Neuron, Vol. 35, 1029–1041, September 12, 2002, Copyright ©2002 by Cell Press

BMP Signaling Is Required Locally to Pattern the Dorsal Telencephalic Midline

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Summary

BMPs have been proposed to pattern the medial-lateral axis of the telencephalon in a concentration-dependent manner, thus helping to subdivide the embryonic telencephalon into distinct forebrain regions. Using a CRE/*loxP* genetic approach, we tested this hypothesis by disrupting the *Bmpr1a* gene in the telencephalon. In mutants, BMP signaling was compromised throughout the dorsal telencephalon, but only the most dorsalmedial derivative, the choroid plexus, failed to be specified or differentiate. Choroid plexus precursors remained proliferative and did not adopt the fate of their lateral telencephalic neighbors. These results demonstrate that BMP signaling is required for the formation of the most dorsal telencephalic derivative, the choroid plexus, and that BMP signaling plays an essential role in locally patterning the dorsal midline. Our data fail to support a more global, concentration-dependent role in specifying telencephalic cell fates.

Introduction

The embryonic telencephalon begins as a simple sheet of neuroepithelial cells surrounding two symmetrical ventricles at the anterior end of the neural tube. It develops into the many important parts of the adult cerebral hemispheres: ventrally, as the basal ganglia (which functions in body movement and coordination) and dorsally and laterally, as the cerebral cortex (which is used for our highest cognitive functions). Cells generated near the midline from the most telencephalic dorsal domain differentiate into the only nonneural tissue of the telencephalon, the choroid plexus, which is responsible for secreting the cerebrospinal fluid into the lateral and third ventricles. How the areas of the telencephalon destined to form these very different adult brain structures become specified during development remains a poorly understood process. The mechanisms involved are likely to be intrinsic to the developing telencephalon itself since its progenitor cells acquire positional identities prior to the arrival of neuronal projections from other parts of the brain, as evidenced by the restricted expression patterns of several genes (reviewed by Ragsdale and Grove, 2001).

At least two models can explain how progenitor cells can acquire positional identities in a sheet of cells. The first is a classical morphogen model, whereby the signaling center (a group of cells at one end of a tissue) secretes a signaling factor that diffuses to form a concentration gradient along the length of the tissue. In this model, different concentrations of signaling factor induce progenitor cells to adopt distinct positional identities and cell fates. Evidence that such a mechanism participates in patterning the mammalian CNS comes primarily from work done on spinal cord development. For example, SHH secreted from the notochord specifies cell fates along the dorsoventral axis of the neural tube in a concentration-dependent fashion (Roelink et al., 1995). An alternative model for the acquisition of positional identities within a tissue postulates that signaling factors, instead of diffusing over the length of the tissue, only act locally to specify cell identities. In the mammalian forebrain, several secreted signaling factors (including members of the WNT, FGF, and BMP families) are expressed in restricted groups of cells that could potentially act as signaling centers to pattern the telencephalon (reviewed by Ragsdale and Grove, 2001). It is not yet clear whether each of these ligands plays an essential role in specifying telencephalic cell fates, nor is it clear whether they act purely locally or over longer distances. Indeed, there is as yet no evidence that these ligands diffuse away from their source; they may instead be sequestered by proteoglycans shortly after being secreted (Perrimon and Bernfield, 2000). If the signals act locally, other areas of the telencephalon may be specified independently by other factors or might arise through the action of a signaling cascade in which the initial action of one signaling factor induces the expression of another in neighboring cells. The two models described here can in principle be distinguished simply by lowering the level of signaling in vivo, which can be accomplished by disrupting expression of a single gene encoding either a ligand or its receptor, and assessing whether or not there is a global shift in positional identities or only a local loss of cell fate specification. In this report, we focus on BMP signaling as an example of a potential inducer of positional identities in the telencephalon.

The effects of BMP proteins on neural progenitor cells in culture has been studied intensively, although little has been done to directly address their role in brain development in vivo. BMPs have been implicated in patterning the medial-lateral (M-L) axis of the telencephalon by regulating the development of the dorsal midline (the M-L axis is also referred to as the dorsal-ventral axis). Several *Bmp* family members, including *Bmp2*, *4*, *5*, *6*, and *7*, are expressed along the dorsal telencephalic midline, a potential signaling center for M-L patterning (Furuta et al., 1997). Application of BMP-soaked beads to explants of lateral telencephalic tissue induces midline properties in vitro, including the induction of the dorsal midline marker *Msx1*, repression of a more later-

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ally expressed gene (*Foxg1*), the inhibition of proliferation, and the promotion of apoptosis (Furuta et al., 1997). Recent studies have also suggested that the dorsal telencephalic midline, and in particular BMPs, pattern the dorsal telencephalon in part by regulating expression of the LIM homeodomain transcription factor *Lhx2*. *Lhx2* is required to specify lateral cell fates; in *Lhx2*-deficient mice, much of the cerebral cortex is lost at the expense of the more medial choroid plexus (Bulchand et al., 2001; Monuki et al., 2001). In addition, experiments using cultured explants suggest that BMP2 and BMP4, but not BMP6, can repress *Lhx2* expression at high concentrations and promote *Lhx2* expression at lower concentrations (Monuki et al., 2001). Whether BMPs are required in vivo for the normal development of the dorsal midline and whether they act in a concentration-dependent manner has not yet been determined.

Another unresolved question is whether the different BMP ligands or their receptors have redundant or distinct functions in telencephalic development. BMPs bind and signal through heteromeric receptor complexes composed of type I and type II BMP receptors (BMPRI and BMPRII). If either BMPRI or BMPRII is missing, BMP signaling is abolished (reviewed by Massagué, 1996). Recent studies have suggested that the two type I BMP receptors that are expressed in the telencephalon, *Bmpr1a* and *Bmpr1b*, have distinct functions. Results from overexpressing activated and dominant-negative forms of these receptors suggest that BMPRIA activation can respecify lateral cells to adopt medial fates, is required to induce expression of *Bmpr1b*, and promotes proliferation of neural progenitor cells, whereas BMPRIB inhibits progenitor cell proliferation (Panchision et al., 2001). In contrast, at least some of the BMP ligands appear to have overlapping functions. For example, BMP2 and BMP4 have similar activities in the induction of midline properties in vitro (Furuta et al., 1997), and the loss of either *Bmp5* or *Bmp7* on its own has little effect on development, whereas embryos deficient for both *Bmp5* and *Bmp7* die with severe CNS defects (Solloway and Robertson, 1999). The *Bmp5;Bmp7* compound mutants have, however, revealed little about the role of BMP ligands in telencephalic patterning because an analysis of brain development was confounded by early defects in neural tube closure and in other embryonic tissues. In fact, genetic evidence demonstrating a direct requirement for BMP signaling in telencephalic patterning or development is notably absent.

Because there are at least five *Bmp* ligand genes expressed in the dorsal telencephalic midline and at least some of them are likely to overlap functionally (Furuta et al., 1997; Solloway and Robertson, 1999), targeted disruption of any individual gene may not reveal a role for BMP signaling in this tissue. In addition, disrupting several *Bmp* ligand genes is likely to generate early lethalities that preclude a study of their function in telencephalic development (e.g., Solloway and Robertson, 1999). Perhaps a better way to directly test whether BMP signaling acts locally or globally to pattern the M-L axis of the telencephalon is to significantly reduce BMP signaling in the telencephalon, using a conditional allele of a gene encoding a primary BMP receptor. This approach confers the additional advantage of addressing whether the different BMP receptors have distinct or overlapping functions. Both type I receptors, BMPR1A and BMPR1B, are expressed in telencephalic precursor cells, although *Bmpr1a* is expressed uniformly throughout the telencephalon and at a higher level than *Bmpr1b* (Dewulf et al., 1995; Panchision et al., 2001; see also Figures 1E and 2A). Mice deficient for *Bmpr1b* are viable with no apparent CNS phenotype (Yi et al., 2000), whereas mice deficient for *Bmpr1a* die at gastrulation (Mishina et al., 1995) and are therefore uninformative as to the role of this gene in telencephalic development.

To test the role of BMP signaling in the telencephalon, we have conditionally disrupted *Bmpr1a* using a *CRE/loxP* approach. We have previously described a genetic approach for knocking out genes in the telencephalon using the Foxg1-Cre mouse line (Hébert and McConnell, 2000). In mice that carry the *Foxg1-Cre* allele and a gene flanked by *lox* sites (floxed), recombination occurs efficiently throughout the telencephalon from its earliest stages of development, including the dorsal midline, even though *Foxg1* is only expressed transiently in this region prior to its invagination between the lateral ventricles. We find that reduced BMP signaling leads to loss of the choroid plexus, the most dorsal derivative of the developing telencephalon, without affecting the fates of more lateral cell types or the expression boundaries of more laterally localized gene products. Our data provide evidence that BMPs act locally to specify the dorsal midline of the telencephalon.

