Embryonic signaling centers expressing BMP, WNT and FGF proteins interact to pattern the cerebral cortex

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Summary

Recent findings implicate embryonic signaling centers in patterning the mammalian cerebral cortex. We used mouse in utero electroporation and mutant analysis to test whether cortical signaling sources interact to regulate one another. We identified interactions between the cortical hem, rich in Wingless-Int (WNT) proteins and bone morphogenetic proteins (BMPs), and an anterior telencephalic source of fibroblast growth factors (FGFs).

Expanding the FGF8 domain suppressed *Wnt2b*, *Wnt3a* and *Wnt5a* expression in the hem. Next to the hem, the hippocampus was shrunken, consistent with its dependence for growth on a hem-derived WNT signal. Maintenance of hem WNT signaling and hippocampal development thus require a constraint on the FGF8 source, which is likely to be supplied by BMP activity. When endogenous BMP signaling is inhibited by noggin, robust *Fgf8* expression appears ectopically in the cortical primordium.

Abnormal signaling centers were further investigated in mice lacking the transcription factor EMX2, in which FGF8 activity is increased, WNT expression reduced, and the hippocampus defective. Suggesting that these defects are causally related, sequestering FGF8 in *Emx2* homozygous mutants substantially recovered WNT expression in the hem and partially rescued hippocampal development.

Because noggin can induce *Fgf8* expression, we examined noggin and BMP signaling in the *Emx2* mutant. As the telencephalic vesicle closed, *Nog* expression was expanded and BMP activity reduced, potentially leading to FGF8 upregulation. Our findings point to a cross-regulation of BMP, FGF, and WNT signaling in the early telencephalon, integrated by EMX2, and required for normal cortical development.

Key words: Cortical area map, In utero electroporation, *Emx2* mutant mouse

Introduction

Normal functioning of the mammalian cerebral cortex depends on the partition of the cortical sheet into different types of cortex, and further subdivision into specialized areas (Nauta and Feirtag, 1986). These divisions form a map that is highly similar from one individual to another in the same species, and has common general features across species (Krubitzer, 1995). Considerable current research focuses on identifying the developmental mechanisms that generate this complex, reproducible pattern.

In a classic model of neocortical development (Rakic, 1988), a ‘protomap’ of neocortical areas is set up in the cortical neuroepithelium. More recent findings suggest how such early cortical pattern might be achieved. It has been proposed that signaling centers in and around the cortical neuroepithelium secrete members of a select set of powerful embryonic signaling molecule families. These signaling molecules confer positional information and regulate regional growth (Assimacopoulos et al., 2003; Garel et al., 2003; Grove and Fukuchi-Shimogori, 2003; O’Leary and Nakagawa, 2002; Ohkubo et al., 2002; Ragsdale and Grove, 2001; Rubenstein et al., 1999; Shimamura and Rubenstein, 1997).

Two likely signaling centers for the mouse cortical primordium have been investigated in some depth. First, an anterior telencephalic source of FGF proteins, including FGF8 and FGF17 (Bachler and Neubuser, 2001; Crossley et al., 2001; Maruoka et al., 1998), imparts position along the anteroposterior (AP) axis of the developing area map (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003; Garel et al., 2003). If a new FGF8 source is introduced at the posterior pole of the cortical primordium, posterior cortex is induced to take on a more anterior fate (Fukuchi-Shimogori and Grove, 2001). As might be expected from a classic patterning theory (Wolpert, 1996), the two opposing AP signaling sources induce formation of apparently mirror-image partial area duplicates (Fukuchi-Shimogori and Grove, 2001).

The dorsomedial edge of each cerebral cortical hemisphere, which includes the ‘cortical hem’, is a source of BMP and WNT proteins (Furuta et al., 1997; Grove et al., 1998; Lee et al., 2000). Without a canonical WNT signal from the hem, the adjacent hippocampus fails to develop (Galceran et al., 2000; Lee et al., 2000). Furthermore, when telencephalic BMP activity is partially blocked, few choroid plexus epithelial cells (CPe) are generated from the medial neuroepithelium (Hebert et al., 2002). Conversely, continuous activation of BMP signaling transforms the entire cortical primordium into CPe

(Panchision et al., 2001). These findings indicate a potential for medial/lateral (M/L) patterning of the cortical neuroepithelium by signals from the dorsomedial telencephalon.

A devastated cerebral cortex is predicted if cortical signaling centers are too large, too small, or incorrectly positioned. In the present study, we therefore searched for mechanisms that could control the size and position of signaling sources. Classic embryonic signaling centers, studied longer and more fully, include those in the vertebrate limb bud, which organize the major axes of the limb and the size of limb components. These classic signaling centers regulate one another to coordinate limb patterning (Capdevila and Izpisua Belmonte, 2001; Dahn and Fallon, 2000; Niswander, 2002; Riddle et al., 1993; Sun et al., 2002; Wolpert, 1996), suggesting that the recently identified cortical patterning centers may also be controlled by a network of interactions.

More specific guidance comes from studies of dorsal telencephalic signaling centers in non-mammalian vertebrates (Crossley et al., 2001; Ohkubo et al., 2002; Shanmugalingam et al., 2000). Major dorsal telencephalic circuitry is comparable among mammals, reptiles and birds (Karten, 1997; Medina and Reiner, 2000), suggesting homologies at the level of cell type, circuitry and, and in at least some cases, function. Moreover, telencephalic signaling centers identified in mouse and chick are strikingly alike, suggesting a further consistency between the two species in the basic patterning of the telencephalic AP and ML axes.

The chick telencephalon, like that of the mouse, is exposed to an anterior source of FGF8 and a dorsomedial source of BMP proteins, both of which regulate morphological and gene expression patterning (Crossley et al., 2001; Golden et al., 1999; Ohkubo et al., 2002). In addition, *Wnt7b* and *Wnt8b* are expressed in the medial pallium, including a position comparable with that of the mouse cortical hem (Garda et al., 2002). Furthermore, interactions between signaling sources have been analyzed in the chick: *Fgf8* expression is suppressed by BMP signaling, and enhanced by BMP inhibition (Crossley et al., 2001; Ohkubo et al., 2002). Thus, chick studies have already provided evidence of regulation between dorsal telencephalic signaling sources. We sought to extend this investigation to the mouse cerebral cortex.

Materials and methods

In utero micro- and macroelectroporation

At all ages, embryos were visualized by transillumination inside the intact uterus using a fiber optic light source. Expression plasmid DNA was mixed with fast green (Sigma) and injected into the cerebral ventricle via a glass capillary. Two alternative forms of electroporation were employed, depending on the age of the embryos, and differing in the type of electrode. At embryonic day (E) 10 and above, microelectroporation was used as previously described (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003), except that a CUY21 Electroporator (Nepagene) allowed the passage of current to be monitored. At E9.5, the fine electrodes needed for microelectroporation were difficult to introduce into the small embryo forebrain, and macroelectroporation was used instead. After DNA injection, platinum electrodes (Nepagene) were positioned outside the uterus and a series of three square-wave current pulses (30V, 100 mseconds×3) delivered, as before. After electroporation, the surgical incision in the mother was closed and embryos developed in utero with a survival rate of about 60%. As previously, 50% of surviving embryos showed effective electroporation, as indicated by strong expression of a transgene. The site and efficiency of transfection were monitored, whenever possible, by direct visualization of transgene expression. In some cases, this was aided by the co-electroporation of a marker gene encoding enhanced green fluorescent protein (EGFP).

