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Frontal Cortex Subdivision Patterning Is Coordinately Regulated by *Fgf8*, *Fgf17*, and *Emx2*

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Abstract

The frontal cortex (FC) plays a major role in cognition, movement and behavior. However, little is known about the genetic mechanisms that govern its development. We recently described a panel of gene expression markers that delineate neonatal FC subdivisions and identified FC regionalization defects in *Fgf17*^{-/-} mutant mice (Cholfin and Rubenstein [2007] *Proc. Natl. Acad. Sci. U. S. A.* [in press]). In the present study, we applied this FC gene expression panel to examine regionalization phenotypes in *Fgf8*^{neo/neo}, *Emx2*^{-/-}, and *Emx2*^{-/-}; *Fgf17*^{-/-} newborn mice. We report that *Fgf8*, *Fgf17* and *Emx2* play distinct roles in the molecular regionalization of FC subdivisions. The changes in regionalization are presaged by differential effects of rostral patterning center *Fgf8* and *Fgf17* signaling on the rostral cortical neuroepithelium, revealed by altered expression of *Spry1*, *Spry2*, and “rostral” transcription factors *Er81*, *Erm*, *Pea3*, and *Sp8*. We used *Emx2*^{-/-}; *Fgf17*^{-/-} double mutants to provide direct evidence that *Emx2* and *Fgf17* antagonistically regulate the expression of *Erm*, *Pea3*, and *Er81* in the rostral cortical neuroepithelium and FC regionalization. We have integrated our results to propose a model for how fibroblast growth factors regulate FC patterning through regulation of regional transcription factor expression within the FC anlage.

Keywords

arealization; regionalization; forebrain; protomap; neocortex; fibroblast growth factor; prefrontal

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The frontal cortex (FC) is an anatomically and functionally heterogeneous brain structure that has a central role in higher cognitive function, behavior, and movement in mammals (Dalley et al., 2004; Fuster, 2001; Heidbreder and Groenewegen, 2003; Price, 2006; Uylings et al., 2003). The FC can be subdivided functionally into motor, premotor, and prefrontal cortices and anatomically into a number of distinct areas (Dalley et al., 2004; Heidbreder and Groenewegen, 2003; Krettek and Price, 1977; Uylings et al., 2003; Zilles and Wree, 1995). Although adult FC anatomy and function has been well studied, little is known about how FC subdivisions are patterned during development. To work toward solving this problem, we have recently identified a panel of gene expression markers that delineate FC subdivisions in neonatal mice that correlate with adult cytoarchitectonic areas (Cholfin and Rubenstein, 2007).

The prevailing model of rostral neocortical patterning suggests that members of the fibroblast growth factor (FGF) family, secreted from the rostral patterning center, provide positional information by controlling the expression of transcription factors and other regulatory molecules in the cortical neuroepithelium, consistent with the protomap model of cortical development (for review see Garel and Rubenstein, 2004; Grove and Fukuchi-Shimogori, 2003; O'Leary and Nakagawa, 2002; Rakic, 1988; Sur and Rubenstein, 2005). Four *FGF* genes, *Fgf8*, *Fgf15*, *Fgf17*, and *Fgf18*, are known to be expressed in the rostral patterning center (Bachler and Neubuser, 2001; Crossley and Martin, 1995; Crossley et al., 2001; Hoshikawa et al., 1998; Maruoka et al., 1998; Xu et al., 1999).

Several transcription factors are implicated in cortical patterning, including *COUP-TF1*, *Emx2*, *Foxg1*, *Lhx2*, and *Pax6* (Bishop et al., 2000, 2002, 2003; Dou et al., 1999; Hamasaki et al., 2004; Mallamaci et al., 2000; Monuki et al., 2001; Muzio et al., 2002a,b; Shinozaki et al., 2004; Toresson et al., 2000; Zhou et al., 2001); among these, the most information is known about *Emx2*. Both loss-of-function and gain-of-function experiments demonstrate that the level of *Emx2* expression in the cortical neuroepithelium provides positional information that contributes to regional differences in cortical identity (Bishop et al., 2000, 2002; Hamasaki et al., 2004; Mallamaci et al., 2000).

Previous work has identified functions for the *FGF* s and *Emx2* in cortical patterning. Manipulations of *Fgf8* levels in utero or in ovo show that *Fgf8* can act as an anterior-posterior patterning molecule in the cortex, in part through repression of *Emx2* expression (Cholfin and Rubenstein, 2007; Crossley et al., 2001; Fukuchi-Shimogori and Grove, 2001, 2003). *Fgf8* (mild: *Fgf8^{neo/neo}*; severe: *Fgf8^{neo/null}*) hypomorphic mice (Meyers et al., 1998) have decreased FC size and a rostral shift of caudal cortical areas, associated with rostral expansion of *Emx2* expression (Garel et al., 2003; Storm et al., 2003, 2006). *Fgf17* mutant mice (*Fgf17^{-/-}*) display a selective decrease in dorsal FC size and rostral shift of caudal cortical areas (Cholfin and Rubenstein, 2007). Conversely, *Emx2* null (*Emx2^{-/-}*) mice have an expanded rostral cortex and reduced caudal cortex (Bishop et al., 2000, 2002; Mallamaci et al., 2000). *Emx2* can both negatively regulate *Fgf8* signaling and directly specify the identity of neural progenitors (Fukuchi-Shimogori and Grove, 2003; Hamasaki et al., 2004). Thus, *Fgf8* and *Emx2* have reciprocal repressive interactions.