Results

Foxg1-Cre;floxed-Bmpr1a Mice Lack *Bmpr1a* in the Telencephalon

BMP signaling has been postulated to pattern the telencephalon in a concentration-dependent manner along its M-L axis. Embryos homozygous for a null allele of *Bmpr1a* die at gastrulation (Mishina et al., 1995) before the establishment of the telencephalon and are thus uninformative as to the role of this gene in brain development. To explore directly the role of *Bmpr1a* in telencephalic development and patterning, we crossed floxed-Bmpr1a (Bmpr1a^{fx}) mice to Foxg1-Cre mice, which have previously been shown to efficiently recombine floxed alleles in the telencephalon (Hébert and McConnell, 2000). The Bmpr1a^{fx} mice carry a *Bmpr1a* allele in which exon 2 is flanked by *loxP* sites ("floxed") and which behaves as wild-type until recombined, at which point it behaves as a null (Mishina et al., 2002). For all experiments described below, mutant mice were generated by crossing homozygous Bmpr1a$^{fx/fx}$ mice to Foxg1-Cre;Bmpr1a^{null} mice (null allele: Mishina et al., 1995).

To test the efficiency with which CRE expressed from the *Foxg1-Cre* allele recombines the *Bmpr1a^{fx}* allele, we analyzed DNA isolated from embryonic day (E)12.5 Foxg1-Cre;Bmpr1a$^{fx/+}$ embryos. No unrecombined *Bmpr1a^{fx}* allele could be detected in telencephalic DNA for 7/7 embryos by either Southern blot or PCR analyses (Figures 1A and 1B). In addition, RNA in situ hybridization analysis using an exon-2-specific probe indicates a loss of *Bmpr1a* transcript specifically in the anterior prosencephalon of E10 Foxg1-Cre;Bmpr1a$^{fx/null}$ embryos and the telencephalon of E12.5 embryos (Figures 1C–1F). By

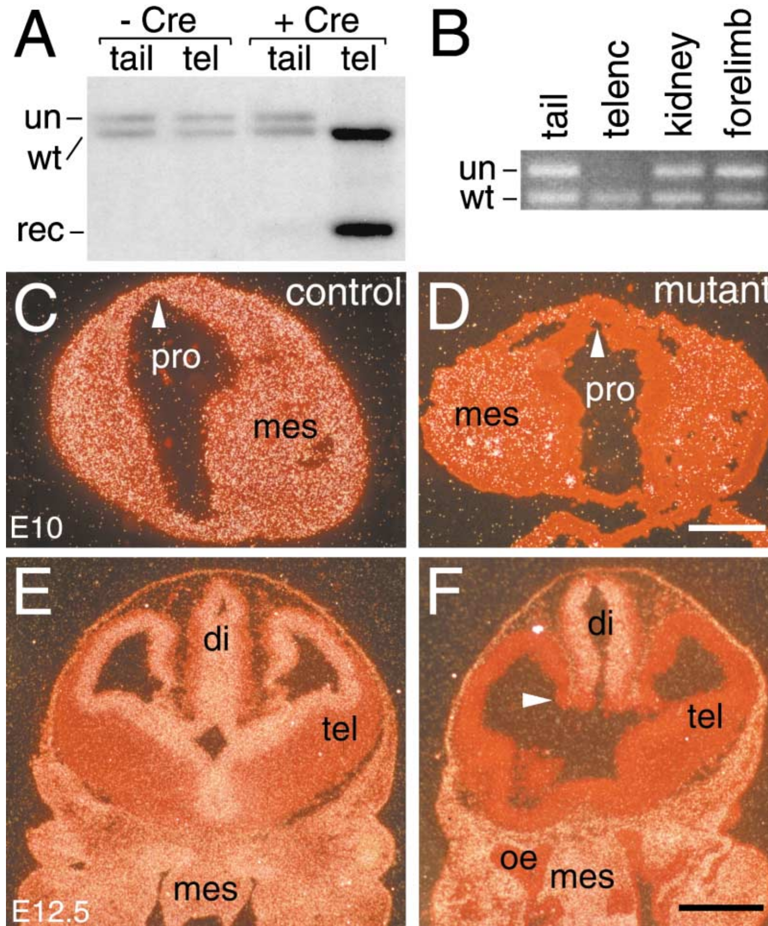

Figure 1. *Bmpr1a* Expression Is Efficiently Abolished in the Telencephalon of Foxg1-Cre;floxed-Bmpr1a Mice

(A) Southern blot analysis of DNA taken from the telencephalon (tel) or tail of E12.5 Bmpr1a$^{fx/+}$ and Foxg1-Cre;Bmpr1a$^{fx/+}$ embryos. Only the unrecombined *Bmpr1a*^{fx} allele (un) (4.4 kb) and the wild-type allele (wt) (4.0 kb) are observed in Bmpr1a$^{fx/+}$ tissues, whereas in the telencephalon of Foxg1-Cre;Bmpr1a$^{fx/+}$ mice, only the recombined (rec) (2.2 kb) and wild-type alleles are observed, indicating complete recombination.

(B) PCR analysis of tissues from the tail, telencephalon (telenc), kidney, or forelimb of E12.5 Bmpr1a$^{fx/+}$ and Foxg1-Cre;Bmpr1a$^{fx/+}$ embryos. The telencephalon is the only tissue in which the unrecombined allele was not detected by PCR.

(C) RNA in situ hybridization analysis for *Bmpr1a* expression in a coronal section through the anterior prosencephalon of an E10 control embryo. *Bmpr1a* is expressed throughout the neuroepithelium lining the prosencephalic ventricle (pro) as well as mesencephalic tissue (mes).

(D) In an E10 Foxg1-Cre;Bmpr1a$^{fx/null}$ animal, *Bmpr1a* expression is lost from the anterior prosencephalon, including the dorsal midline (arrowhead). Scale bar, 0.25 mm.

(E) Coronal section through the head of an E12.5 control embryo. *Bmpr1a* expression is widespread throughout the ventricular zone of the telencephalon (tel) and diencephalon (di), as well as more ventral mesencephalic tissues.

(F) In an E12.5 Foxg1-Cre;Bmpr1a$^{fx/null}$ animal, *Bmpr1a* expression is lost from the telencephalon, including the midline (arrowhead), but not from the diencephalon or head mesenchyme. Recombination is also observed in the olfactory epithelium (oe), as described previously (Hébert and McConnell, 2000). Scale bar, 1 mm.

E10, *Bmpr1a* expression is already lost in the dorsal midline of mutants, consistent with previous observations on the timing of Cre-mediated recombination using Foxg1-Cre mice (Hébert and McConnell, 2000). At this stage, the dorsal midline (where *Bmp* gene expression and signaling are likely to be strongest) has not yet started to show any signs of invagination (Figures 1C and 1D, arrowheads).

To assess the viability of Foxg1-Cre;Bmpr1a$^{fx/null}$ mice, litters from crosses between Foxg1-Cre;Bmpr1a^{null} and Bmpr1a^{fx} mice were genotyped from E11.5 to postnatal day (P)0 (Table 1). Reduced numbers of Foxg1-Cre; Bmpr1a$^{fx/null}$ mutants are apparent by E12.5, and the majority of the mutants die between E11.5 and P0. This variability in the time of death is presumably due to variable recombination in tissues other than the telencephalon, such as the heart, which is expected in mice of a mixed genetic background (Hébert and McConnell, 2000). Only mutant mice showing no signs of necrosis, such as broken blood vessels, blood clots, or lack of circulation, were used for further analysis of forebrain development. The brains of mutant animals appeared overall to be grossly normal. The only visible phenotype found in mutants of all ages was a recessed lower jaw, presumably due to a requirement for *Bmpr1a* in branchial arch development (data not shown).

Loss of *Bmpr1a* Results in Significantly Reduced Levels of BMP Signaling in the Telencephalon

Bmpr1a is one of two type I BMP receptors that are expressed in the telencephalon; the other is *Bmpr1b* (Dewulf et al., 1995; Panchision et al., 2001). BMP signal-

Table 1. Genotype of Recovered Embryos

	Bmpr1a$^{fx/+}$	Bmpr1a$^{fx/null}$	Cre;Bmpr1a$^{fx/+}$	Cre;Bmpr1a$^{fx/null}$
E11.5	10	4	8	5
E12.5	106	74	100	30[a]
E13.5	9	14	14	5
E15.5–16.5	23	18	20	6[b]
P0	16	15	19	2[c]

[a] Nine of the 30 embryos showed signs of necrosis and were not used for experiments.
[b] Five of the six embryos were necrotic.
[c] Both pups were born dead or died within 24 hr.

