, J.L., and Grinspan, J.B. (1999). Ectopic bone morphogenetic proteins 5 and 4 in the chicken forebrain lead to cyclopia and holoprosencephaly. *Proc. Natl. Acad. Sci. USA* 96, 2439–2444.
- Graham, A., Francis-West, P., Brickell, P., and Lumsden, A. (1994). The signalling molecule BMP4 mediates apoptosis in the rhombencephalic neural crest. *Nature* 372, 684–686.
- Grieshammer, U., Lewandoski, M., Prevette, D., Oppenheim, R.W., and Martin, G.R. (1998). Muscle-specific cell ablation conditional upon Cre-mediated DNA recombination in transgenic mice leads to massive spinal and cranial motoneuron loss. *Dev. Biol.* 197, 234–247.
- Grove, E.A., Tole, S., Limon, J., Yip, L., and Ragsdale, C.W. (1998). The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. *Development* 125, 2315–2325.
- Hauptmann, G., and Gerster, T. (1994). Two-color whole-mount in situ hybridization to vertebrate and *Drosophila* embryos. *Trends Genet.* 10, 266.
- Ikeya, M., Lee, S.M., Johnson, J.E., McMahon, A.P., and Takada, S. (1997). Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 389, 966–970.
- Kaufman, M.H. (1992). *The Atlas of Mouse Development* (London, San Diego: Academic Press).
- Knecht, A.K., and Harland, R.M. (1997). Mechanisms of dorsal-ventral patterning in noggin-induced neural tissue. *Development* 124, 2477–2488.
- Lavdas, A.A., Grigoriou, M., Pachnis, V., and Parnavelas, J.G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* 19, 7881–7888.
- Lee, K.J., and Jessell, T.M. (1999). The specification of dorsal cell fates in the vertebrate central nervous system. *Annu. Rev. Neurosci.* 22, 261–294.
- Lee, K.J., Mendelsohn, M., and Jessell, T.M. (1998). Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. *Genes Dev.* 12, 3394–3407.
- Lee, K.J., Dietrich, P., and Jessell, T.M. (2000a). Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. *Nature* 403, 734–740.
- Lee, S.M., Tole, S., Grove, E., and McMahon, A.P. (2000b). A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127, 457–467.
- Lendahl, U., Zimmerman, L.B., and McKay, R.D. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell* 60, 585–595.
- Lewandoski, M., Meyers, E.N., and Martin, G.R. (1997). Analysis of Fgf8 gene function in vertebrate development. *Cold Spring Harb. Symp. Quant. Biol.* 62, 159–168.
- Liem, K.F., Jr., Tremml, G., Roelink, H., and Jessell, T.M. (1995). Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* 82, 969–979.
- Liem, K.F., Jr., Tremml, G., and Jessell, T.M. (1997). A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91, 127–138.
- Mabie, P.C., Mehler, M.F., and Kessler, J.A. (1999). Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. *J. Neurosci.* 19, 7077–7088.
- Marchant, L., Linker, C., Ruiz, P., Guerrero, N., and Mayor, R. (1998). The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. *Dev. Biol.* 198, 319–329.
- Mercer, E.H., Hoyle, G.W., Kapur, R.P., Brinster, R.L., and Palmiter, R.D. (1991). The dopamine beta-hydroxylase gene promoter directs expression of *E. coli lacZ* to sympathetic and other neurons in adult transgenic mice. *Neuron* 7, 703–716.
- Messing, A., Behringer, R.R., Hammang, J.P., Palmiter, R.D., Brinster, R.L., and Lemke, G. (1992). P0 promoter directs expression of reporter and toxin genes to Schwann cells of transgenic mice. *Neuron* 8, 507–520.
- Millonig, J.H., Millen, K.J., and Hatten, M.E. (2000). The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS. *Nature* 403, 764–769.
- Misson, J.P., Edwards, M.A., Yamamoto, M., and Caviness, V.S., Jr. (1988). Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker. *Brain Res. Dev. Brain Res.* 44, 95–108.
- Nakagawa, Y., Johnson, J.E., and O'Leary, D.D. (1999). Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input. *J. Neurosci.* 19, 10877–10885.