Analysis of compound *Fgf;Emx2* mutants would be an effective approach to demonstrate directly their *in vivo* interactions in FC subdivision patterning. Unfortunately, *Fgf8* and *Emx2* are closely linked on mouse chromosome 19, making it difficult to generate the double mutant. We have partially circumvented this problem by assessing the FC phenotype of *Emx2;Fgf17* double mutants, in parallel with *Fgf8^{neo/neo}*, *Fgf17^{-/-}*, and *Emx2^{-/-}* single mutants. Here we demonstrate that *Fgf8*, *Fgf17*, and *Emx2* have distinct roles in FC patterning. At birth, *Fgf8^{neo/neo}* brains display reduced dorsal and orbital FC, *Fgf17^{-/-}* mutants have a selective loss of dorsal FC molecular properties (Cholfin and Rubenstein, 2007), and *Emx2^{-/-}* mutants have an expansion of dorsal and orbital FC molecular subdivisions. Our analysis demonstrates that loss of *Fgf17* rescues a specific subset of cortical defects in *Emx2* mutants. By examining changes in regionally expressed transcription factors (*COUP-TFI*, *Emx2*, *Er81*, *Erm*, *Pea3*, and *Sp8*) and other FGF-responsive genes (*Spry1* and *Spry2*), we provide evidence for the mechanisms through which *Fgf8*, *Fgf17*, and *Emx2* regulate FC patterning.

MATERIALS AND METHODS

Mouse lines and genotyping

All mice were housed and handled in accordance with the Institutional Animal Care and Use Committee of the University of California, San Francisco. The *Fgf17* (Xu et al., 2000) allele was maintained on a mixed 129Sv/C57BL/6 background; *Fgf8* (Meyers et al., 1998) and *Emx2* (Pellegrini et al., 1996) alleles were maintained on a C57BL/6 background. Heterozygotes were crossed to generate homozygous mutants. For the *Emx2^{-/-};Fgf17^{-/-}* genotype, double heterozygous mice were generated by an intercross of *Fgf17^{+/-};Emx2^{+/-}* mice, which are viable and fertile. PCR genotyping was performed as described elsewhere (Cholfin and Rubenstein, 2007; Garel et al., 2003; Pellegrini et al., 1996). For embryo staging, noon on the day of the vaginal plug was considered embryonic day 0.5 (E0.5).

In situ hybridization, immunohistochemistry, and TUNEL

Embryos and brains were fixed overnight in 4% paraformaldehyde in PBS (pH 7.4) at 4°C. In situ hybridization was performed on 10–20-μm cryostat sections as described previously (Cholfin and Rubenstein, 2007; Rubenstein et al., 1999). Digoxigenin (DIG)-labeled riboprobes were generated for the following genes: *Cadherin-6* and *Cadherin-8* (Rubenstein et al., 1999), *COUP-TFI* (gift from M. Tsai), *Emx2* (gift from A. Simeone), *EphrinA5* (Bishop et al., 2002), *Er81* (gift from T. Jessell), *Erm* (gift from A. Chotteau-Lelievre), *Fgf8* (Crossley and Martin, 1995), *Fgf15* (Gimeno et al., 2003), *Fgf17* (Xu et al., 1999), *Fgf18* (Maruoka et al., 1998), *Id-2* (Rubenstein et al., 1999), *Lmo3* and *Lmo4* (Bulchand et al., 2003), *Neurogenin-2* (Fode et al., 1998), *Neurotrophin-3* (gift from L. Ma), *Pea3* (gift from A. Chotteau-Lelievre), *Rzr-β* (Rubenstein et al., 1999), *Sp8* (gift from J.C. Belmonte), *Sprouty* (*Spry*) 1 and 2 (gift from G. Martin), and *Steel* (gift from E. Grove). See Table S1 for more details.

Immunohistochemistry was performed on 10–16-μm cryostat sections as described previously (Rubenstein et al., 1999). Primary antibodies were as follows. For rabbit antiphosphohistone-3 (PH3; Millipore, Bedord, MA; No. 06-570), the immunogen was

peptide (ARKpSTGGKA-PRKQLC), corresponding to amino acids 7–20 of human histone H3. The manufacturer tested the specificity of this antibody with immunoblot on acid extracts of colcemid-treated and untreated HeLa cells and demonstrated a single band of appropriate molecular weight (~17 kD) specifically in the colcemid-treated group. This antibody has been effectively used by immunohistochemistry to study phosphohistone 3 expression in mitotic cells (Oike et al., 2003). For rabbit antiphosphorylated Erk1/2 (p44/42 MAP kinase; Cell Signaling, Beverly, MA; No. 9101), the immunizing sequence was TGFLtEyVATRKYRC, where the lowercase t and y represent phosphorylated Thr202 and Tyr204, respectively, of human Erk1/p44. In Western blots, the antibody was shown by the manufacturer to react specifically with as little as 0.25 ng of phosphorylated p42 MAP kinase and not to cross-react with up to 4 µg of nonphosphorylated p42 MAP kinase. Specificity was confirmed by using immunocytochemistry on NIH/3T3 cells treated with either MEK1/2 inhibitor U0126 or platelet-derived growth factor (PDGF), labeled with phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody. Cells treated with the MEK1/2 inhibitor showed no labeling, whereas cells treated with PDGF showed labeling in the nuclei. Western blot analysis of whole extracts of unstarved wild-type mouse embryonic fibroblasts showed that growth factors (basic fibroblast growth factor and PDGF) can increase the amount of protein (bands of appropriate molecular weight) detected by the phospho-p44/42 antibody from basal level, without affecting the total amount of p44/42 MAPK protein. This increase in phosphorylated p44/42 MAPK was blocked by the addition of MAPK pathway inhibitors U0126 or PD98059. Flow cytometric analysis of Jurkat cells showed an increased population of phospho-p44/42-positive cells in cells treated with PMA (a phosphorylation inducer), compared with untreated controls and a population of cells treated with a nonspecific negative control antibody. Additional flow cytometric studies in human peripheral blood lymphocytes showed an increased population of phospho-p44/42-positive cells treated with PMA compared with unstimulated controls. This effect was blocked by BAY 37-951 and U0126, which inhibit the MAPK pathway. This antibody has been utilized previously for immunohistochemistry (Vasioukhin et al., 2001). For rabbit antigreen fluorescent protein (GFP; Chemicon, Temecula, CA; No. AB3080), the immunogen was highly purified native GFP from *Aequorea victoria*. According to the manufacturer, this anti-body was reactive with GFP from both native and recombinant sources and was purified by protein A chromatography. We tested the specificity by using this antibody on tissue that does not have GFP expressed, and we observed no staining. This antibody has been utilized previously for immunohistochemistry (Knott et al., 2006). TUNEL was performed on 16-µm cryostat sections using the ApopTag Plus Peroxidase kit (Chemicon; No. S7101).