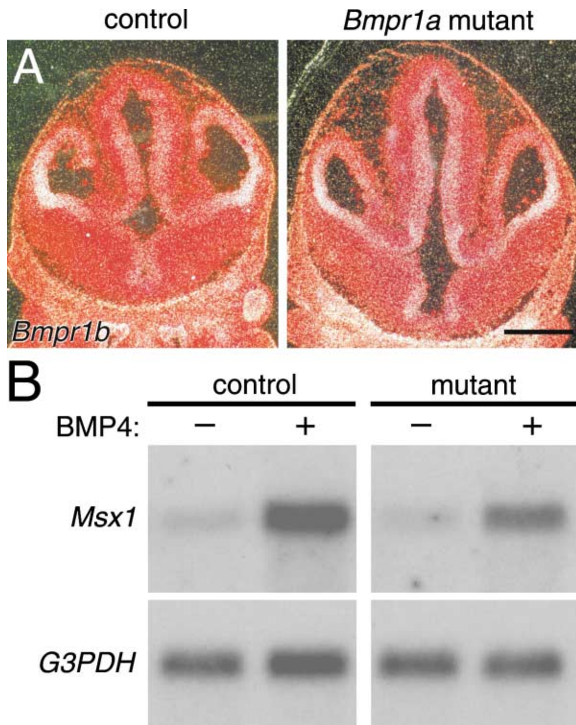

Figure 2. The Lateral Telencephalon, which Expresses *Bmpr1b*, Is Still Responsive to Exogenous BMP4 in *Bmpr1a*-Deficient Telencephalons, Although to a Lesser Degree Than in Controls

(A) RNA in situ hybridization analysis of *Bmpr1b* in control and mutant brains. *Bmpr1b* is expressed within the ventricular zone of both the telencephalon and diencephalon. Within the telencephalon, *Bmpr1b* expression is highest in lateral regions (the lateral ganglionic eminence) and shows a decreasing gradient of expression from lateral to medial. Little or no *Bmpr1b* signal is present in the most medial regions of the telencephalon. Scale bar, 1 mm.

(B) Northern analysis of *Msx1* expression by cortical cells that were removed from the lateral telencephalon of E12.5 embryos and incubated with BMP4 (100 ng/ml) for 1 hr. *G3PDH* expression is shown as a control for loading. Results from three separate assays were pooled. Control cells (left) responded to the addition of BMP4 by a large (10.6-fold) induction of *Msx1* expression. Cells from *Bmpr1a*-deficient embryos also showed an induction of *Msx1* expression in response to BMP4, but the response was quantitatively smaller (3.1-fold).

ing may still occur in the *Bmpr1a*-deficient telencephalon if *Bmpr1b* can compensate for lack of *Bmpr1a*. However, a previous report has suggested that the two receptors are not redundant and that signaling through BMPR1A and BMPR1B exert distinct effects on gene expression, proliferation, and cell survival (Panchision et al., 2001). To explore BMP signaling in *Bmpr1a*-deficient animals, we first examined more closely the expression of *Bmpr1b* in control and mutant E12.5 embryos. Interestingly, *Bmpr1b* expression occurs in a gradient with levels highest in the most lateral telencephalon that decrease medially and are undetectable above background in the dorsal midline (Figure 2A). This pattern is maintained in Foxg1-Cre;Bmpr1a$^{fx/null}$ mutants (Figure 2A). Therefore, in Foxg1-Cre;Bmpr1a$^{fx/null}$ mutants, neither of the type I BMP receptor genes (*Bmpr1a* and *Bmpr1b*) is expressed in the dorsal midline.

To ascertain whether *Bmpr1a*-deficient lateral telencephalic cells are still responsive to BMP protein, cells from the lateral telencephalon of E12.5 and E13.5 mutant and control embryos were dissociated and cultured in the presence or absence of BMP4. Responsiveness to BMP4 was assessed by examining the expression of *Msx1*, a gene whose expression can be induced by BMP2/4 (Furuta et al., 1997; Shimamura and Rubenstein, 1997). As expected, cells from the lateral telencephalon of control animals responded to BMP4 by expressing *Msx1* (Figure 2B). Cells from mutant brains also showed a strong induction of *Msx1* in response to BMP4 compared with control cells, albeit at significantly reduced levels. The density of the *Msx1* band was normalized and quantified for three separate assays (see Experimental Procedures), revealing that control cells showed a 10.6-fold induction ($\pm$2.5), whereas mutant cells showed only a 3.1-fold induction ($\pm$1.6). It is unlikely that this responsiveness in mutants is due to residual *Bmpr1a* because recombination is complete by E10 (Figure 1).

Collectively, these results suggest three conclusions. First, although it has previously been suggested that the normal regulation of *Bmpr1b* expression requires signaling through BMPRIA (Panchision et al., 2001), our data demonstrate that this is not the case. *Bmpr1b* continues to be expressed in its normal gradient pattern even in the absence of *Bmpr1a*. Second, our results suggest that BMP signaling is likely to have been lost completely in the dorsal midline where *Bmpr1a* is deleted and *Bmpr1b* is not expressed. Third, our data show that *Bmpr1b* can partially compensate for loss of *Bmpr1a* in the lateral telencephalon but that the level of signaling transmitted by the remaining receptor is markedly dampened compared to that in control embryos. The production of embryos in which BMP signaling is reduced, but not abrogated completely, can thus be utilized to distinguish between models in which gradient signaling specifies cell fates in a concentration-dependent manner versus those in which BMPs act purely locally in the development of midline fates.

Patterning in the Dorsolateral and Ventral Telencephalon Is Normal in Mutants

To test the hypothesis that BMP signaling patterns the M-L axis of the telencephalon, we examined the expression of genes that mark the different regions of the telencephalon by RNA in situ hybridization analysis of control and *Bmpr1a*-deficient embryos. Several ventral markers are expressed similarly in E12.5 mutant and control mice. These include *Dlx2*, which marks both the medial and lateral ganglionic eminences (Figure 3B); *Nkx2.1*, which marks the medial ganglionic eminence (Figure 3A); and *Shh*, which marks the differentiating field of the medial ganglionic eminence (data not shown). These data indicate that ventral patterning in *Bmpr1a*-deficient telencephalons is normal, as expected if BMP signaling is crucial for dorsal, but not ventral, development.

Evidence from explant cultures suggest that expression of *Foxg1* and *Lhx2* is regulated by BMP signaling in the developing dorsal telencephalon (Furuta et al., 1997; Monuki et al., 2001). Expression of both genes in normal animals is excluded from the dorsal midline where the *Bmp* genes are most strongly expressed. *Lhx2*, which is known to confer lateral cell fates (Bul-

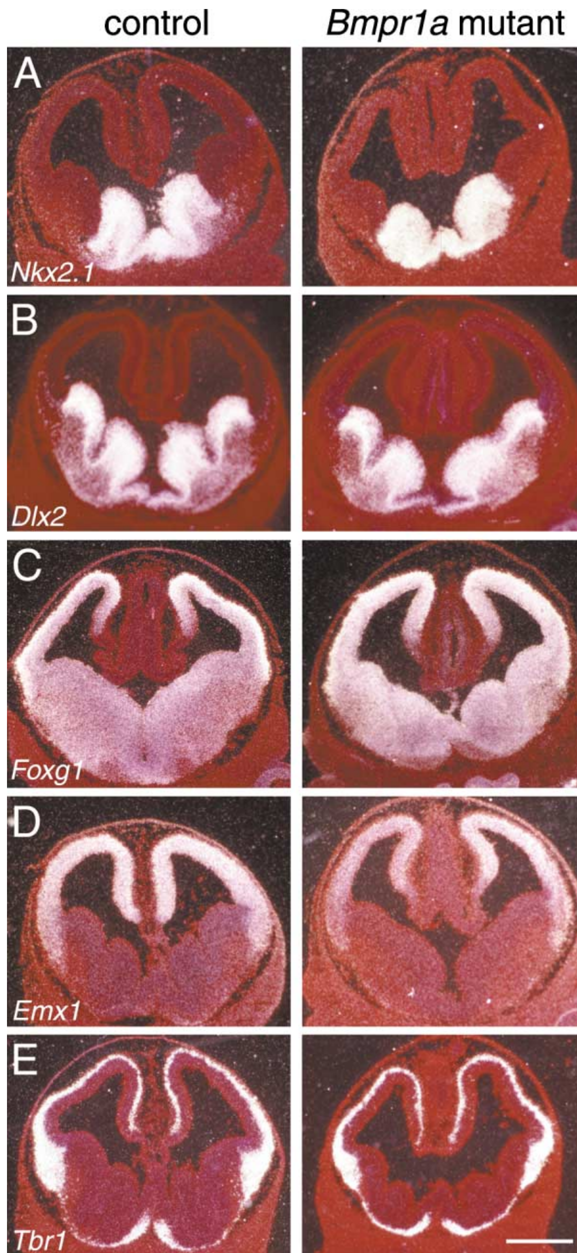

Figure 3. Patterns of Gene Expression that Mark the Ventral and Dorsolateral Telencephalon Are Normal in *Bmpr1a*-Deficient Brains