Rescue of the *Emx2* mutant cortex

For rescue experiments, the *Emx2* mutant mouse line was maintained on a mixed CD1/C57BL/J6 background. In comparison with mice on a C57BL/J6 background, the addition of the CD1 outbred strain appeared to increase survival of electroporated embryos. Mice were genotyped as previously (Pellegrini et al., 1996). The neocortical and hippocampal phenotype, judged by morphology and gene expression, did not differ from the *Emx2* mutant maintained on a C57BL/J6 background (Fukuchi-Shimogori and Grove, 2003). To test the hypothesis that excess FGF8/17 reduces WNT expression, leading in turn to hippocampal abnormalities, a construct encoding a truncated FGF8 receptor (see below) was electroporated into the anterior cortical primordium of *Emx2* mutants at E9.5.

Constructs and histology

Expression plasmids carrying cDNA encoding human placental alkaline phosphatase (Ap), mouse FGF8 (isoform b) or the soluble, truncated FGFR receptor 3c (sFGFR3c) were as used previously (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003). *EGFP-N1* (Clontech), chick *Noggin* and human *BMP4* were cloned into the expression vector pEFX (Agarwala et al., 2001). Brains or whole embryos were fixed in 4% paraformaldehyde, and, where needed, sectioned in the coronal plane with a Leica sliding microtome. Section and whole-mount in situ hybridization followed described procedures (Agarwala et al., 2001; Grove et al., 1998).

Results

Excess FGF8 suppresses WNT gene expression in the cortical hem

At E11.5 (Fig. 1A), expression domains of *Wnt3a* and *Fgf8* almost abut along the medial wall of the cortical hemisphere, suggesting a mutual antagonism between WNT3A and FGF8. To test this possibility, we overexpressed *Ap*, *Fgf8* or *Wnt3a* at E11.5, directing the site of transfection to the anterior cerebral hemisphere. Brains were assessed at E13.5. In the electroporated hemisphere only, excess FGF8 regionally suppressed *Wnt3a* (Fig. 1B,C, n=4/4, brains selected from those that showed strong, correctly targeted *Fgf8* expression), *Fgf2b* (Fig. 1E,F, n=6/9) and *Fgf5a* expression (Fig. 1I,J, n=3/7). Control electroporation of *Ap* had no effect (Fig. 1E,I, n=12/12).

Downregulation of WNT genes was not due to general tissue deterioration caused by excess FGF8, or by electroporation itself. The hem appeared morphologically normal as a characteristically translucent band of tissue. *Foxg1* expression, which marks the boundary between the cortical hem and the rest of the cortical neuroepithelium, was unchanged after electroporation with *Fgf8* (Fig. 1K,L), as was *Zic3* expression, which appeared in its normal anterior telencephalic domain, near the standard anterior site of electroporation (n=4/4 for *Ap* and 5/5 for *Fgf8*).

Electroporated *Fgf8* had no observable effect on expression of *Bmp4* in the hem region (Fig. 1D), on expression of *Bmp6* or *Bmp7* (data not shown), or on BMP activity assessed by expression of *Msx1*, a direct reporter of BMP2 and BMP4

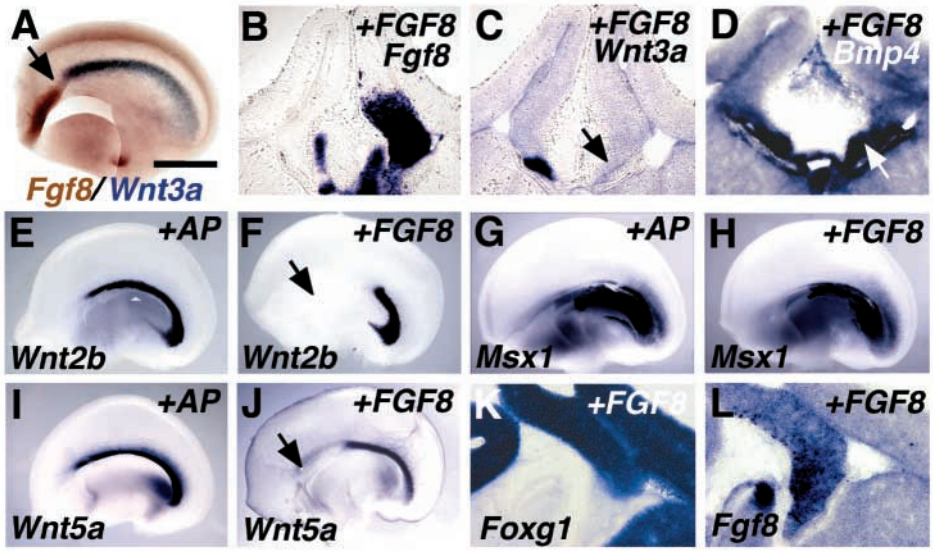

Fig. 1. Excess anterior FGF8 downregulates WNT gene expression in the hem. (A) E11.5 cerebral hemisphere viewed from the medial face, anterior towards the left, processed for two color in situ hybridization. The cortical hem is marked by *Wnt3a* expression (blue); *Fgf8* expression (brown) is separated from the hem by a short gap (arrow). (B-L) Brains electroporated anteriorly at E11.5 with *Ap* or *Fgf8* and analyzed at E13.5. B-D and K,L are coronal sections; E-J are hemispheres viewed from the medial face. (B,C) In adjacent sections from the same brain, ectopic expression of FGF8 (B) obliterates *Wnt3a* expression in the hem (C, black arrow indicates normal *Wnt3a* expression site). (Note endogenous *Fgf8* expression ventrally in B.) *Wnt2b* expression fills the cortical hem of an *Ap* electroporated brain (E) but is lost anteriorly in a brain electroporated with *Fgf8* (arrow, F). *Wnt5a* shows a similar loss of expression (I, arrow in J). In contrast to WNT gene expression, *Bmp4* expression is not downregulated at this age by excess FGF8 (D, white arrow indicates site of excess FGF8), nor is expression of *Msx1*, a indicator of BMP activity (G,H). Overexpression of FGF8 (L) has no apparent effect on *Foxg1* expression at this age, indicating that the experimental protocol does not cause general damage. The boundary between the hem and the rest of the cortical neuroepithelium not obviously affected (K). Scale bar: 0.8 mm for A,E-J; 0.17 mm for B-D; 0.12 mm for K,L.

activity (Fig. 1G,H, *n*=3/3 for *Ap* and 5/5 for *Fgf8*). Reciprocal effects of WNT3A on *Fgf8* expression were also not detected. However, electroporation of *Wnt3a* at E10.5 or 11.5 promoted general growth of the cortical hemisphere (Fukuchi-Shimogori and Grove, 2001) (data not shown).

Excess FGF8 produces an abnormal hippocampus

Hippocampal development requires a WNT signal from the cortical hem (Galceran et al., 2000; Lee et al., 2000). We therefore examined the hippocampus at E15.5 in brains electroporated with *Ap* or *Fgf8*. Substantial expression of the *Ap* transgene (Fig. 2B,H) did not affect the expression patterns of *Wnt3a*, *Ka1*, an early marker of hippocampal field CA3 (Grove et al., 1998; Tole et al., 1997), *Prox1*, an early marker of the dentate gyrus (DG) (Pleasure et al., 2000), or *Wnt5a*, which is expressed in the hem and the distal hippocampus (Grove et al., 1998) (Fig. 2A,C,G-I). By contrast, brains efficiently electroporated with *Fgf8* showed little or no expression of *Wnt3a* or *Wnt5a*, or the hippocampal field markers *Ka1* and *Prox1* (Fig. 2D-F, *n*=6/6 for *Wnt3a* and *Ka1*; and Fig. 2J-L, *n*=5/5 for *Wnt5a* and *Prox1*). Efficiency and site of transfection were checked for all brains by transgene expression. Hippocampal defects in *Fgf8*-electroporated mice therefore correlate with ongoing depletion of WNT signaling at the hem.