Image acquisition and analysis

Images were acquired with Spot (Diagnostic Instruments, Sterling Heights, MI) and Olympus digital cameras and imaging software. Contrast was adjusted and images were resized equally across genotypes in Adobe Photoshop CS2 (Adobe Systems, Inc, San Jose, CA).

RESULTS

Abnormal FC molecular properties in cortical patterning mutants

We have developed a panel of gene expression markers to define immature FC subdivisions in the neonatal mouse (Table 1; Cholfin and Rubenstein, 2007). By using these markers, we demonstrated that *Fgf17*^{-/-} mutants show a selective deficit in the dorsal FC (Cholfin and Rubenstein, 2007). Here we applied the same panel to *Fgf8*^{neo/neo} and *Emx2*^{-/-} postnatal day 0 (P0) mutants. For purposes of comparison, we also show the wild type (Figs. 1, S1) and *Fgf17*^{-/-} mutant (Figs. 1, S2).

Fgf8^{neo/neo} mutants were described previously to have a small FC based on changes in *Id2* and *Cad6* expression (Garel et al., 2003); at that time, we did not have the tools to define region-specific defects within the FC. Here we characterized the FC regionalization defects by examining expression of *Lmo4*, *Cad8*, *Id2*, *Steel*, *Nt3*, *Ngn2*, *Rzr-β*, *Cad6*, *Lmo3*, and *EphrinA5* (Figs. 1, S3). *Fgf8*^{neo/neo} mutants displayed medially (dorsally) shifted dorsal expression borders of *Lmo4*, *Cad8*, *Id2*, *Steel*, and *Nt3* (Figs. 1B,G, S3). These changes were complemented by a rostromedial expansion of *Lmo3*, *Cad6*, *Rzr-β*, and *EphrinA5* expression in the parietal cortex (Figs. 1L,Q,V, S3). The medial aspect of the FC (formerly regions dM and MO) had molecular and histological features that resemble more caudal structures (caudal dorsomedial FC; dMc); we postulate that much of this region is transformed into dorsal and ventral anterior cingulate cortex.

Fgf8^{neo/neo} mutants maintain expression of orbital cortex markers (*Lmo4*, *Cad8*, *Id2*, *Steel*, *Nt3*, *Lmo3*, *Rzr-β*, and *Ngn2*), although this region is much smaller than in wild-type brains (Fig. S3). For example, *Lmo4* is normally expressed strongly in the orbital cortex (Fig. S1). This domain extends caudally throughout the orbital cortex for three sections (20 μm each, 180 μm space between sections), suggesting that the orbital cortex spans at the very least 400 μm in distance along the rostral-caudal axis (Fig. S1). In the *Fgf8*^{neo/neo} mutant, orbital cortex *Lmo4* expression is present only in the first section and is missing in the next section (200 μm away; Fig. S3, first and second columns). Thus, reduction in *Fgf8* dosage reduces the overall size of the FC and leads to caudalization of the dorsal and dorsomedial FC.

Previous studies have established that in *Emx2*^{-/-} mutants the rostral cortex expands caudally at the expense of a reduced occipital (visual) cortex (Bishop et al., 2000, 2002; Mallamaci et al., 2000); an analysis of FC patterning has not been performed in these mutants. The *Emx2*^{-/-} FC showed expanded dorsal expression of *Lmo4*, *Cad8*, *Id2*, and *Steel* and laterally (ventrally) shifted dorsal *Nt3* expression (Figs. 1D,I, S4; note: *Emx2*^{-/-} and *Emx2*^{+/-}; *Fgf17*^{+/-} mutants were indistinguishable; data not shown). This suggests that dorsal FC subdivisions (regions dM, D, and dL) were expanded and/or shifted caudolaterally. Strong expression of *Lmo3*, *Cad6*, and *EphrinA5* (parietal cortex markers) was not present in rostral sections (Figs. 1N,S, S4), consistent with the known caudal shift of the parietal cortex that is complementary to the expansion/shift of the dorsal FC.

It was surprising that *Emx2*^{-/-} mutants lose orbital FC expression of *Lmo4*, *Cad8*, *Id2*, *Steel*, *Nt3*, *Lmo3*, *Rzr-β*, and *Ngn2* (Figs. 1D,I,N,S,X,CC, S4). Although this initially suggested a loss of orbital cortex, more detailed analysis provided evidence for an alternative

explanation. Strong *Ngn2* expression is normally limited to the ventro-medial FC (orbital cortex; Figs. 1Z, S1). However, in the mutants, this strong expression was present in a more dorsal location (Figs. 1CC, S4). This suggests that the medial orbital cortex is shifted to a more dorsal position. We are uncertain about the identity of the tissue that remains in the position of the orbital cortex.

Given the complementary FC phenotypes between the *Fgf* and the *Emx2* mutants, we tested whether the genetic programs downstream of these genes interact. To do this, we generated *Emx2*^{-/-};*Fgf17*^{-/-} double mutants. Indeed, *Emx2*^{-/-};*Fgf17*^{-/-} P0 mutants had FC regionalization phenotypes intermediate to *Fgf17*^{-/-} and *Emx2*^{-/-} single mutants for all FC markers examined (Figs. 1E,J,O,T,Y,DD, S5, S6), demonstrating that loss of *Fgf17* can partially rescue the *Emx2*^{-/-} FC regionalization defects (and vice versa). Together, these results provide strong evidence that endogenous *Fgf17* and *Emx2* antagonistically regulate regionalization of FC subdivisions.