RNA is situ hybridization analysis of coronal sections through E12.5 control (left) and mutant (right) heads. (A) *Nkx2.1* expression marks the medial ganglionic eminence, (B) *Dlx2* marks both medial and lateral ganglionic eminences, (C) *Foxg1* marks the entire telencephalon except the dorsal midline, (D) *Emx1* marks the presumptive cerebral cortex, and (E) *Tbr1* marks all telencephalic neurons. Scale bar, 1mm.

chand et al., 2001; Monuki et al., 2001), is also expressed at its highest levels immediately adjacent to the dorsal midline. It has been postulated that high concentrations of BMP inhibit *Lhx2* expression, whereas lower concentrations strongly promote the expression of *Lhx2* (Monuki et al., 2001). To test the hypothesis that BMP signaling represses expression of *Foxg1* and *Lhx2* in the dorsal midline and induces higher levels of *Lhx2* immediately adjacent to the midline, we examined the expression of these genes, as well as others that are normally expressed in the dorsal telencephalon. Surprisingly, *Foxg1*, *Lhx2*, *Emx1*, and *Ngn2*, whose medial boundary of expression stops just short of the cortical hem in E12.5 control animals, do not show a medial shift of this boundary in mutants (Figures 3C, 3D, and 4B [arrows], and data not shown). A shift in the mutants would be expected given a loss of BMP signaling in the midline and the hypothesis that BMP signaling inhibits the expression of these genes in this region. Furthermore, the peak of *Lhx2* expression adjacent to the dorsal midline shows neither a loss nor a shift in its position, which would be expected if lower levels of BMP signaling normally induce high levels of *Lhx2* expression. These results illustrate that *Bmpr1a* is not required outside of the dorsal midline for patterning the M-L axis of the telencephalon, nor is it required to regulate expression of *Foxg1* and *Lhx2*.

Bmpr1a Is Required for Formation of Dorsal Midline Structures

BMP signaling has been hypothesized to induce dorsal midline properties, such as expression of the transcription factor *Msx1*, in the telencephalon (Furuta et al., 1997). To ascertain the function of *Bmpr1a* in the dorsal midline of the telencephalon, we examined this region in mutant Foxg1-Cre;Bmpr1a$^{fx/null}$ and control brains at E11.5 and E12.5. Somewhat surprisingly, expression of *Msx1* in this region appears fairly normal in mutants, although the levels of expression in some embryos seemed to be reduced (Figure 4A). This suggests either that other factors alone or in combination with BMPs regulate *Msx1* expression in the dorsal midline or that signaling through BMPRIB can still occur in the midline despite an expression level that is too low to be detected by RNA in situ hybridization (Figure 2A).

The part of the telencephalic dorsal midline that separates the hippocampal primordium from the most medial tissue, that of the choroid plexus primordium, has been called the cortical hem (Grove et al., 1998). This region expresses members of the *Wnt* family of secreted molecules (Grove et al., 1998). We examined *Wnt2b* expression in E11.5 and E12.5 *Bmpr1a*-deficient telencephalons and found that its pattern is normal compared with controls (Figure 4C and 4D), indicating that the formation of the cortical hem does not require *Bmpr1a*.

Although analysis of *Wnt2b* expression at E12.5 did not reveal obvious differences between control and mutant embryos, Figure 4 does reveal a striking morphological difference in the dorsal midline of mutant E12.5 embryos. In control animals, the choroid plexus (the most medial telencephalic structure) has begun to differentiate from a thick pseudostratified into a thin cuboidal epithelium (Figure 4D, arrowhead). In contrast, all of the mutant embryos examined exhibit few or none of the morphological changes associated with normal choroid plexus development (Figure 4D, arrowhead). We therefore examined embryos at E12.5 for expression of *transthyretin* (*Ttr*), a gene encoding a thyroxine transport protein that is expressed in choroid plexus epithelial cells from the earliest stage these cells can be distinguished (Thomas et al., 1988). *Ttr* expression is greatly reduced

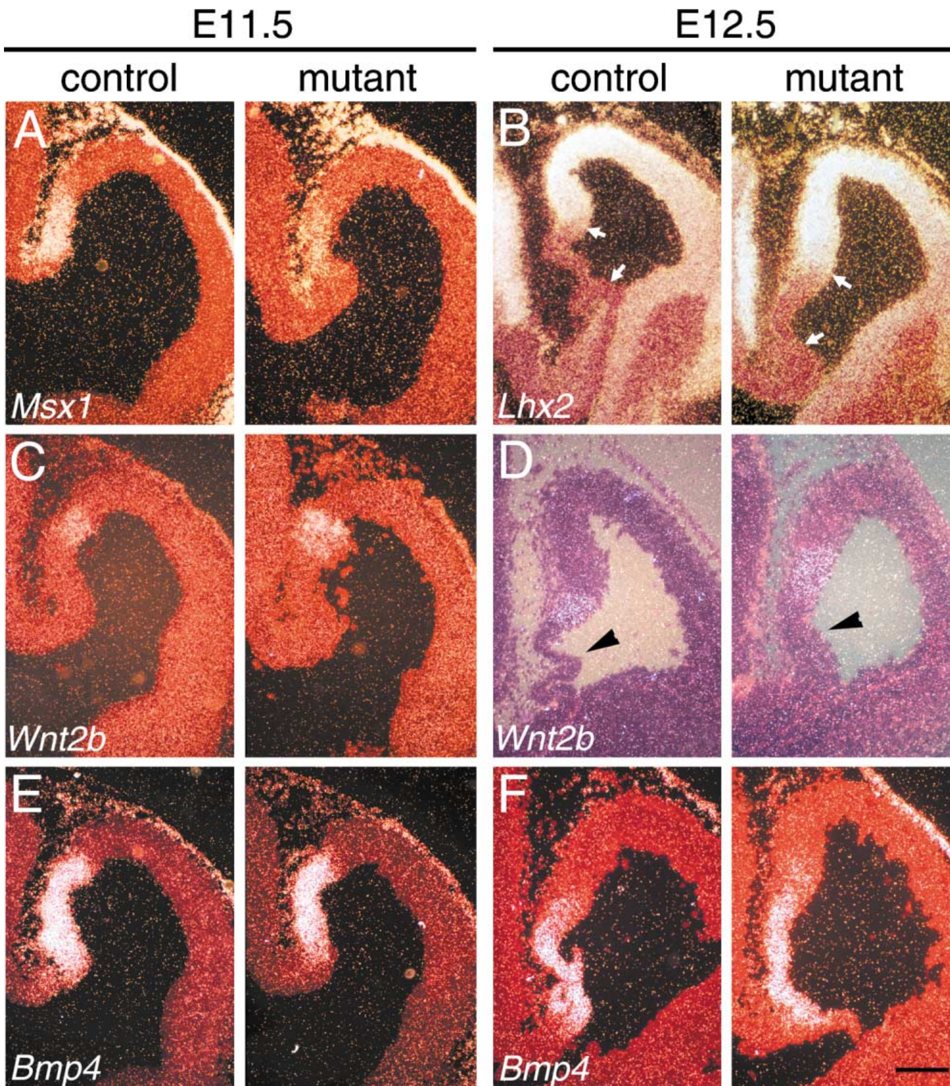

Figure 4. Patterns of Gene Expression that Distinguish Medial and Lateral Telencephalon Are Normal in *Bmpr1a*-Deficient Mutants

Each panel shows in situ hybridization results from the telencephalon, focusing on the dorsal midline. (A, C, and E) E11.5 embryos. (B, D, and F) E12.5 embryos.

(A) *Msx1* expression defines a spatial domain that encompasses the cortical hem and the future choroid plexus. The domain of expression is similar in control and mutant embryos.

(B) *Lhx2* is expressed in the telencephalon lateral to the cortical hem but is excluded from the hem and choroid plexus in both control and mutant embryos. The arrows mark the dorsal midline region that includes the cortical hem and the area that normally gives rise to the choroid plexus.