Size and position of the endogenous FGF8 source is constrained by time-limited BMP signaling

In chick, *Fgf8* expression in the anterior telencephalon is downregulated by BMP4 and expanded by the BMP inhibitor noggin (Ohkubo et al., 2002). In mice lacking chordin (*Chrd*) and with only a single copy of *Nog*, BMP activity is increased and *Fgf8* expression is reduced in the anterior neural ridge (ANR) and anterior telencephalon (Anderson et al., 2002). We therefore tested whether inhibiting endogenous BMP signaling in living mouse embryos leads to ectopic expression of *Fgf8* in the cerebral cortical primordium.

Electroporation at E11.5 of either *Bmp4* or *Nog* had little or no effect on *Fgf8* expression. However, unlike WNT gene expression in the hem, which is not fully established until E11.5, an anterior FGF8 source is evident very early in telencephalic development, initially at the interface between ectoderm and neurectoderm, the ANR (Shimamura and Rubenstein, 1997). Thus, although we found that WNT expression in the hem is malleable as late as E11.5, anterior *Fgf8* expression may not be. Consequently, mice were electroporated earlier, at E9.5, with *EGFP* as a control, or *Nog* together with *EGFP*. Embryos were collected 24 hours later and the site of transgene expression was detected by EGFP fluorescence in each brain (Fig. 3 insets). As in previous experiments, about half of the brains showed efficient transfection (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003), which is evident from dense patches of EGFP fluorescence. In co-electroporated brains with efficient transfection of *EGFP*, and presumably also *Nog*, *Fgf8* expression was robustly upregulated. In some cases, noggin overexpression induced patches of ectopic *Fgf8* in the cortical primordium lateral to the midline (Fig. 3B, Fig. 4A, *n*=3). Sections through such a brain showed ectopic *Fgf8* expression extending through the entire thickness of the cortical neuroepithelium (Fig. 4B,C). Normal midline expression of *Fgf8* was also enhanced or extended posteriorly (Fig. 3B, Fig. 4A). Diminished BMP activity after *Nog* electroporation was inferred by downregulated expression of *Msx2* (Fig. 3G,H, *n*=9 for *Ap* and *n*=5 for *Fgf8*) and *Msx1* (data not shown), representing a functional readout of BMP signaling. Consistent with findings above, increased *Fgf8* expression after *Nog* electroporation was accompanied by decreased *Wnt3a* expression in the cortical hem (Fig. 3E, *n*=6). Notably, the effects described were seen in all brains showing efficient transfection. No such effects were observed in brains in which EGFP fluorescence could not be detected, or appeared only in scattered cells; nor were effects seen in brains electroporated with EGFP alone (Fig. 3A,D,G).

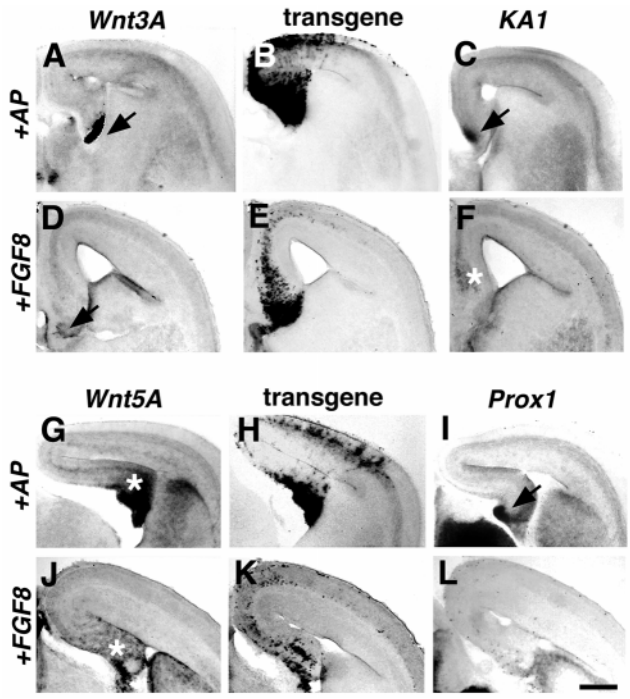

Fig. 2. Anterior overexpression of FGF8 causes prolonged downregulation of WNT expression in the hem, and a defective hippocampus. (A-L) Coronal sections of E15.5 brains processed for in situ hybridization. Medial is towards the left. Brains are electroporated with *Ap* (A-C,G-I) or *Fgf8* (D-F,J-L) at E11.5. Each row illustrates three near-adjacent sections from the same brain. (A-C) A brain electroporated with *Ap* (transgene in B) shows normal expression of *Wnt3a* at the hem remnant (arrow, A), and a dense patch of *Ka1* in the developing hippocampal CA3 field (arrow, C). A brain electroporated with *Fgf8* (E) shows a little *Wnt3a* expression (D), and diffuse *Ka1* expression in the hippocampus (asterisk, F). (G-L) Similarly a brain electroporated with *Ap* displays robust *Wnt5a* expression in the distal region of the hippocampus (G, asterisk), compared with very sparse and diffuse expression in a brain electroporated with *Fgf8* (J, asterisk). *Prox1* marks the developing DG in I, but not in L. Scale bar: 0.25 mm.

Early inhibition of BMP activity in the EMX2 homozygote mutant telencephalon

The patterns of *Fgf8* and WNT gene expression following overexpression of noggin are reminiscent of those reported in mice that lack the transcription factor EMX2 (Fukuchi-Shimogori and Grove, 2003; Muzio et al., 2002). We therefore compared *Nog*-electroporated brains directly with *Emx2* homozygote mutant brains at the same age. At E10.5, the *Emx2* mutant and *Nog*-electroporated wild-type brains showed similarities. The *Fgf8* expression domain was expanded in the *Emx2* mutant, as previously reported (Fukuchi-Shimogori and Grove, 2003), and WNT gene expression in the cortical hem was reduced (Muzio et al., 2002) (Fig. 3C,F; *n*=9 for each of *Fgf8* and *Wnt3a*). Expression of *Msx2* appeared slightly reduced (Fig. 3I, *n*=3), which suggests lower levels of BMP activity. These findings suggest that the *Emx2* mutant brain can profitably be studied, in part, as an illustration of abnormal cortical signaling centers.

At E8.75, in wild-type embryos, *Nog* was expressed at the anterodorsal telencephalic midline, close to the *Fgf8*-expressing anterior pole (Fig. 5A,I). In *Emx2* homozygote mutants, matched to controls for overall development, we observed consistently increased expression of *Nog* (Fig. 5B,J,K,M, *n*=4/4). The anterior to posterior extent of *Nog* expression was roughly three times greater than in wild-type controls (compare Fig. 5B,J,K with A and I), and expression levels appeared increased (Fig. 5L,M).