Rostral patterning center signaling is differentially regulated by *Fgf8*, *Fgf17*, and *Emx2*

To investigate the mechanisms by which *Fgf8*, *Fgf17*, and *Emx2* regulate FC regionalization, we first expanded on previous findings (Bachler and Neubuser, 2001; Gimeno et al., 2003; Maruoka et al., 1998) by examining the expression domains of *Fgf8*, *Fgf17*, *Fgf15*, and *Fgf18* mRNA in the rostral telencephalon of E10.5 and E12.5 embryos. Furthermore, we assessed responses to FGF signaling in the rostral neuroepithelium by examining the expression of FGF-induced signaling antagonists *Spry1* and *Spry2* (Fukuchi-Shimogori and Grove, 2001, 2003; Storm et al., 2003; Zhang et al., 2001).

At E10.5, *Fgf8* and *Fgf18* were expressed in similar domains in the commissural plate (Fig. 2A,D). By contrast, *Fgf17* was expressed in a broader domain, particularly in the dorsal dimension (Fig. 2G). *Fgf15* was also expressed more broadly than *Fgf8* and *Fgf18*; unlike *Fgf17*, *Fgf15* expression extends ventrally (Fig. 2J, and data not shown). *Spry1* and *Spry2* expression correlated with *Fgf8*, *Fgf17*, and *Fgf18* expression, but not with the ventral domain of *Fgf15*; their expression was strongest at the midline and slightly weaker dorsally (Fig. 2M,P). Thus, *Spry1* and *Spry2* were expressed at the highest levels where there was overlap with *Fgf8* /*Fgf18* expression, and at lower levels in neuroepithelium that was positive for *Fgf17* and negative for *Fgf8* mRNA.

At E12.5, *Fgf8* and *Fgf18* were expressed in very similar domains in the presumptive septum (Figs. 3A,D, S7). *Fgf17* expression overlapped with *Fgf8* and *Fgf18*, but extended into regions that were ~200 μ m more rostral than *Fgf8* (Figs. 3G, S7, S12). *Fgf15* expression overlapped with the other FGFs in a small region of the septum and extended into a distinct domain in the rostroventral telencephalon that appears to include the olfactory bulb rudiment (Figs. 3J, S7). *Fgf15* was also expressed in small domains at the boundaries between the pallium and subpallium and the lateral ganglionic eminence and medial ganglionic eminence (Figs. 3J, S7). *Spry1* and *Spry2* expression overlapped with *Fgf8*, *Fgf17*, and *Fgf18* expression and extended more broadly into the rostradorsal neuroepithelium than these FGFs (Figs. 3M,P, S7, S12). Therefore, *Fgf8* and *Fgf18* are expressed in a core domain within the septum, from which *Fgf17* expression extends rostradorsally and *Fgf15* extends

rostroventrally. The *Spry1/2* transcriptional response to FGF signaling is highest within the core domain and extends outward into the rostral telencephalon.

We examined *Fgf* and *Spry* expression in *Fgf17*^{-/-} and *Fgf8*^{neo/neo} E10.5 and E12.5 mutants. For *Fgf17*^{-/-} mutants, we found no overt change in *Fgf8*, *Fgf15*, or *Fgf18* expression. (Figs. 2B,E,K, 3B,E,K, S8). However, for E10.5, we found a reduction in *Spry1* and *Spry2* expression selectively in the rostradorsal neuroepithelium (Fig. 2N,Q), but not in the core domain of the septum anlage in E10.5 or E12.5 embryos (Figs. 2N,Q, 3N,Q, S8). Therefore, *Fgf17*^{-/-} mutants retain the central septal domain of FGF signaling, whereas the more rostral and dorsal neuroepithelia have decreased FGF signaling.

By contrast, E10.5 and E12.5 *Fgf8*^{neo/neo} mutants had a severe reduction of *Fgf18* expression and a reduction in the size of the *Fgf17* expression domain; we did not detect an obvious change in *Fgf15* expression (Figs. 2F,I,L, 3F,I,L, S9). At E10.5, the *Fgf17* expression domain was reduced to approximately the size of the wild-type *Fgf8* domain (compare Fig. 2A, G, and I); correspondingly, *Spry1* and *Spry2* expression was reduced (Fig. 2O,R). At E12.5, both *Spry1* and *Spry2* were reduced in the rostradorsal neuroepithelium (Figs. S9, S12). Thus, *Fgf8*^{neo/neo} mutants have both a shrunken FGF core domain and smaller rostradorsal penumbra of *Fgf17* expression and signaling.

Previously, *Emx2* was found to repress *Fgf8* and *Fgf17* expression in experiments that studied whole-mount embryos and tissue explants (Fukuchi-Shimogori and Grove, 2003). We carefully examined the spatial relationships of *Fgf8*, *Fgf15*, *Fgf17*, and *Spry1* expression in adjacent coronal sections from E12.5 *Emx2*^{-/-} mutants (Figs. 4, S10). *Fgf8* and *Fgf17* expression appeared more intense and extended more dorsally (Figs. 4B,D, S10). *Fgf15* expression was increased in intensity and expanded dorsally into the rostral cortex (Figs. 4F, S10). More caudally, *Fgf15* expression in the pallial/subpallial boundary was greatly increased and extended farther dorsally into the cortical neuroepithelium (Fig. S10). The expanded *Fgf8/17* expression was correlated with increased extent of *Spry1* expression, suggesting an increase in FGF signaling (Figs. 4H, S10).

Finally, we found that the expanded *Fgf8* and *Fgf15* expression domains were not rescued in E12.5 *Emx2*^{-/-}; *Fgf17*^{-/-} double mutants (Figs. 4J,L, S11), suggesting that removing *Fgf17* expression does not affect the increase/ expansion of *Fgf8* and *Fgf15* expression.

***Fgf8*, *Fgf17*, and *Emx2* differentially regulate transcription factor expression in the presumptive FC neuroepithelium**

To explore how changes in FGF expression and signaling transduce changes in FC fate and growth, we focused on the expression pattern of transcription factors in the FC primordium that have been previously implicated in cortical patterning and arealization (*Emx2*, *COUP-TFI*; Bishop et al., 2000, 2002; Hamasaki et al., 2004; Mallamaci et al., 2000; Zhou et al., 2001) in coronal sections of E12.5 brains. In parallel, we examined the expression of genes that are induced by FGF signaling (*Sp8*, *Erm*, *Pea3*, *Er81*; Bell et al., 2003; Fukuchi-Shimogori and Grove, 2003; Kawakami et al., 2004; Storm et al., 2006).