(C) *Wnt2b* is expressed within the cortical hem region of control embryos at E11.5. Mutant embryos show a similar pattern of expression, suggesting that cortical hem induction has occurred.

(D) *Wnt2b* expression is similar between control and mutant embryos at E12. In mutant embryos, however, the spatial domain in which the choroid plexus normally develops (marked by arrowheads) has failed to undergo the morphological changes associated with the differentiation of cells in this region.

(E and F) *Bmp4* expression encompasses the cortical hem and the choroid plexus region in control embryos. The domain of *Bmp4* expression is similar in the mutant telencephalon. Scale bar, 250 μm.

in all mutants (Figure 5B) and in some cases is absent. Later in development, the choroid plexus normally extends from the telencephalic/diencephalic boundary into the lateral ventricles (Figure 5C, arrowhead). However, in the only mutant E16.5 embryo recovered without any signs of necrosis (Table 1), careful examination revealed the complete absence of the choroid plexus (Figure 5C). In addition, the lateral ventricles were collapsed, consistent with a lack of functional choroid plexus, which normally secretes cerebrospinal fluid into the lateral ventricles. Thus, *Bmpr1a* is required for the choroid plexus to form.

To address the question of whether the choroid plexus ever begins to form in the mutant telencephalon, we assessed the expression of *Ttr* at E11.5, when the choroid plexus is just beginning to be morphologically dis-

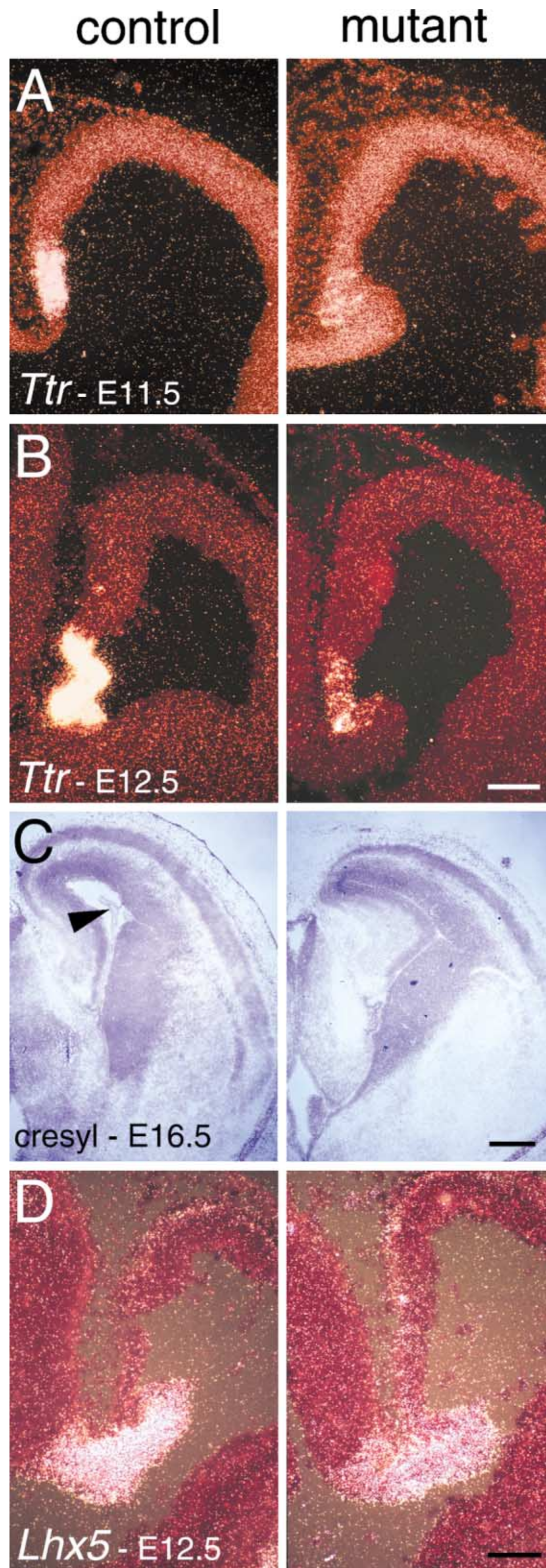

Figure 5. The Choroid Plexus Fails to Form in the *Bmpr1a*-Deficient Telencephalon

Each panel shows in situ hybridization results from the dorsal telencephalon.

(A and B) *Ttr* expression marks the differentiation of cells in the choroid plexus. Expression is greatly diminished in the mutant telencephalon at both E11.5 (A) and E12.5 (B).

(C) Cresyl violet-stained sections through the telencephalon at E16.5. In control animals, the choroid plexus (arrowhead) is highly differentiated and forms an epithelial sheet that extends into the lateral ventricle. In the telencephalon of the single *Bmpr1a* mutant that survived until E16.5 without signs of necrosis, the choroid plexus is completely absent and the lateral ventricles have collapsed.

(D) *Lhx5* expression defines the eminentia thalami, which physically link the telencephalic choroid plexus to the diencephalon. *Lhx5* expression is normal in mutant brains, suggesting that cells normally fated to become choroid plexus do not differentiate into eminentia thalamic cells. Scale bar, 250 μm., except in (C) where it is 0.5 mm.

tinguishable (Sturrock, 1979; Zaki, 1981). In E11.5 control brains, *Ttr* expression is detected throughout the primordium of the choroid plexus (Figure 5A), whereas in mutant embryos, expression is confined to a few scattered cells (Figure 5A). These results suggest that *Bmpr1a* is required for the initial formation of the choroid plexus.

We suspect that the presence of scattered cells that express *Ttr* in the dorsal telencephalic midline of mutant embryos is due to incomplete recombination of the *Bmpr1a*^{fx} allele. Previous studies in which Foxg1-Cre were crossed to mice carrying reporter alleles revealed that not all cells in the choroid plexus undergo recombination (Hébert and McConnell, 2000). Our efforts to ascertain whether cells expressing *Ttr* have failed to recombine the *Bmpr1a*^{fx} allele using in situ RNA hybridization analysis of serial sections have failed, probably because *Bmpr1a* is normally downregulated as cells begin to express *Ttr* (data not shown).

Cells that Normally Become Choroid Plexus Remain Partially Specified Progenitors in *Bmpr1a*-Deficient Telencephalons

If BMP signaling patterns the M-L axis of the telencephalon, with the highest concentrations of BMP specifying medial cell fates and lower concentrations specifying more lateral fates, a shift toward more lateral fates may be anticipated in the *Bmpr1a*-deficient telencephalon. During development, the telencephalic cells immediately lateral to the choroid plexus primordium are those of the cortical hem (Grove et al., 1998). At both E11.5 and E12.5, the expression domain of *Wnt2b* in the mutant telencephalon does not expand or shift significantly to cover the area normally destined to become choroid plexus (Figures 4C and 4D). This suggests that cells normally destined to form choroid plexus do not adopt a more lateral fate in the absence of *Bmpr1a*.

The telencephalic choroid plexus is physically linked to the diencephalon via the eminentia thalami, which express *Lhx5*. To ascertain whether cells that are normally destined to form choroid plexus instead become eminentia thalami in *Bmpr1a*-deficient telencephalon, we examined the expression of *Lhx5*. The border of *Lhx5* expression in mutants does not shift to include the cells normally destined to form choroid plexus (Figure 5D), indicating that the mutant telencephalic cells do not adopt a diencephalic fate. Thus, in the absence of *Bmpr1a*, cells normally destined to form choroid plexus neither become choroid plexus nor seem to adopt a neighboring cell fate. This is not to say that *Bmpr1a*-

deficient cells are completely unspecified, since they do express genes whose expression normally marks the dorsal midline, including the cortical hem and presumptive choroid plexus. For example, cells that would normally become choroid plexus still express *Msx1*, *Bmp4*, and *Bmp2* in E11.5 and E12.5 *Bmpr1a*-deficient telencephalons (Figures 4A, 4E, and 4F, and data not shown). Hence in the mutants, cells normally fated to become choroid plexus have at least been specified as dorsal midline cells, but do not appear to have been further specified to one particular fate.