Bmp4 and *Bmp7* were expressed along the telencephalic dorsal midline without a detectable difference between wild type and mutant (Fig. 5C-F, *n*=3 for each gene in wild type and mutant). However, indicating a difference in BMP activity, *Msx2* expression appeared at the dorsal midline in wild type mice (Fig. 5G) but was depleted in the mutant (Fig. 5H, *n*=3

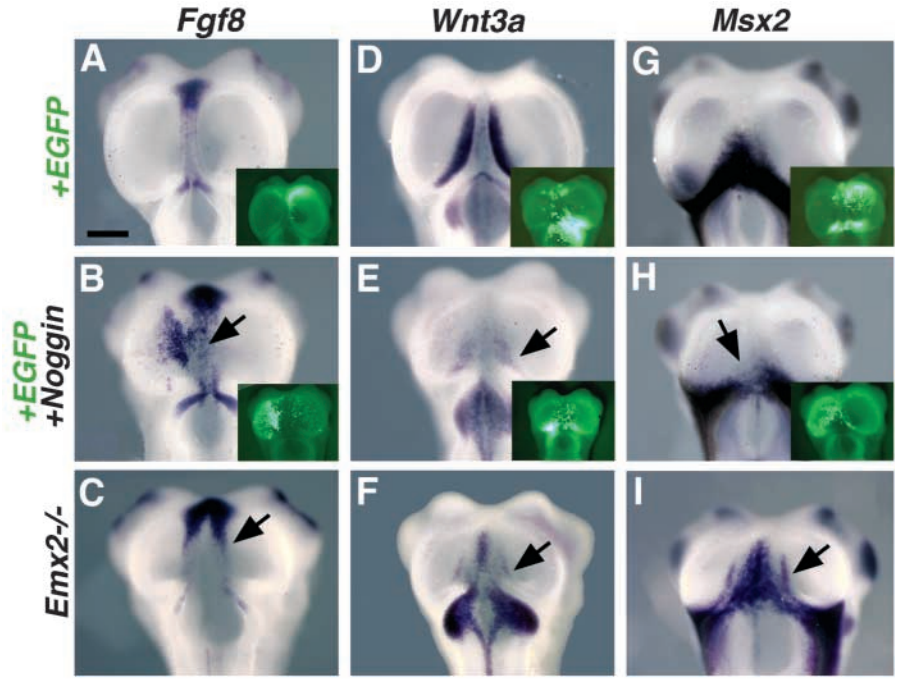

Fig. 3. Similar changes in *Fgf8* and *Wnt3a* expression by BMP inhibition or loss of EMX2. (A-I) E10.5 forebrains viewed dorsally, anterior towards the top, processed for whole-mount in situ hybridization. *EGFP* alone (A,D,G) or *EGFP* and *Nog* together (B,E,H) were electroporated into the telencephalic vesicle at E9.5. EGFP fluorescence indicates the position of the electroporated site (insets). Brains electroporated with *EGFP* alone show wild-type expression patterns of *Fgf8*, *Wnt3a* and *Msx2* (a reporter of BMP activity). Co-electroporation of *Nog* results in inhibition of BMP activity, reflected in decreased *Msx2* expression (arrow in H), increased and ectopic *Fgf8* expression in the cortical primordium (B), and lowered expression of *Wnt3*a in the cortical hem (arrow in E). The forebrains of *Emx2* homozygote mutant mice are not identical to *Nog*-electroporated brains, but, like the latter, show expanded *Fgf8* expression (C, arrow) and reduced expression of *Wnt3a* in the hem (F, arrow). A slight decrease in *Msx2* expression is most evident in the cortical hem (I, arrow). The latter region is buried in a dense mass of *Msx2* expression in control forebrains (G). Scale bar: 0.4 mm.

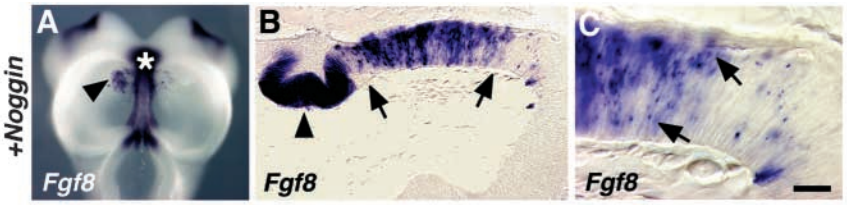

Fig. 4. Noggin induces ectopic *Fgf8* expression throughout the depth of the cortical neuroepithelium. (A) E10.5 forebrain viewed from the dorsal side. Noggin misexpression has induced a patch of ectopic *Fgf8* (arrowhead). In addition, the normal domain of *Fgf8* expression (asterisk) is expanded along the two sides of the dorsal midline. (B) Coronal section through a different cortical hemisphere, processed in the same way. *Fgf8* expression is evident at the midline (arrowhead) posterior to its normal limit. A patch of ectopic expression extends further laterally, filling the width of the neuroepithelium. (C) A higher magnification of *Fgf8* expression in B. Typical of dividing neuroepithelial cells, labeled cell bodies lie near both the pial and ventricular surfaces (arrows in C) and show polarized processes. Scale bar: 0.35 mm for A; 0.1 mm for B; 0.03 mm for C.

wild type and mutant). Reduced BMP activity could explain why the anterior FGF8 domain expands past its normal borders in the *Emx2* mutant. Supporting this possibility, reduced BMP activity at E8.75 in the mutant preceded expansion of *Fgf8* expression, detectable by E9.5 (data not shown). Our data further suggest that reduction of BMP activity in the mutant telencephalic dorsal midline is transient. That is, by E10.5 (Fig. 3I), *Msx2* expression in the *Emx2* mutant appeared only slightly reduced compared with wild type.

Sequestering FGF8 in *Emx2* mutants partially rescues hem WNT expression and hippocampal development

In addition to the defects noted above, the *Emx2* mutant shows dramatic anterior to posterior shifts in neocortical regional pattern (Bishop et al., 2000; Bishop et al., 2002; Mallamaci et al., 2000), similar to those seen after augmentation of the endogenous FGF8 source in wild-type mice (Fukuchi-Shimogori and Grove, 2001). In a previous study, we tested the effect on neocortical patterning of reducing excess FGF8 in the *Emx2* mutant. We found that this reduction partially rescued neocortical patterning (Fukuchi-Shimogori and Grove, 2003). Based on the previous study, and on findings described above, we hypothesized that excess FGF8 also causes decreased WNT expression in the hem of *Emx2* mutants and consequent abnormalities of the hippocampus.

To test this possibility, we reduced FGF8 activity, as before, by electroporation of a truncated FGF receptor. FGFR3c is a high-affinity receptor for FGF8 and for closely related FGF family members such as FGF17, which is also overexpressed in the anterior telencephalon of the *Emx2* mutant (Fukuchi-Shimogori and Grove, 2003). A soluble, truncated form of the receptor, sFGFR3c, is presumed to sequester FGF8 and related FGFs, preventing them from binding to their endogenous receptors (Fukuchi-Shimogori and Grove, 2001; Ye et al., 1998).

The soluble receptor construct, *sFGFR3c*, and EGFP were co-electroporated into the anterior cortical primordium of *Emx2* mutants at E9.5 – the age at which *Wnt3a* expression is initiated at the hem (Lee et al., 2000). Supporting the hypothesis, 4 days after electroporation, *Emx2* mutant brains that were efficiently transfected showed increased expression of *Wnt3a* and *Wnt2b* in the cortical hem (Fig. 6C,F, *n*=8/8). Brains with sparse or no EGFP-fluorescence continued to show diminished expression of both WNT genes (Fig. 6B,E).

A prominent defect in the *Emx2* mutant hippocampus is the absence of a morphologically distinct dentate gyrus (DG) (Pellegrini et al., 1996; Yoshida et al., 1997; Tole et al., 2000a). Given that reducing excess FGF8 in the mutant substantially restored *Wnt3a* in the cortical hem, we examined the DG in the 'rescued' mutant at E18.5. In wild-type mice at this age, *Prox1*-expressing cells were dense in the distal hippocampus, beginning to form the blades of the DG (Fig. 7A,D). By contrast, in homozygote *Emx2* mutants, *Prox1*-expressing cells formed only tiny patches in the distal hippocampus – the presumptive region of the DG (Fig. 7B,E). In addition, abnormal *Prox1* expression appeared along the ventricular surface of the hippocampus (Fig. 7E, asterisk). When homozygote *Emx2* mutants were electroporated with *sFGFR3c* at E9.5, a dense domain of *Prox1*-expressing DG cells was restored, suggesting partial rescue of DG development (Fig. 7C,F, *n*=8/17). A similar partial recovery of expression (Fig.