In wild-type E12.5 embryos, *Sp8*, *Erm*, and *Pea3* were expressed in high-rostromedial to low-caudolateral gradients in the medial wall of the rostral telencephalic neuroepithelium (Figs. 5A,F,K, S7). *Sp8* was the most broadly expressed of these “rostral” transcription factors; in addition to its expression in the medial wall of the FC, its dorsal expression extended caudally throughout the entire dorsomedial wall, including the anlagen of the cingulate cortex and hippocampus (Fig. S7). The expression of *Erm* and *Pea3* was largely localized to the rostral neuroepithelium (Fig. S7). *Sp8*, *Erm*, *Er81*, and *Fgf15* were also expressed in overlapping domains within rostroventral regions that we postulate are subpallial regions related to ventral parts of the septum and its extension into the olfactory bulb (Figs. 5, S7; Long et al., 2007).

The expression of *Emx2* and *COUP-TF1* differed in the rostral cortical neuroepithelium. *Emx2* was expressed in a high-dorsomedial to low-ventrolateral gradient, whereas *COUP-TF1* was expressed in an opposing gradient (Figs. 5U,Z, S7). Consistently with previous observations (Bishop et al., 2000; Garel et al., 2003; O’Leary and Nakagawa, 2002), both *Emx2* and *COUP-TF1* were expressed in high-caudal to low-rostral gradients that extended to the rostral pole (Fig. S7).

Fgf17^{-/-} E12.5 brains displayed a selective reduction in the dorsomedial expression of *Sp8*, *Erm*, *Pea3*, and *Er81*, which was most evident in the most rostral sections (Figs. 5B,G,L,Q, S8). Expression of these genes appeared unaltered in rostroventral (subpallial) regions that overlap with *Fgf15* expression (Figs. 3K, S8). On the other hand, *COUP-TF1* expression was up-regulated slightly in the most rostral sections (Figs. 5V, S8). We did not detect an overt difference in *Emx2* expression (Figs. 5AA, S8).

Fgf8^{neo/neo} E12.5 mutants exhibited related, but distinct, changes in transcription factor expression in the rostral cortical primordium. Unlike the case in the *Fgf17*^{-/-} mutants, *Sp8* expression was not appreciably altered (Figs. 5C, S9). *Erm* expression was reduced in the dorsal ventricular zone, whereas its expression in the medial wall was preserved (unlike the case in *Fgf17*^{-/-} mutants; Figs. 5H, S9). *Pea3* expression was reduced in the ventromedial ventricular zone (Fig. 5M, S9). *Er81* was more subtly reduced in the cortical ventricular zone (Figs. 5R, S9). The *COUP-TF1* and *Emx2* gradients in *Fgf8*^{neo/neo} mutants were shifted rostrally, with increased expression in the dorsomedial ventricular zone (Figs. 5W,BB, S9), consistent with previous findings in *Fgf8*^{neo/neo} and *Fgf8*^{null/n} mutants (Garel et al., 2003; Storm et al., 2006).

Emx2^{-/-} E12.5 mutants showed increased dorsolateral ventricular zone expression of *Sp8* and *Erm* (Figs. 5D,I,N, S10). *Pea3* expression was up-regulated in the medial wall (Figs. 5N, S10). *Er81* expression was up-regulated in the lateral, dorsal, and medial cortical ventricular zone and was ectopically expressed in the dorsolateral cortical plate (Figs. 5S, S10). Conversely, *COUP-TF1* displayed complementary reduced expression in the dorsomedial cortical progenitor zone (Figs. 5X, S10), consistent with the caudolateral shift in its gradient described previously (Muzio and Mallamaci, 2003).

Finally, we examined *Sp8*, *Erm*, *Pea3*, *Er81*, and *COUP-TF1* expression in E12.5 *Emx2*^{-/-};*Fgf17*^{-/-} double mutants to test directly whether *Emx2* and *Fgf17* have opposing

functions in regulating the expression of transcription factors in the FC primordium. *Erm*, *Pea3*, and *Er81* expression in the rostral cortical progenitor zone was more similar to the that in wild-type brain than either the *Emx2*^{-/-} or the *Fgf17*^{-/-} mutants (Figs. 5J,O,T, S11). By contrast, *Sp8* and *COUP-TF1* expression was not overtly rescued, nor was the ectopic expression of *Er81* in the cortical plate (Figs. 5E,T,Y, S11). These results provide strong evidence for genetic antagonism between *Fgf17* and *Emx2* in regulating *Erm*, *Pea3*, and *Er81* expression in FC progenitors.

Cell proliferation, apoptosis, and MAPK activation are not affected in *Fgf17*^{-/-} mutants

Previous work has showed that *Fgf8* has a dosedependent role in regulating cell proliferation and death in the rostral patterning center and cortical neuroepithelium (Storm et al., 2003, 2006). To examine whether smaller reductions in *FGF* signaling have an overt effect on proliferation or apoptosis, we studied E9.5 and E10.5 *Fgf17*^{-/-} embryos with phosphohistone H3 (PH3) immunohistochemistry and TUNEL. No genotype difference in either PH3 or TUNEL staining was observed (Fig. S13), suggesting that proliferative and apoptotic mechanisms are not overtly sensitive to small perturbations in rostral patterning center *FGF* signaling.

FGF signaling leads to activation of the MAPK signaling pathway in the telencephalon (Shinya et al., 2001). To determine whether activation of the MAPK pathway is affected by loss of *Fgf17*, we performed phosphorylated-Erk (P-Erk) immunohistochemistry on sections from E9.5 and E10.5 *Fgf17*^{-/-} embryos. Although cells along the ventricle were labeled strongly, there was no discernible difference in P-Erk staining between genotypes (Fig. S13).