Normally, as the choroid plexus matures into a thin cuboidal epithelium, its cells become postmitotic. Addition of BMP ligands to telencephalic cells has previously been shown to inhibit the proliferation of cortical progenitor cells (Furuta et al., 1997; Li et al., 1998; Mehler et al., 2000), suggesting that this may be a role for BMP signaling in vivo. In the E12.5 *Bmpr1a*-deficient telencephalon, this region remains thick (Figures 4B, 4D, and 4F). To ascertain whether mutant cells in this region become postmitotic or remain proliferative, E12.5 embryos were exposed to BrdU in utero for 1 hr before being collected and then analyzed in situ for BrdU incorporation. The percentage of BrdU-positive cells in the area that would have normally given rise to choroid plexus remains high in mutants (40.3% $\pm$ 8.3% standard deviation, compared with 11.7% $\pm$ 9.3% in controls; Figures 6A and 6B). In fact, the percentage of BrdU-positive cells in the mutant choroid plexus area is comparable to that in the cerebral cortex of either mutants or controls (42.4% $\pm$ 3.8% and 40.4% $\pm$ 5.6%, respectively; Figures 6C, 6D, and 6I). Hence, in the *Bmpr1a*-deficient telencephalon, the region that normally becomes choroid plexus remains in a proliferative, partially specified state.

The Role of *Bmpr1a* in Telencephalic Apoptosis and Neurogenesis

The dorsal telencephalic midline has a high rate of apoptosis compared to all other telencephalic regions. BMP signaling may be responsible for inducing apoptosis in the dorsal midline, since BMP-soaked beads placed on lateral telencephalic explants in culture induce higher rates of apoptosis compared with controls (Furuta et al., 1997). Moreover, addition of BMPs to cultured cortical cells promotes cell death (Mehler et al., 2000). To assess the role of *Bmpr1a* in promoting dorsal midline apoptosis in vivo, we examined control and *Bmpr1a*-deficient E12.5 telencephalons for cell death using TUNEL staining. No significant difference in the rates of cell death could be observed in either the choroid plexus region or the cerebral cortex in mutants versus controls (Figures 6E–6H). The percentage of TUNEL-positive cells in the choroid plexus region was 0.3% $\pm$ 0.5% for controls and 2.3% $\pm$ 3.5% for mutant brains; whereas in the cerebral cortex, 3.0% $\pm$ 2.4% of cells were TUNEL positive in controls and 1.9% $\pm$ 1.0% in mutants. These results indicate that *Bmpr1a* on its own is not required to promote apoptosis in the telencephalon.

Finally, previous studies have suggested that BMP signaling regulates neurogenesis in the cerebral cortex. Addition of BMP to cortical cell cultures promoted the differentiation of neurons (Li et al., 1998; Mehler et al., 2000) and expression of a dominant-negative BMP receptor in E14 cortical explants by retrovirus-mediated gene transfer blocked neurogenesis (Li et al., 1998). To test the effect on neurogenesis of deleting *Bmpr1a* in the telencephalon, we examined embryos for expression of *Tbr1*, a gene expressed in postmitotic telencephalic neurons (Bulfone et al., 1995), No difference in the levels or patterns of *Tbr1* expression is observed between control and mutant E12.5 telencephalons (Figure 3E). In addition, staining of E16.5 embryos with the TuJ1 antibody, a marker for neurons, showed no difference between mutant and control brains (data not shown). Therefore, signaling through *Bmpr1a* is not required for the production or differentiation of telencephalic neurons. Consistent with this finding, rates of cell proliferation in the telencephalon outside of the dorsal midline are similar in control and mutant embryos at E12.5 (Figures 6C, 6D, and 6I), as well as in a control and a mutant embryo at E16.5 (data not shown).

Discussion

In this report, we demonstrate an essential role for BMP signaling in the forebrain. By crossing Foxg1-Cre mice to mice that carry a *Bmpr1a* allele in which the essential exon 2 is flanked by *lox* sites, we generated embryos in which the telencephalon lacks a functional copy of *Bmpr1a*. In these mutants, the choroid plexus is greatly reduced or fails to form, as evidenced by a diminished expression of *Ttr* and by a lack of morphological differentiation of the most dorsal telencephalic midline cells from a pseudostratified epithelium into a cuboidal one. In addition, we show that without *Bmpr1a*, the cells that normally become choroid plexus remain proliferative and are specified to some extent as dorsal midline cells since they express midline markers such as *Msx1* and *Bmp4*, but that they do not acquire a more specified or differentiated fate. The rest of the telencephalon appears normal in its patterning, proliferation, and neurogenesis.

The Role of BMP Signaling in Patterning the Telencephalon

The patterning of developing tissues by BMPs and related factors is likely to be a widely utilized process in many organisms. For example, compelling evidence indicates that DPP, a BMP-related factor, acts as a diffusible morphogen to pattern the wing imaginal disc in flies (reviewed by Podos and Ferguson, 1999). In mammalian CNS development, BMPs have not yet been demonstrated to act as classical morphogens to specify cell fates in a concentration-dependent manner, although a secreted signaling molecule related to the BMPs, GDF7, is required for the generation of D1A neurons in the dorsal spinal cord (Lee et al., 1998). In the forebrain, at least five *Bmp* genes are expressed in the dorsal midline, a region that could act as a signaling center (Furuta et al., 1997). BMP signaling has been hypothesized to pattern the M-L axis of the dorsal telencephalon where it could act in one of two ways. The first is by specifying cell fates in a concentration-dependent manner throughout the dorsal telencephalon, and the second is by locally patterning only the dorsal midline itself. The results

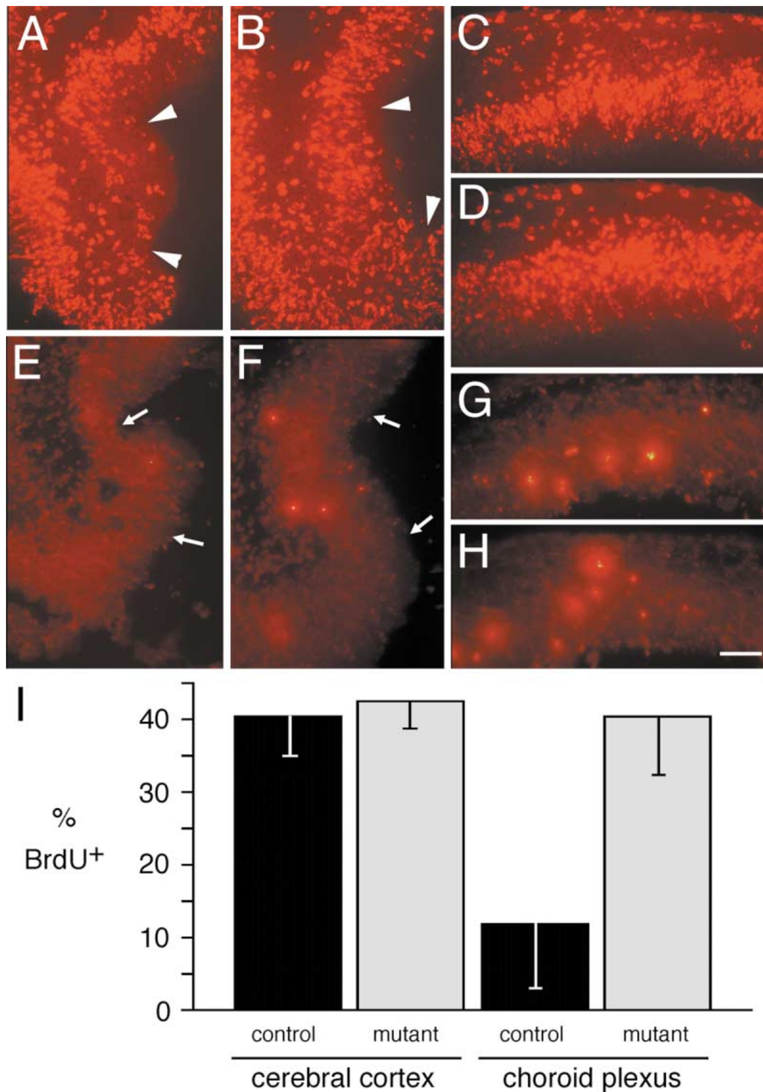

Figure 6. Cells in the *Bmpr1a*-Deficient Telencephalon that Would Normally Become Choroid Plexus Remain as Proliferative Cells

(A–D) BrdU incorporation in E12.5 telencephalons. In control embryos, cells in the differentiating choroid plexus become postmitotic ([A], arrowheads), whereas in mutants, cells in this region continue to incorporate BrdU ([B], arrowheads). BrdU incorporation in control (C) and mutant (D) cerebral cortex is similar.

(E–H) TUNEL staining in E12.5 telencephalons. The number of cells staining positive for TUNEL is similar in the choroid plexus region of control ([E], arrows) and mutant ([F], arrows) embryos, as well as in the cerebral cortex of control (G) and mutant (H) embryos.