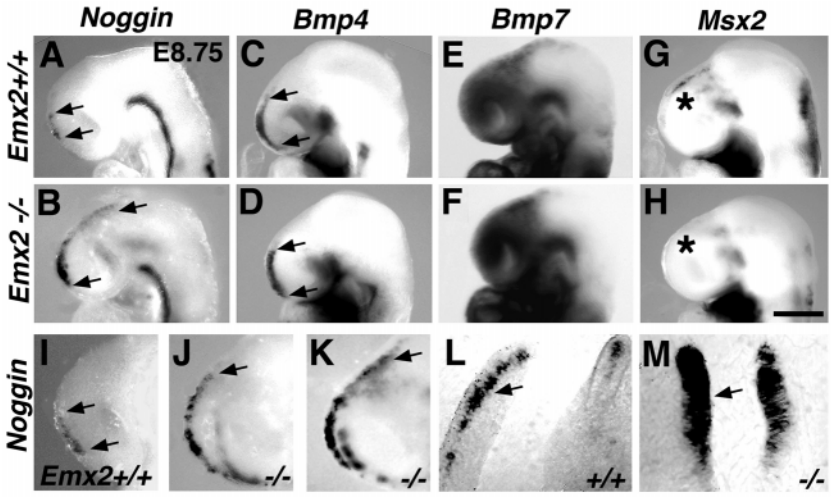

Fig. 5. Decreased BMP activity in the *Emx2* mutant forebrain. (A-K) E8.5 forebrains viewed from the lateral side just after closure of the neural tube, anterior is towards the left. At this stage of telencephalic development in wild-type mice, *Nog* expression appears in a restricted domain at the anterior dorsal midline (A,I, arrows). In three *Emx2* homozygous mutant mice (*Emx2*$^{-/-}$), this domain of *Nog* expression extends about three times as far posteriorly along the midline (B,J,K). In addition, *Nog* expression appears stronger than in wild type (arrows in L,M). Mice represented in A and B, I and J, and L and M are paired littermates. No detectable differences were seen in *Bmp4* or*7* expression (C-F), but BMP activity appears deficient in the *Emx2* mutant, assessed by the loss of *Msx2* expression (G,H, asterisks). Scale bar: 0.3 mm for A-H; 0.17 mm for I-J; 0.06 mm for L,M.

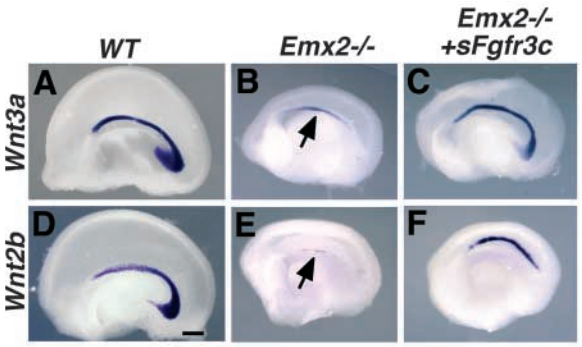

Fig. 6. Substantial rescue of WNT gene expression in the *Emx2* mutant cortical hem. (A-F) E13.5 hemispheres viewed from the medial side. Wild-type expression of *Wnt3a* and *Wnt2b* (A,D). (B,C,E,F) Hemispheres electroporated anteriorly with *sFGFR3c* at E.9.5. Each brain was co-electroporated with *EGFP*. WNT gene expression remains depleted in *Emx2* mutant brains with sparse or undetected EGFP (inefficiently transfected) (B,E), but expression is substantially restored in two brains that were efficiently transfected with *sFGFR3c*, assessed by high-density EGFP fluorescence (C,F). Scale bar: 0.4 mm.

7I, *n*=8/17) was seen for the ephrin receptor gene *EphB1*, which fills the distal half of the hippocampus in wild-type mice (Fig. 7G), but is reduced in the *Emx2* mutant (Fig. 7H). Efficiency of transfection could not be monitored directly in these brains because 9 days after electroporation the transgene was no longer expressed. Given that the rate of efficient transfection is about 50% in surviving mice (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003) (present study), the number of brains with a distinct DG (about 50%) suggests partial rescue in all efficiently electroporated *Emx2* mutant brains.

The hippocampal DG appears exquisitely sensitive to the level of canonical WNT signaling available during development (Galceran et al., 2000; Zhou et al., 2004). This may be because the primordium of the DG is closest to the cortical hem and requires the highest level of WNT signaling for normal expansion. Both Wnt2b and Wnt3a appear in the hem (Grove et al., 1998), signal through the canonical WNT pathway (Kawakami et al., 2001; Takada et al., 1994), and are depleted in the *Emx2* mutant. Present findings suggest that the DG is almost lost in the *Emx2* mutant because of reduced WNT signaling from the hem.

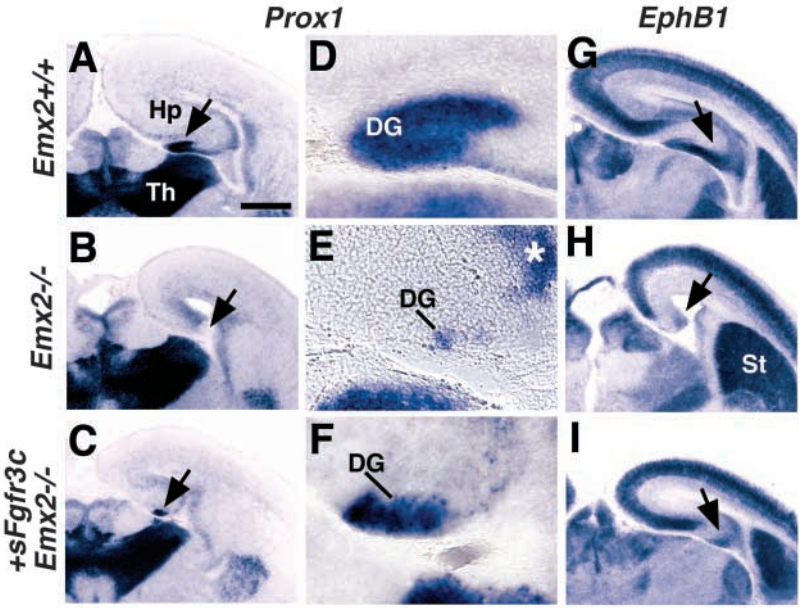

Fig. 7. Partial rescue of the dentate gyrus in *Emx2* mutant hippocampus. (A-I) Coronal sections through the hippocampus of E18.5 wild-type mice (A,D,G), *Emx2* homozygote mice (B,E,H) and *Emx2* homozygotes electroporated at E9.5 with *sFGFR3c* (C,F,I). (D-F) High-power micrographs of a different set of brains from those in (A-C). (A,D,G) *Prox1* expression reveals the developing V-shaped DG in the hippocampus (A,D, arrow in A); *EphB1* expression marks the distal end of the hippocampus, including the DG (G, arrow). (B,E,H) *Emx2* homozygote mutants show no (B, arrow) or only tiny patches of (E) *Prox1*-expressing cells in the region of the presumptive DG, and greatly reduced *EphB1* expression (arrow in H). (C,F,I) When excess FGF8 is reduced in *Emx2* mutant brains, the DG expands, forming a dense band not unlike the ventral DG blade (C, arrow, F). Distal *EphB1* expression is also partially restored (arrow, I). Scale bar: 0.75 mm for A-C,G-I; 0.125 mm for D-F.