DISCUSSION

We have found that *Fgf8*, *Fgf17*, and *Emx2* have unique contributions to patterning FC subdivisions (Figs. 1, S1–S6). By applying a panel of in situ hybridization probes that selectively marks expression in distinct combinations of FC subdivisions (Cholfin and Rubenstein, 2007), we have determined which parts of the neonatal FC depend on *Fgf8*, *Fgf17*, and *Emx2* function. In the absence of sulci and gyri, neuroanatomists have traditionally used cytoarchitectonic criteria to define subdivisions of the rodent frontal cortex (Dalley et al., 2004; Heidbreder and Groenewegen, 2003; Uylings et al., 2003; Zilles and Wree, 1995). Our approach of using gene expression boundaries to define FC subdivisions complements these traditional methods (Table 1), represents an extension of work that has defined larger primary cortical areas in terms of gene expression boundaries (Bishop et al., 2000; Fukuchi-Shimogori and Grove, 2001; Miyashita-Lin et al., 1999; Rubenstein et al., 1999), and has the advantage of detecting immature prefrontal subdivisions. In particular, our work extends genetic analysis to the rodent prefrontal areas that subserve functions that are common among all mammals (Dalley et al., 2004; Heidbreder and Groenewegen, 2003; Price, 2006; Uylings et al., 2003).

Whereas *Fgf17*^{-/-} mutants have a selective reduction of the dorsomedial FC (Cholfin and Rubenstein, 2007), *Fgf8*^{neo/neo} mutants have features consistent with a transformation of the dorsomedial FC into a cingulate cortexlike structure and hypoplasia of the orbital cortex. In

addition, both *Fgf* mutants show a rostral expansion of parietal cortex markers; this phenotype is more severe in the *Fgf8^{neo/neo}* mutants. We propose that the molecular changes in the *Fgf17^{-/-}* mutant represent a reduction in the size of dorsomedial prefrontal areas (Fr2/PrCm, Cg1/Cg2/Cg3/ACd/v) and a shift of motor cortex (Fr1/PrCl), which normally lies more dorsolaterally, to more medial territory (Cholfin and Rubenstein, 2007). This has implications for functional and behavioral studies, insofar as *Fgf17^{-/-}* mice lack motor deficits but instead have a circumscribed set of higher-order social deficits (Searce-Levie et al., 2007).

Emx2^{-/-} mutants also show dorsal FC defects. Their dorsal FC is ventralized; markers of both the ventromedial orbital cortex (*Erm*, *Pea3*, *Ngn2*) and the ventrolateral FC (*Er81*) expand dorsally. Remarkably, many of these severe phenotypes are rescued in *Emx2^{-/-};Fgf17^{-/-}* mutants. To identify the mechanisms that cause the phenotypes in the neonatal single and compound mutants, we investigated the effects of the *Fgf8*, *Fgf17*, and *Emx2* mutations on the molecular features of the neuroepithelium in the embryonic FC.

Fgf8* is upstream of *Fgf17* and *Fgf18

We propose that *Fgf8* promotes the nested expression of *Fgf17* and *Fgf18* in the rostral patterning center (Fig. 5). Reduced expression of *Fgf8* in the *Fgf8^{neo/neo}* hypomorph resulted in decreased expression of *Fgf17* and *Fgf18*. *Fgf15* expression was only weakly affected in *Fgf8^{neo/neo}* hypomorphs. Although *Fgf17* expression was reduced ~200 μ m rostral to the patterning center, its expression remained robust within ~100 μ m of the *Fgf⁺* core domain (Fig. S12). We propose that the residual *Fgf17* expression has a key role in maintaining aspects of rostral identity in the *Fgf8^{neo/neo}* mutant (see below). Unlike the case in the *Fgf8^{neo/neo}* hypomorph, we did not detect an overt change of *Fgf8*, *Fgf15*, and *Fgf18* expression in the *Fgf17^{-/-}* mutant, showing that these genes are not strongly regulated by *Fgf17*. Thus, *Fgf8* lies upstream of the other three *Fgf*'s expressed in the rostral patterning center.

***Fgf8* and *Fgf17* have overlapping and distinct functions in FC patterning**

Both *Fgf8^{neo/neo}* and *Fgf17^{-/-}* mutants show reduced expression of two FGF-responsive genes, *Spry1* and *Spry2*. *Fgf8^{neo/neo}* mutants show a greater reduction in *Spry* expression than *Fgf17^{-/-}* null mutants, showing that this aspect of FGF signaling is particularly sensitive to *Fgf8* expression, given that ~40% of *Fgf8* expression persists in the *Fgf8^{neo/neo}* mild hypomorphs (Garel et al., 2003; Meyers et al., 1998). Further reduction of *Fgf8* expression in the *Fgf8^{neo/null}* severe hypomorph and in the conditional *Fgf8* null leads to a progressive decrease in *Spry1* expression and hypoplasia of the rostral telencephalon (Storm et al., 2003, 2006).

Fgf8 is believed to regulate telencephalic patterning by affecting the expression of several transcription factors, including *COUP-TFI*, *Emx2*, and *Sp8* (Crossley et al., 2001; Fukuchi-Shimogori and Grove, 2003; Storm et al., 2003, 2006). Here we compared transcription factor expression in the *Fgf8^{neo/neo}* and *Fgf17^{-/-}* mutants. Both show rostradorsal expansion of *COUP-TFI* expression, although the increase in *COUP-TFI* was subtle in the *Fgf17^{-/-}* mutants. Overexpression of *COUP-TFI* suppresses rostral cortical fate (Faedo et al., 2007),

which is consistent with loss-of-function analysis (Armentano et al., 2007; Zhou et al., 2001). Thus, we propose that both *Fgf8* and *Fgf17* contribute to rostral cortical fate through repression of *COUP-TFI*, although *Fgf8* appears to have a more prominent role in this process (Fig. 5).