(I) Quantitative analysis of BrdU incorporation in the spatial domain of the lateral cerebral cortex and choroid plexus region of control and mutant brains at E12.5. Six separate fields were counted for each region (two fields from each of three separate embryos). Roughly 40% of cells in the ventricular zone of the cerebral cortex have incorporated BrdU when the embryos are sacrificed 1 hr after injection. The levels of BrdU incorporation in the choroid plexus region of control embryos is markedly lower (roughly 12%). In the mutant, cells in the region that would normally produce the choroid plexus have continued to incorporate BrdU at levels characteristic of neighboring cortical tissue. Error bars show standard deviations. Scale bar, 100 μm for (A)–(H).

presented here support the second model by demonstrating an essential role for BMP signaling in patterning the dorsal midline but fail to support a more global, concentration-dependent role in specifying more lateral telencephalic cell fates.

Previous reports presented evidence suggesting that BMPs can act in a concentration-dependent manner to regulate gene expression and specify cell fates. Regulation of the expression pattern of the LIM homeodomain gene *Lhx2* is important in telencephalic development because this gene specifies the fates of dorsal telencephalic cells outside of the midline region; loss of *Lhx2* leads to a transformation of most of the lateral telencephalic cells into dorsal midline cell types, specifically choroid plexus and cortical hem (Porter et al., 1997; Bulchand et al., 2001; Monuki et al., 2001). *Lhx2* is normally expressed throughout the lateral telencephalon, with lower levels of expression in the most lateral regions and increasing levels of expression going toward the dorsal midline (Monuki et al., 2001; Figure 4B). However, at the dorsal midline itself, *Lhx2* expression forms a sharp boundary and is excluded from this region. In explant cultures BMP2 and BMP4 were shown to regulate expression of *Lhx2*, with high concentrations repressing it and lower concentrations promoting it, providing a possible explanation for why *Lhx2* expression in vivo might be excluded from the midline and be expressed at its highest levels immediately adjacent to the midline (Monuki et al., 2001; Figure 7A).

However, the data presented here do not substantiate a model in which specific levels of BMP signaling regulate expression of *Lhx2* or the fate of cells outside of the dorsal midline. A prediction of this model is that lowering the level of BMP signaling in the telencephalon should cause a medial shift in the peak of *Lhx2* expression, normally found immediately lateral to the midline. Despite having significantly reduced the responsiveness of lateral telencephalic cells to BMP4 (Figure 2B), no shift in the peak of *Lhx2* expression is observed. This is consistent with the lack of shift observed in the medial expression boundaries of three other genes, *Ngn2*, *Emx1*, and *Foxg1* (Figures 3C and 3D, and data not shown). Another prediction of the model depicted in Figure 7A is that loss of BMP signaling in the dorsal

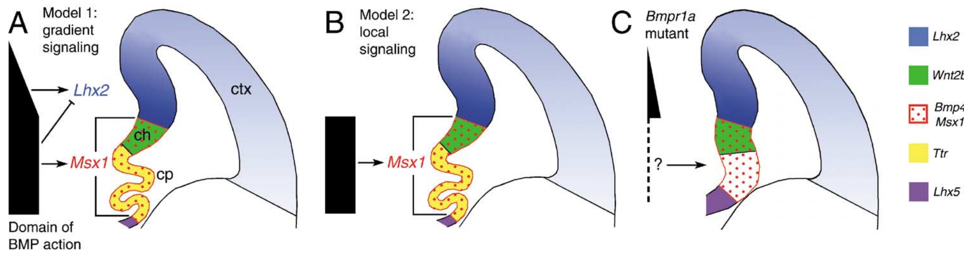

Figure 7. Global versus Local Patterning of the Telencephalon by BMP Signaling

(A) Model in which BMP signaling not only patterns the dorsal telencephalic midline (red dots), but also patterns more lateral cells. In this model, BMPs act in a concentration-dependent manner to promote *Msx1* expression, inhibit *Lhx2* expression in the midline, and promote high levels of *Lhx2* expression (dark blue) adjacent to the midline.

(B) Model in which BMP signaling acts locally to pattern the dorsal midline by promoting expression of *Msx1* and formation of the choroid plexus.

(C) In the *Bmpr1a*-deficient telencephalon, the choroid plexus fails to form and the peak of *Lhx2* expression adjacent to the midline is unaffected, consistent with a local role for BMP signaling. Surprisingly, *Msx1* is expressed in its normal spatial domain, albeit at somewhat lower levels compared to wild-type animals. Abbreviations: ch, cortical hem; cp, choroid plexus; ctx, cerebral cortex.

midline should lead to a derepression of *Lhx2* expression in this region. In the *Bmpr1a*-deficient telencephalon, *Bmpr1a* is deleted from the dorsal midline and *Bmpr1b*, the only other type I *Bmpr* gene, is not expressed in this region at detectable levels (Figure 2A), suggesting that BMP signaling has been effectively lost in this area. Despite this, *Lhx2* expression remains absent from the midline of mutants. *Foxg1* expression, which is also repressed by BMP signaling in vitro (Furuta et al., 1997), is similarly unaffected in the midline (Figure 3C). Our results suggest either that BMP signaling does not regulate the expression of *Lhx2* and *Foxg1* in the midline or that other medially expressed factors, such as WNTs, can also repress expression of these genes.

The data presented here are more consistent with a local role for BMP signaling in patterning the telencephalon (Figure 7B) in which BMPs act to specify only fates of cells within the dorsal midline. Other areas of the telencephalon appear to be specified by other factors, acting either independently or through a signaling cascade that does not rely on a specific concentration or amount of BMP signal. In the dorsal midline of *Bmpr1a*-deficient embryos, loss of BMP signaling leads to loss of the most dorsal-medial cell type, the choroid plexus (Figure 7C). Instead of differentiating into a cuboidal epithelium that expresses *Ttr*, the cells in this area remain in a proliferative state as partially specified midline cells. Mutant animals show a dramatic difference in the morphology of the midline, which remains a thick pseudostratified epithelium similar to that normally seen in neighboring regions. Our results do not distinguish directly between a role for BMP signaling in specifying the choroid plexus primordium, in differentiating an already specified primordium, or both. However, previous evidence has shown that expression of a constitutively active *Bmpr1a* transgene under the control of the nestin neural enhancer leads to a transformation of lateral telencephalon into choroid plexus (Panchision et al., 2001). This study indicates that lateral telencephalic cells have the potential to become choroid plexus and suggests that BMPRIA activation does more than promote choroid plexus differentiation, but that in fact it induces and specifies this tissue. The results presented here support this model in revealing that BMP signaling through BMPRIA is necessary for choroid plexus formation.

Somewhat surprisingly, although the most medial structure fails to form in the *Bmpr1a*-deficient telencephalon, many aspects of dorsal midline patterning do occur normally. In these mutants, *Msx1* and *Bmp4* expression still spans the cortical hem and the region that would give rise to the choroid plexus, although the level of *Msx1* expression may be diminished (Figures 4A, 4E, and 4F). Moreover, the cortical hem itself appears normal, as assessed by expression of *Wnt2b* (Figures 4C and 4D). Two nonmutually exclusive explanations can account for these findings. One is that patterning of the dorsal midline also involves signaling through some other pathway that does not involve *Bmpr1a*. Another possibility is that some patterning of the dorsal midline in the experiments described here has occurred prior to Cre-mediated deletion of *Bmpr1a*. The latter possibility is difficult to exclude because patterning events that can influence formation of the dorsal midline can in principle occur even before a forebrain primordium is morphologically distinguishable. In fact, patterning is likely to be a continuous process that spans the stages of neural induction, which occurs shortly after gastrulation, through the formation of the mature forebrain. *Bmp* gene expression is first detected in the anterior embryonic ectoderm at E8.5, prior to neural tube closure (Furuta et al., 1997; Shimamura and Rubenstein, 1997). The anterior neural plate is responsive to BMPs in culture, suggesting that BMPs may begin to pattern the forebrain even before neural tube closure (Shimamura and Rubenstein, 1997). Nevertheless, here we show that Cre-mediated recombination of *Bmpr1a* occurs prior to several important aspects of dorsal midline development. Recombination of *Bmpr1a* in the anterior prosencephalon is complete by E10 (Figure 1D) and is likely to have occurred efficiently even before this time since recombination of floxed reporter alleles starts as early as E8.5 in the anterior neural plate and neural ridge in Foxg1-Cre mice (Hébert and McConnell, 2000). At E10, when *Bmpr1a* has been completely deleted, the dorsal midline has not yet begun to invaginate or to express high levels of *Msx1* (Furuta et al., 1997), sug-

gesting, at the very least, that many important events of dorsal midline patterning have not yet occurred in the *Bmpr1a*-deficient telencephalon.