Discussion

Regulation of BMP, FGF and WNT signals in the cortical primordium

Candidate patterning centers have been identified previously in the mouse cortical primordium, and existing evidence indicates that these regulate development of the primordium along both the AP and ML axes (Fukuchi-Shimogori and Grove, 2001; Garel et al., 2003; Hebert et al., 2002; Lee et al., 2000). The present study provides evidence that the cerebral cortical patterning centers interact to regulate one another, as do signaling centers in chick dorsal telencephalon (Crossley et al., 2001; Ohkubo et al., 2002) and classic signaling centers elsewhere in the embryo.

Specifically, we find an antagonism between the WNT and BMP-rich cortical hem, and the anterior telencephalic source of FGF8. FGF8 suppresses WNT gene expression in the cortical hem, and may therefore normally set the anterior boundary of the WNT-rich hem. In turn, *Fgf8* expression is suppressed by BMP signaling, which constrains the boundaries of the anterior telencephalic FGF8 source. A particularly striking finding is that a large part of the cortical primordium is competent to express *Fgf8*. That is, noggin can induce sites of *Fgf8* expression far from the telencephalic midline, where *Fgf8* is normally expressed. The implication is that BMP activity in the cortical primordium normally provides an extensive and crucial block of *Fgf8* expression. The ability of BMP activity to regulate the FGF8 source appears to be time limited, perhaps because the lateral cortical primordium loses competence to express *Fgf8*.

Cross species comparisons

Our observations of mouse dorsal telencephalic signaling centers fit well with previous studies in the chick (Crossley et al., 2001; Ohkubo et al., 2002). As detailed above,

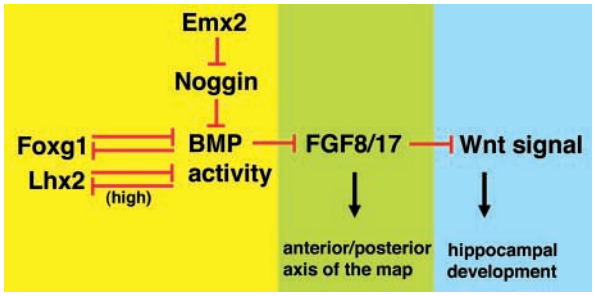

Fig. 8. Preliminary model of signaling interactions that pattern the mouse cerebral cortex. Transcription factors EMX2, LHX2 and FOXG1 expressed in the early cortical primordium regulate *Bmp* or *Nog* expression and thereby influence BMP4/7 activity. BMP signaling is active early in choroid plexus development, perhaps specifying the choroid plexus epithelial cell fate. BMP4 signaling further regulates expression of *Fgf8/17*. FGF8/17 signaling is crucial for neocortical area patterning by regulating the AP axis of the cortical primordium, and at least indirectly, for hippocampal development. FGF8/17 inhibits WNT gene expression; canonical WNT signaling at the cortical hem is required for hippocampal development (Boncinelli et al., 1993; Bulchand et al., 2001; Dou et al., 1999; Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003; Galceran et al., 2000; Garel et al., 2003; Hebert et al., 2002; Lee et al., 2000; Mallamaci et al., 1998; Monuki et al., 2001; Panchision et al., 2001; Shimamura et al., 1995; Shimamura and Rubenstein, 1997; Storm et al., 2003).

telencephalic sources of FGF, WNT and BMP proteins appear in comparable positions in both species. We confirm in mouse the negative regulation of an anterior FGF8 source by BMP activity, as well as the broad competence of the telencephalic primordium to express *Fgf8* (Crossley et al., 2001; Ohkubo et al., 2002). Previous findings indicate shared AP expression gradients of several genes associated with cerebral cortical pattern, including *Emx2* (Crossley et al., 2001; Ohkubo et al., 2002; Fukuchi-Shimogori and Grove, 2003; Garel et al., 2003). *Fgfr3*, and *COUP-TFI* and *COUP-TFII* (*Nr2f1* and *Nr2f2* – Mouse Genome Informatics) (Tsai and Tsai, 1997; Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003) (C. W. Ragsdale and E. A. Grove, unpublished observations). The AP gene expression gradients are regulated by FGF8 (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003; Ohkubo et al., 2002). These observations suggest that similar mechanisms confer initial positional information to the dorsal telencephalon in both species. Indeed, the anterior telencephalic FGF8 source in zebrafish (Shanmugalingam et al., 2000) suggests conservation of patterning mechanism across an even wider range of vertebrates. Further investigation of dorsal telencephalic patterning in a variety of species should continue to provide general insights.

Equally important, however, will be determining when and how patterning strategies diverge, given that the dorsal telencephalon has a profoundly different final organization in chick and mouse. In the cerebral cortex of mice or other mammals, functional specialization is organized by subdivision of the cortical sheet into areas. Basic cortical circuitry is organized by cortical lamination. In general, neurons in layer four receive thalamic input, layers two and three project to other cortical areas, and five and six to subcortical structures.

In the chick, as in other birds, basic pallial circuitry is comparable with that in mammals but its components are distributed as distinct cell types in different pallial nuclei (Karten, 1997). A particular functional specialization therefore co-opts parts or all of several nuclei. The avian Wulst is proposed to be most similar to neocortex (Medina and Reiner, 2000), containing somatosensory and visual representations. Its circuitry is organized in pseudolayers, which give a cortex-like appearance. However, the pseudolayers are in actuality radially adjacent slab-shaped nuclei (Medina and Reiner, 2000), so the Wulst also fits the scheme of connected nuclei, in a particularly orderly manner. Thus, chick and mouse show homology at the level of dorsal telencephalic cell types, circuits and sensorimotor representations (Karten, 1997; Medina and Reiner, 2000; Reiner et al., 2004), but not at the level of anatomical organization of circuitry.

Intense debate continues regarding homologies between specific avian and mammalian dorsal telencephalic structures (Reiner et al., 2004). A clearer comparison requires further connectional and physiological investigation of the avian brain. Comparative studies of embryonic patterning should also be helpful, at both early (as in the present study) and later stages of development, as final morphology and connectivity emerge.

Effects of abnormal signaling sources; the *Emx2* mutant mouse

Gene transfer experiments with wild-type mice uncover dramatic cortical patterning defects that occur when signaling centers are misregulated (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003). Augmenting anterior FGF8 shifts the neocortical area map, suppresses WNT expression in the cortical hem and compromises hippocampal development. In humans, hippocampal and other cortical abnormalities accompany certain forms of thanatophoric dysplasia (TDs), devastating disorders caused by constitutive activation of FGFR3 (Ho et al., 1984; Neilson and Friesel, 1996; Wongmongkolrit et al., 1983). A human hem has been identified (Abu-Khalil et al., 2004); thus, a prediction to be tested is whether defects in hem WNT signaling appear in TDs and underlie the hippocampal abnormalities.

The *Emx2* mutant mouse line provides an informative illustration of the consequences of signaling center defects. Homozygous mutants display an expanded FGF8 domain, and predictably, given the present findings, a partial loss of WNT gene expression in the hem (Muzio et al., 2002). A previous study provided evidence that shifts in region-specific gene expression in the *Emx2* mutant neocortex are in part caused by excess FGF8 (Fukuchi-Shimogori and Grove, 2003). Findings from the present study indicate that the expanded FGF8 source in the mutant reduces WNT signaling from the cortical hem, which in turn could contribute to defective development of the hippocampus.