Fgf8^{neo/neo} mutants also have a rostroventral expansion of *Emx2* expression (Figs. 4F, S9; Garel et al., 2003); we do not detect increased *Emx2* expression in the *Fgf17*^{-/-} mutants (Figs. 4F, S8). Overexpression of *Emx2* suppresses rostral cortical fate (Hamasaki et al., 2004). We propose that an increase of *Emx2* in the rostromedial FC accounts for the rostral expansion of molecular features of the posterior parts of the dorsomedial FC (cingulate cortex Cg1 and Cg2) in the *Fgf8*^{neo/neo} mutant (Fig. 1). The *Fgf17*^{-/-} mutants do not have this phenotype, perhaps because *Fgf8* expression is preserved, which prevents rostral expansion of *Emx2* into this region. Thus, we propose that *Fgf8* and *Fgf17* have distinct roles in suppressing the caudalizing function of *Emx2* (Fig. 5). Previously, we demonstrated that *Fgf8* represses rostral expression of *Wnt8b* (Storm et al., 2006). Thus, the rostral expansion of *Emx2* in the *Fgf8*^{neo/neo} mutant could be due to increased Wnt expression/signaling, in that *Emx2* is known to be Wnt-regulated (Theil et al., 2002).

Whereas *COUP-TFI* and *Emx2* expression is increased more in *Fgf8*^{neo/neo} than in *Fgf17*^{-/-}, expression of *Erm*, *Pea3* and *Sp8* are reduced more in *Fgf17*^{-/-} than in *Fgf8*^{neo/neo} mutants (Fig. 4). In *Fgf8*^{neo/neo} mutants, *Fgf17* expression remains strong near the regions that express *Er81*, *Erm*, and *Pea3*, suggesting that *Fgf17* is responsible for preserving the expression of these transcription factors. Thus, *Fgf17* may regulate local patterning within dorsomedial parts of the FC through positive regulation of “rostral” transcription factors (*Erm*, *Pea3*, *Sp8*), whereas *Fgf8* function may be responsible for regulating the gradients of more broadly expressed transcription factors, such as *COUP-TFI* and *Emx2*.

Recent evidence suggests that *Sp8* contributes to cortical patterning at least in part by modulating expression of *Emx2* and *Pax6* (Sahara et al., 2007; Zembrzycki et al., 2007). *Fgf8* and *Sp8* have been shown to affect each other's expression reciprocally (Kawakami et al., 2004; Sahara et al., 2007; Storm et al., 2006). Thus, it is surprising that *Fgf17*^{-/-} embryos display a striking decrease in *Sp8* expression specifically in the dorsomedial neuroepithelium, whereas *Fgf8*^{neo/neo} mutants do not. This may be explained if both *Fgf8* and *Fgf17* are capable of positively regulating *Sp8* expression. In *Fgf17*^{-/-} mutants, the selective decrease in *Sp8* expression occurs rostrally, whereas, more caudally, closer to where *Fgf8* expression is maintained, *Sp8* expression is less affected. By contrast, there is residual *Fgf8* and *Fgf17* expression in *Fgf8*^{neo/neo} mutants that may be sufficient to support *Sp8* expression.

***Emx2* represses FGF expression/signaling and FC patterning**

Emx2^{-/-} mutants have more intense and expanded expression domains of *Fgf8* and *Fgf17*, supporting previous findings (Fukuchi-Shimogori and Grove, 2003). *Spry1* expression was also increased rostradorsally in these mutants. In addition, *Fgf15* expression is up-regulated in several telencephalic structures: septum, rostral subpallium, pallial/subpallial boundary, LGE/MGE boundary, and caudoventral cortical primordium. Therefore, *Emx2* has a

widespread role in repressing *Fgf* expression and signaling in the telencephalon. The increased expression of *Fgf8*, *Fgf15*, and *Fgf17* then presumably contributes to modifying cortical fate through changes in transcription factor expression; our analysis of *Emx2*^{-/-};*Fgf17*^{-/-} mutants provides direct evidence for this hypothesis.

Emx2 promotes caudodorsal cortical fate (Bishop et al., 2000, 2002, 2003; Hamasaki et al., 2004; Mallamaci et al., 2000; Muzio and Mallamaci, 2003; Muzio et al., 2005). Consistently with this, *Emx2*^{-/-} mutants had more extensive expression of *Er81*, *Erm*, *Pea3*, and *Sp8* in the rostradorsal cortical neuroepithelium. We propose that *Erm*, *Pea3*, and *Sp8* have a direct role in defining dorsal FC identity, insofar as the rostral neocortex of neonatal *Emx2*^{-/-} mutants has expanded expression of FC markers (*Lmo4*, *Cad8*, *Id2*, and *Steel*; Figs. 1, S4). Furthermore, we propose that *Er81* and *Pea3* promote ventrolateral and orbital FC identity, because expression of these genes spreads dorsally from the ventral parts of the FC neuroepithelium (Figs. 4C,D, S10); they may contribute to the dorsal shift in *Ngn2* expression (Figs. 1F, S4). *Emx2* may also contribute to specifying ventrolateral FC by promoting *COUP-TFI* expression.

***Fgf17-Emx2* interactions antagonistically regulate FC patterning**

To what extent are the increases in rostral cortical identity mediated by the increased *Fgf17* signaling in the *Emx2*^{-/-} mutant? The expression of transcription factors *Er81*, *Erm*, and *Pea3* in progenitor cells is normalized in *Emx2*^{-/-};*Fgf17*^{-/-} double mutants relative to both single mutants, which correlates with the normalization of FC marker expression in the neonatal dorsal and ventral/ orbital FC (Figs. 1, 4B,C,D, 6, S5, S11). On the other hand, for one double mutant brain, we observed only a partial rescue of FC molecular features, suggesting that other genetic factors (such as *Fgf8*) may play a modifying or functionally redundant role. We propose that restoration of *Erm*, *Er81*, and *Pea3* expression accounts at least in part for the rescue of neonatal FC molecular features in the *Emx2*^{-/-};*Fgf17*^{-/-} double mutants (Fig. 1).

Despite the robust rescue of many FC features, some phenotypes of the *Emx2*^{-/-} mutants were not rescued in *Emx2*^{-/-};*Fgf17*^{-/-} double mutants. This includes the elevated expression of *Fgf8* and *Fgf15*, a result that underscores the importance of reducing *Fgf17* in the rescue of FC patterning (Figs. 3K,L, S5, S6). In addition, the double mutants exhibit persistently elevated expression of *Sp8*, reduced expression of *COUP-TFI*, and ectopic expression of *Er81* in the cortical plate. It merits comment that the persistent increase in *Sp8* expression in the *Emx2*^{-/-};*Fgf17*^{-/-} double mutants could be due to elevated expression of *Fgf8*, insofar as *Sp8* is known to be positively regulated by *Fgf8* (Storm et al., 2006).