The Roles of Different BMP Ligand and Receptor Family Members in Telencephalic Development

The precise role of each BMP ligand and receptor in telencephalic patterning and development remains to be fully elucidated. The targeted disruption of individual *Bmp* genes expressed in the dorsal midline has so far been uninformative as to their role, if any, in telencephalic patterning. This is due either to early embryonic lethality, as for *Bmp4* and *Bmp2* mutants (Winnier et al., 1995; Zhang and Bradley, 1996), or to a lack of a forebrain phenotype due to possible functional overlap, as for mutations in *Bmp5*, *6*, or *7* (Kingsley et al., 1992; Dudley et al., 1995; Luo et al., 1995; Solloway et al., 1998). There is good evidence for both functional overlap and differences in function for each BMP ligand. Embryos deficient for either *Bmp5* or *Bmp7* are viable, whereas loss of both *Bmp5* and *Bmp7* leads to early lethality and demonstrates functional overlap for these two ligands (Solloway and Robertson, 1999). On the other hand, in explant cultures, different ligands have distinct properties: BMP2 and 4, but not 6 and 7, can induce midline properties (Furuta et al., 1997) and BMP2 and 4, but not 6, can regulate *Lhx2* expression (Monuki et al., 2001).

Both of the known type I BMP receptors are expressed in the telencephalon. *Bmpr1a* is expressed throughout the telencephalic ventricular zone (Figure 1E), whereas *Bmpr1b* is expressed more laterally and in a countergradient to the ligands (Figure 2A). Based on experiments in which activated and dominant-negative forms of these two receptors were overexpressed, it was concluded that *Bmpr1a* and *Bmpr1b* have distinct functions in the telencephalon. Namely, BMPRIA activation can respecify lateral cells to adopt medial fates, is required to induce expression of *Bmpr1b*, and promotes proliferation of neural progenitor cells, whereas BMPRIB inhibits progenitor cell proliferation (Panchision et al., 2001). Consistent with the finding that BMPRIA activation leads to a transformation of the lateral telencephalon into the most dorsal-medial cell type, the choroid plexus, we find that loss of *Bmpr1a* leads to the opposite phenotype. However, our data do not support the hypothesis that *Bmpr1a* promotes proliferation of neural progenitor cells and is required for *Bmpr1b* expression. On the contrary, the data presented here demonstrates that *Bmpr1a* is required to reduce proliferation of telencephalic precursor cells, at least in the dorsal midline (Figure 6), and that *Bmpr1a* is not required for normal *Bmpr1b* expression. Furthermore, mice deficient for *Bmpr1b* show no gross defects in the forebrain (Yi et al., 2000), so it is likely that either *Bmpr1b* plays no role in telencephalic development or that *Bmpr1a* can compensate for loss of *Bmpr1b*. Consistent with a functional overlap between *Bmpr1a* and *Bmpr1b*, we find that neural precursor cells deficient for *Bmpr1a* can still respond to BMP4 in culture, presumably through BMPRIB. It is also possible in principle that BMP signaling might occur through more distantly related members of the type I TGFβ receptor family, such as ACTRIA.

In the telencephalon of older embryos, BMP signaling has been proposed to inhibit proliferation of cerebral cortex progenitor cells and to promote their apoptosis or differentiation into neurons. In E14.5 cortical explant cultures, expression of a dominant-negative *Bmpr1a* in progenitor cells by retroviral delivery inhibited neurogenesis (Li et al., 1998). In addition, BMP added to either cultured cortical explants or dissociated cortical cells inhibited proliferation and promoted apoptosis or differentiation into neurons or glia (Li et al., 1998; Mehler et al., 2000). BMPs were also shown to inhibit the production of oligodendrocytes (Mehler et al., 2000). Together these results suggest that subsequent to general M-L patterning, BMP signaling may be required to regulate lineage decisions in the cerebral cortex. Unfortunately, Foxg1-Cre;Bmpr1a$^{fx/null}$ mutants die prior to gliogenesis and the generation of oligodendrocytes. The results presented here therefore do not address the role of BMP signaling in cortical lineage diversification. *Bmpr1a*-deficient mice do, however, survive long enough to undergo neurogenesis. If BMP signaling plays a role in promoting neurogenesis as has been proposed, then it is likely that *Bmpr1a* and *Bmpr1b* can compensate for each other, since loss of either on its own has no effect on this process. In the *Bmpr1a*-deficient telencephalon, proliferation, neurogenesis, and cell death appear normal at E12.5 (Figures 3E and 6) and E16.5 (data not shown). These processes are also grossly normal in the *Bmpr1b*-deficient embryo (Yi et al., 2000). Therefore, it may be necessary to abolish expression of both *Bmpr1a* and *Bmpr1b* to test the role of BMP signaling in telencephalic neurogenesis.

Experimental Procedures

Maintenance of Mouse Lines

Foxg1-Cre and floxed-Bmpr1a mice were maintained in a mixed 129SvJ:C57BL/6 (~75:25) background. Mice were genotyped as described (Hébert and McConnell, 2000; Mishina et al., 2002). Because Foxg1-Cre mice were generated by targeting *Cre* to the *Foxg1*-locus, creating a *Foxg1* null allele, only heterozygous Foxg1-Cre mice are used in the experiments described here. Although mice that are heterozygous for *Foxg1* show no phenotype on their own (Xuan et al., 1995; this report), it should be noted that the severity of the phenotypes observed with loss of *Bmpr1a* could in theory be affected by loss of one functional copy of *Foxg1*.

RNA In Situ Hybridization Analysis

Frozen sections were prepared and hybridized as previously described (Frantz et al., 1994). A minimum of three mutant and three control embryos were analyzed for each probe at each age. Plasmids used to make probes were kindly provided by William Blaner (*Ttr*), Elizabeth Grove (*Wnt2b*), John Rubenstein (*Dlx2*, *Nkx2.1*, *Tbr1*), Heiner Westphal, (*Lhx5*), Juan Botas (*Lhx2*), and Ryan Rountree (*Bmpr1b*).

BrdU and TUNEL Analysis

Females pregnant with E12.5 or E16.5 embryos received an intraperitoneal injection with BrdU and were euthanized 1 hr later. Embryos were collected and frozen in OCT. Fresh frozen sections were used for either BrdU staining, as previously described (O'Rourke et al., 1997), or for TUNEL analysis according to the manufacturer's specifications (Roche, Cat. # 2 156 792). Sections were counterstained with Syto11 (Molecular Probes). The fraction of BrdU- or TUNEL-positive cells was determined by counting the number of these cells in a radial segment of choroid plexus or cerebral cortex spanning from the ventricular surface to the pial surface and dividing by the total number of cells in the segment. The number of cells

per unit area appeared roughly equivalent in mutants versus controls and was therefore not quantified. At least two segments from each of three separate embryos were counted in each case. A higher rate of apoptosis was observed in the lateral and ventral telencephalon of embryos carrying the *Foxg1-Cre* allele (Foxg1-Cre;Bmpr1a$^{fx/+}$ embryos) than of controls not carrying the *Foxg1-Cre* allele (*Bmpr1a*$^{fx/+}$, data not shown). Expression of Cre in mouse cells has previously been reported to increase the frequency of chromosomal abnormalities both in mice (Schmidt et al., 2000) and in cultured cells (Loonstra et al., 2001). In our studies, it appeared that the increased rate of apoptosis due to Cre was not in itself sufficient to cause a phenotype. Only embryos that carried the *Foxg1-Cre* allele were used as controls in the TUNEL and BrdU incorporation assays presented here, and all other experiments presented in this report included Foxg1-Cre;Bmpr1a$^{fx/+}$ amongst the controls.

Msx1 Induction Assay

Lateral cortical tissue was dissected from the brains of control or *Bmpr1a*-deficient embryos at E12.5. Cells were dissociated for 10 min with trypsin EDTA and allowed to recover 5 hr in DMEM supplemented with N2. BMP4 was added at 100 ng/ml to samples for 1 hr before cells were collected for Northern blot analysis. The intensity of Northern blot bands from three separate assays was quantified by measuring the integrated density using NIH Image 1.62. The intensity of bands obtained with *Msx1* was normalized to the intensity of bands obtained with *G3PDH*. Fold induction in levels of *Msx1* expression was calculated by dividing the normalized intensity for *Msx1* bands in (+) BMP4 samples by those in (−) BMP4 samples.

Acknowledgments

We thank Mike Porter and Chris Kaznowski for outstanding technical support, John Rubenstein for thoughtful discussions, and Elizabeth Alcamo and Ami Okada for critically reading the manuscript. We are also grateful to Lubert Stryer and Bill Mobley for indispensable input. This work was supported by NIH grants MH51864 and NS12151 and a McKnight Investigator Award.

Received: December 31, 2001
Revised: June 13, 2002

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