- Noctor, S.C., Flint, A.C., Weissman, T.A., Dammerman, R.S., and Kriegstein, A.R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409, 714–720.
- Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H., and Mikoshiba, K. (1995). The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* 14, 899–912.
- Palmiter, R.D., Behringer, R.R., Quafe, C.J., Maxwell, F., Maxwell, I.H., and Brinster, R.L. (1987). Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. *Cell* 50, 435–443.
- Parnavelas, J.G. (2000). The origin and migration of cortical neurons: new vistas. *Trends Neurosci.* 23, 126–131.
- Porter, F.D., Drago, J., Xu, Y., Cheema, S.S., Wassif, C., Huang, S.P., Lee, E., Grinberg, A., Massalas, J.S., Bodine, D., et al. (1997). Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. *Development* 124, 2935–2944.
- Puelles, L., and Rubenstein, J.L. (1993). Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization. *Trends Neurosci.* 16, 472–479.
- Retaux, S., Rogard, M., Bach, I., Failli, V., and Besson, M.J. (1999). Lhx9: a novel LIM-homeodomain gene expressed in the developing forebrain. *J. Neurosci.* 19, 783–793.
- Rincon-Limas, D.E., Lu, C.H., Canal, I., Calleja, M., Rodriguez-Esteban, C., Izpisua-Belmonte, J.C., and Botas, J. (1999). Conservation of the expression and function of apterous orthologs in *Drosophila* and mammals. *Proc. Natl. Acad. Sci. USA* 96, 2165–2170.
- Roberson, M.S., Schoderbek, W.E., Tremml, G., and Maurer, R.A. (1994). Activation of the glycoprotein hormone alpha-subunit promoter by a LIM-homeodomain transcription factor. *Mol. Cell. Biol.* 14, 2985–2993.
- Rubenstein, J.L.R., and Shimamura, K. (1997). Regulation of patterning and differentiation in the embryonic vertebrate forebrain. In *Molecular and Cellular Approaches to Neural Development*, W.M. Cowan, T.M. Jessell and S. L. Zipursky, eds. (New York: Oxford University Press), pp. 356–390.
- Shimamura, K., Hartigan, D.J., Martinez, S., Puelles, L., and Rubenstein, J.L. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* 121, 3923–3933.
- Solloway, M.J., and Robertson, E.J. (1999). Early embryonic lethality within the 60A subgroup. *Development* 126, 1753–1768.
- Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* 21, 70–71.
- Tan, S.S., Kalloniatis, M., Sturm, K., Tam, P.P., Reese, B.E., and Faulkner-Jones, B. (1998). Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. *Neuron* 21, 295–304.
- Tao, W., and Lai, E. (1992). Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. *Neuron* 8, 957–966.
- Trousse, F., Esteve, P., and Bovolenta, P. (2001). Bmp4 mediates

apoptotic cell death in the developing chick eye. *J. Neurosci.* *21*, 1292–1301.

Wilkinson, D.G., and Nieto, M.A. (1993). Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts. *Methods Enzymol.* *225*, 361–373.

Williams, J.A., Paddock, S.W., and Carroll, S.B. (1993). Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete subregions. *Development* *117*, 571–584.

Wilson, S.W., and Rubenstein, J.L. (2000). Induction and dorsoventral patterning of the telencephalon. *Neuron* *28*, 641–651.

Xu, Y., Baldassare, M., Fisher, P., Rathbun, G., Oltz, E.M., Yancopoulos, G.D., Jessell, T.M., and Alt, F.W. (1993). LH-2: a LIM/homeodomain gene expressed in developing lymphocytes and neural cells. *Proc. Natl. Acad. Sci. USA* *90*, 227–231.

Zhao, Y., Sheng, H.Z., Amini, R., Grinberg, A., Lee, E., Huang, S., Taira, M., and Westphal, H. (1999). Control of hippocampal morphogenesis and neuronal differentiation by the LIM homeobox gene *Lhx5*. *Science* *284*, 1155–1158.

Zimmerman, L.B., De Jesus-Escobar, J.M., and Harland, R.M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* *86*, 599–606.