Emx2 is expressed broadly in the cortical primordium, but its loss does not lead to a broad expansion of *Fgf8* expression. Instead, the normally medial and anterior FGF8 domain is enlarged laterally and posteriorly, but retains clear boundaries. Findings from the present study suggest a partial explanation. A likely cause of the expanded FGF8 domain in the *Emx2* mutant is early overexpression of noggin at the telencephalic midline, decreasing local BMP activity. BMP inhibition of *Fgf8* expression is thereby relieved close to the midline, but

not at a distance. Remaining BMP activity may contain further lateral spread of *Fgf8* expression.

We suggest that cortically expressed EMX2 influences signaling centers by direct gene regulation in the cortical primordium. However, an indirect influence by EMX2 function outside the cortical primordium remains a formal possibility. *Emx2* expression appears at E8-8.5 in rostral brain, and continues in both the cortical and subcortical forebrain (Boncinelli et al., 1993), where EMX2 has diverse roles in development (Cecchi and Boncinelli, 2000). These complexities challenge easy interpretation of specific defects in the *Emx2* mutant. For example, a misrouting of thalamocortical axons, first ascribed to the absence of EMX2 in the neocortex, may be partially due to loss of gene function in the ventral telencephalon where the thalamocortical pathway begins (Bishop et al., 2002; Lopez-Bendito et al., 2002).

Ultimately, the timing and sites of *Emx2* expression that are crucial to particular aspects of development will be resolved by appropriate conditional deletions, or regional misexpression, of the gene. A recent advance has been the generation of a mouse that overexpresses *Emx2* under the control of the nestin promotor (Hamasaki et al., 2004). FGF8 levels appear unaffected, perhaps because *Emx2* is overexpressed too late, yet area boundaries are shifted. These findings, together with our own, indicate a primary effect of EMX2 on cortical patterning, and a secondary effect via two signaling sources.

Model of signaling interactions

We propose (Fig. 8) that early in telencephalic development, EMX2 acts directly or indirectly on noggin to derepress BMP activity. BMP activity constrains expansion of the anterior FGF8 source, and keeps the cortical hem clear of FGF8, protecting local WNT gene expression. Meanwhile, normal levels of midline noggin allow the FGF8 source to be established and maintained. Effectively completing a negative feedback loop, FGF8 downregulates *Emx2* expression. These interactions help to ensure FGF and WNT/BMP sources of appropriate size, position and duration to regulate cortical patterning and growth.

Additional transcription factors have been implicated in the regulation of telencephalic signaling centers (Fig. 8). For example, the cortical hem, assessed by expression of BMP and WNT genes, is expanded in mice lacking the Lim homeobox domain transcription factor, LHX2 (Bulchand et al., 2001; Monuki et al., 2001). Restriction of *Bmp4* expression to the hem region also requires the winged-helix transcription factor, FOXG1 (BF1) (Dou et al., 1999). Potential feedback pathways among signaling molecules and transcription factors add further complexity (see Fig. 8). For example, FGF8 and BMPs up- and downregulate *Foxg1* expression, respectively (Furuta et al., 1997; Shimamura and Rubenstein, 1997; Storm et al., 2003); BMP signaling regulates *Lhx2* expression (Monuki et al., 2001); FGF8/17 suppresses *Emx2* expression (Fukuchi-Shimogori and Grove, 2003; Garel et al., 2003; Storm et al., 2003); and both BMP and WNT signaling pathways may control *Emx2* transcription (Theil et al., 2002). A qualification is that in the different studies cited, the mouse dorsal telencephalon is analyzed at different embryonic ages, in different preparations, and in wild-type or engineered mutant mice. A systematic analysis will be needed to fit these observations into a comprehensive model of cortical patterning.

In conclusion, embryonic signaling centers and their interactions appear crucial for normal cerebral cortical patterning. Experiments using in utero electroporation and analysis of the *Emx2* mutant mouse prompt a model in which two signaling centers, the anterior FGF source, and the WNT- and BMP-rich cortical hem interact antagonistically. Both the time dependency of gene misexpression effects and transient gene expression changes in the *Emx2* mutant suggest there is a crucial period in early telencephalic development. During this period, the balance of signaling molecules governs the future functional organization of the cerebral cortex.

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References

Abu-Khalil, A., Fu, L., Grove, E. A., Zecevic, N. and Geschwind, D. H. (2004). *Wnt* genes define distinct boundaries in the developing human brain: implications for human forebrain patterning. *J. Comp. Neurol.* **474**, 276-288.

Agarwala, S., Sanders, T. A. and Ragsdale, C. W. (2001). Sonic hedgehog control of size and shape in midbrain pattern formation. *Science* **291**, 2147-2150.

Anderson, R. M., Lawrence, A. R., Stottmann, R. W., Bachiller, D. and Klingensmith, J. (2002). Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development* **129**, 4975-4987.

Assimacopoulos, S., Grove, E. A. and Ragsdale, C. W. (2003). Identification of a Pax6-dependent epidermal growth factor family signaling source at the lateral edge of the embryonic cerebral cortex. *J. Neurosci.* **23**, 6399-6403.

Bachler, M. and Neubuser, A. (2001). Expression of members of the Fgf family and their receptors during midfacial development. *Mech. Dev.* **100**, 313-316.

Bishop, K. M., Goudreau, G. and O'Leary, D. D. (2000). Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. *Science* **288**, 344-349.

Bishop, K. M., Rubenstein, J. L. and O'Leary, D. D. (2002). Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. *J. Neurosci.* **22**, 7627-7638.

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.

Capdevila, J. and Izpisua Belmonte, J. C. (2001). Patterning mechanisms controlling vertebrate limb development. *Annu. Rev. Cell Dev. Biol.* **17**, 87-132.

Cecchi, C. and Boncinelli, E. (2000). Emx homeogenes and mouse brain development. *Trends Neurosci.* **23**, 347-352.

Crossley, P. H., Martinez, S., Ohkubo, Y. and Rubenstein, J. L. (2001). Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. *Neuroscience* **108**, 183-206.

Dahn, R. D. and Fallon, J. F. (2000). Interdigital regulation of digit identity and homeotic transformation by modulated BMP signaling. *Science* **289**, 438-441.

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.

Fukuchi-Shimogori, T. and Grove, E. A. (2001). Neocortex patterning by the secreted signaling molecule FGF8. *Science* **294**, 1071-1074.

Fukuchi-Shimogori, T. and Grove, E. A. (2003). Emx2 patterns the neocortex by regulating FGF positional signaling. *Nat. Neurosci.* **6**, 825-831.

Furuta, Y., Piston, D. W. and Hogan, B. L. (1997). Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development* **124**, 2203-2212.

Galceran, J., Miyashita-Lin, E. M., Devaney, E., Rubenstein, J. L. and Grosschedl, R. (2000). Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* **127**, 469-482.

Garda, A. L., Puelles, L., Rubenstein, J. L. and Medina, L. (2002). Expression patterns of Wnt8b and Wnt7b in the chicken embryonic brain suggest a correlation with forebrain patterning centers and morphogenesis. *Neuroscience* **113**, 689-698.

Garel, S., Huffman, K. J. and Rubenstein, J. L. R. (2003). Molecular regionalization of the neocortex is disrupted in Fgf8 hypomorphic mutants. *Development* **130**, 1903-1914.

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.

Grove, E. A. and Fukuchi-Shimogori, T. (2003). Generating the cerebral cortical area map. *Annu. Rev. Neurosci.* **26**, 355-380.

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.

Hamasaki, T., Leingartner, A., Ringstedt, T. and O'Leary, D. D. (2004). EMX2 regulates sizes and positioning of the primary sensory and motor areas in neocortex by direct specification of cortical progenitors *Neuron* **43**, 359-372.

Hebert, J. M., Mishina, Y. and McConnell, S. K. (2002). BMP signaling is required locally to pattern the dorsal telencephalic midline. *Neuron* **35**, 1029-1041.

Ho, K. L., Chang, C. H., Yang, S. S. and Chason, J. L. (1984). Neuropathologic findings in thanatophoric dysplasia. *Acta Neuropathol.* **63**, 218-228.

Karten, H. J. (1997). Evolutionary developmental biology meets the brain: the origins of mammalian cortex. *Proc. Natl. Acad. Sci. USA* **94**, 2800-2804.

Kawakami, Y., Capdevila, J., Buscher, D., Itoh, T., Rodriguez Esteban, C. and Izpisua Belmonte, J. C. (2001). WNT signals control FGF-dependent limb initiation and AER induction in the chick embryo. *Cell* **104**, 891-900.

Krubitzer, L. (1995). The organization of neocortex in mammals: are species differences really so different? *Trends Neurosci.* **18**, 408-417.

Lee, S. M., Tole, S., Grove, E. and McMahon, A. P. (2000). A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* **127**, 457-467.

Lopez-Bendito, G., Chan, C. H., Mallamaci, A., Parnavelas, J. and Molnar, Z. (2002). Role of Emx2 in the development of the reciprocal connectivity between cortex and thalamus. *J. Comp. Neurol.* **451**, 153-169.

Mallamaci, A., Iannone, R., Briata, P., Pintonello, L., Mercurio, S., Boncinelli, E. and Corte, G. (1998). EMX2 protein in the developing mouse brain and olfactory area. *Mech. Dev.* **77**, 165-172.

Mallamaci, A., Muzio, L., Chan, C. H., Parnavelas, J. and Boncinelli, E. (2000). Area identity shifts in the early cerebral cortex of Emx2–/– mutant mice. *Nat. Neurosci.* **3**, 679-686.

Maruoka, Y., Ohbayashi, N., Hoshikawa, M., Itoh, N., Hogan, B. M. and Furuta, Y. (1998). Comparison of the expression of three highly related genes, Fgf8, Fgf17 and Fgf18, in the mouse embryo. *Mech. Dev.* **74**, 175-177.

Medina, L. and Reiner, A. (2000). Do birds possess homologues of mammalian primary visual, somatosensory and motor cortices? *Trends Neurosci.* **23**, 1-12.

Monuki, E. S., Porter, F. D. and Walsh, C. A. (2001). Patterning of the dorsal telencephalon and cerebral cortex by a roof plate-lhx2 pathway. *Neuron* **32**, 591-604.

Muzio, L., DiBenedetto, B., Stoykova, A., Boncinelli, E., Gruss, P. and Mallamaci, A. (2002). Emx2 and Pax6 control regionalization of the pre-neuronogenic cortical primordium. *Cereb. Cortex* **12**, 129-139.

Nauta, W. J. H. and Feirtag, M. (1986). *Fundamental Neuroanatomy*. New York, NY: W. H. Freeman.

Neilson, K. M. and Friesel, R. (1996). Ligand-independent activation of fibroblast growth factor receptors by point mutations in the extracellular, transmembrane, and kinase domains. *J. Biol. Chem.* **271**, 25049-25057.

Niswander, L. (2002). Interplay between the molecular signals that control vertebrate limb development. *Int. J. Dev. Biol.* **46**, 877-881.

O'Leary, D. D. and Nakagawa, Y. (2002). Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. *Curr. Opin. Neurobiol.* **12**, 14-25.

Ohkubo, Y., Chiang, C. and Rubenstein, J. L. (2002). Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles. *Neuroscience* **111**, 1-17.

Panchision, D. M., Pickel, J. M., Studer, L., Lee, S. H., Turner, P. A., Hazel, T. G. and McKay, R. D. (2001). Sequential actions of BMP receptors control neural precursor cell production and fate. *Genes Dev.* **15**, 2094-2110.

Pellegrini, M., Mansouri, A., Simeone, A., Boncinelli, E. and Gruss, P. (1996). Dentate gyrus formation requires Emx2. *Development* **122**, 3893-3898.

Pleasure, S. J., Collins, A. E. and Lowenstein, D. H. (2000). Unique expression patterns of cell fate molecules delineate sequential stages of dentate gyrus development. *J. Neurosci.* **20**, 6095-6105.

Ragsdale, C. W. and Grove, E. A. (2001). Patterning the mammalian cerebral cortex. *Curr. Opin. Neurobiol.* **11**, 50-58.

Rakic, P. (1988). Specification of cerebral cortical areas. *Science* **241**, 170-176.

Reiner, A., Perkel, D. J., Bruce, L. L., Butler, A. B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G. et al. (2004). Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J. Comp. Neurol.* **473**, 377-414.

Riddle, R. D., Johnson, R. L., Laufer, E. and Tabin, C. (1993). Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* **75**, 1401-1416.

Rubenstein, J. L. R., Anderson, S., Shi, L., Miyashita-Lin, E., Bulfone, A. and Hevner, R. (1999). Genetic control of cortical regionalization and connectivity. *Cereb. Cortex* **9**, 524-532.

Shanmugalingam, S., Houart, C., Picker, A., Reifers, F., Macdonald, R., Barth, A., Griffin, K., Brand, M. and Wilson, S. W. (2000). Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon. *Development* **127**, 2549-2561.

Shimamura, K. and Rubenstein, J. L. R. (1997). Inductive interactions direct early regionalization of the mouse forebrain. *Development* **124**, 2709-2718.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Storm, E. E., Rubenstein, J. L. and Martin, G. R. (2003). Dosage of Fgf8 determines whether cell survival is positively or negatively regulated in the developing forebrain. *Proc. Natl. Acad. Sci. USA* **100**, 1757-1762.

Sun, X., Mariani, F. V. and Martin, G. R. (2002). Functions of FGF signalling from the apical ectodermal ridge in limb development. *Nature* **418**, 501-508.

Takada, S., Stark, K. L., Shea, M. J., Vassileva, G., McMahon, J. A. and McMahon, A. P. (1994). Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8**, 174-189.

Theil, T., Aydin, S., Koch, S., Grotewold, L. and Ruther, U. (2002). Wnt and Bmp signalling cooperatively regulate graded Emx2 expression in the dorsal telencephalon. *Development* **129**, 3045-3054.

Tole, S., Christian, C. and Grove, E. A. (1997). Early specification and autonomous development of cortical fields in the mouse hippocampus. *Development* **124**, 4959-4970.

Tole, S., Goudreau, G., Assimacopoulos, S. and Grove, E. A. (2000). Emx2 is required for growth of the hippocampus but not for hippocampal field specification. *J. Neurosci.* **20**, 2618-2625.

Tsai, S. Y. and Tsai, M. J. (1997). Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age. *Endocr. Rev.* **18**, 229-240.

Wolpert, L. (1996). One hundred years of positional information. *Trends Genet.* **12**, 359-364.

Wongmongkolrit, T., Bush, M. and Roessmann, U. (1983). Neuropathological findings in thanatophoric dysplasia. *Arch. Pathol. Lab. Med.* **107**, 132-135.

Ye, W., Shimamura, K., Rubenstein, J. L., Hynes, M. A. and Rosenthal, A. (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **93**, 755-766.

Yoshida, M., Suda, Y., Matsuo, I., Miyamoto, N., Takeda, N., Kuratani, S. and Aizawa, S. (1997). *Emx1* and *Emx2* functions in development of dorsal telencephalon. *Development* **124**, 101-111.

Zhou, C. J., Zhao, C. and Pleasure, S. J. (2004). Wnt signaling mutants have decreased dentate granule cell production and radial glial scaffolding abnormalities. *J. Neurosci.* **24** 121-126.