In summary, we have shown that *Fgf8*, *Fgf17*, and *Emx2* play distinct roles in the control of transcription factor gradients in progenitor cells of the FC primordium. We propose that *Fgf8* has a pivotal role in establishing the expression of the other *Fgf* s and in shaping the gradients of transcription factor expression as the telencephalon forms, particularly of *Emx2* and *COUP-TFI*. Then, the other *Fgf* s have the primary role in shaping the local levels of transcription factor expression that determine local regional identity. *Emx2* and the *Fgf* genes share reciprocal functions in regulating cortical patterning; in the FC, this is

accomplished at least in part through controlling the levels of *Erm*, *Er81*, *Pea3*, and *Sp8* expression.

These insights have implications for the protomap model in which neurons are committed to their areal position at the time of their last cell division in the proliferative zones (Caviness and Rakic, 1978; McConnell, 1988; Rakic, 1988). Individual cortical areas may be selectively changed in size during the course of evolution by altered expression of *Fgf* and downstream transcription factorsignaling mechanisms. Further studies will be needed to establish the functions of these *Fgf*-regulated transcription factors in FC development and to determine whether changes in *Fgf* signaling might have contributed to the evolution of the higher cortical areas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. *Fgf8/17* and *Emx2* antagonistically regulate FC regionalization. Top row: Schema showing a summary of changes in FC molecular subdivisions at P0. Dorsal FC is in red, ventral/orbital FC is in blue, and parietal cortex is in yellow. The asterisk over the double mutant indicates that there is variability in the degree of rescue. In situ hybridization (ISH) on P0 wild-type (A,F,K,P,U,Z), *Fgf8*^{neo/neo} (B,G,L,Q,V,AA), *Fgf17*^{-/-} (C,H,M,R,W,BB), *Emx2*^{-/-}; *Fgf17*^{+/-} (D,I,N,S,X,CC), and *Emx2*^{-/-}; *Fgf17*^{-/-} (E,J,O,T,Y,DD) coronal sections for the following genes: *Lmo4*, *Nt3*, *Lmo3*, *Cad6*, *RzrB*, and *Ngn2*; a single plane of section

is shown for each genotype/gene expression; see Figures S1–S6 for full data sets. The wild-type and *Fgf17*^{-/-} series were published previously (Cholfin and Rubenstein, 2007) and are shown here for purposes of comparison. FC phenotypes of *Emx2*^{-/-} and *Emx2*^{-/-};*Fgf17*^{+/-} mutants were comparable; sections from a *Emx2*^{-/-};*Fgf17*^{+/-} brain are shown. Arrows signify shifted borders, whereas arrowheads indicate maintained borders. D, dorsal FC; DP, dorsal peduncular cortex; dLO, dorsolateral orbital cortex; dM, dorsomedial FC; dMc, caudal dorsomedial FC; LO, lateral orbital cortex; MO, medial orbital cortex; OB, olfactory bulb; Par, parietal cortex; VO, ventral orbital cortex. Scale bar = 0.5 mm.

Fig. 2.

Differential regulation of rostral patterning center signaling by *Fgf8* and *Fgf17* at E10.5. In situ hybridization on wild-type (A,D,G,J,M,P), *Fgf17*^{-/-} (B,E,H,K,N,Q), and *Fgf8*^{neo/neo} (C,F,I,L,O,R) horizontal sections for *Fgf8*, *Fgf18*, *Fgf17*, *Fgf15*, *Spry1*, and *Spry2*. Arrowheads point to the limit of the *Fgf8* expression domain, where there is coexpression of *Fgf8/15/17/18* and *Spry1/2*. Boxed areas of adjacent rostradorsal neuroepithelium lack *Fgf8/18* expression, show lower levels of *Spry1/2* expression, and maintain *Fgf17*.

expression. Asterisks adjacent to the boxed region indicate reduced *Spry1* and *Spry2* expression in the *Fgf17*^{-/-} mutant neuroepithelium. Scale bar = 0.5 mm.

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Fig. 3.

Regulation of rostral patterning center signaling by *Fgf8* and *Fgf17* at E12.5. A–R: ISH on coronal sections from wild-type (A,D,G,J,M,P), *Fgf17*^{-/-} (B,E,H,K,N,Q), and *Fgf8*^{neo/neo} (C,F,I,L,O,R) brains for *Fgf8*, *Fgf18*, *Fgf17*, *Fgf15*, *Spry1*, and *Spry2*. Arrows indicate regions of interest in the cortical ventricular zone described in Results. A single plane of section is shown for each genotype/gene expression; see Figures S7–S12 for full data sets. Scale bar = 0.5 mm.

Fig. 4.

Emx2 suppression of rostral patterning center FGF signaling at E12.5. A–H: ISH on coronal sections from wild-type (A,C,E,G) and *Emx2*^{-/-} (B,D,F,H) brains for *Fgf8*, *Fgf17*, *Fgf15*, and *Spry1*. I–L: ISH on coronal sections from wild-type (I,K) and *Emx2*^{-/-};*Fgf17*^{-/-} (J,L) brains for *Fgf8* and *Fgf15*. Arrows indicate regions of interest in the cortical ventricular zone described in Results. A single plane of section is shown for each genotype/gene expression; see Figures S7– S12 for full data sets. Scale bar = 0.5 mm.

Fig. 5.

Fgf8, *Fgf17*, and *Emx2* differentially control expression of transcription factors in the rostral cortical primordium. ISH on E12.5 wild-type (A,F,K,P,U,Z), *Fgf17*^{-/-} (B,G,L,Q,V,AA), *Fgf8*^{neo/neo} (C,H,M,R,W,BB), *Emx2*^{-/-} (D,I,N,S,X), and *Emx2*^{-/-}; *Fgf17*^{-/-} (E,J,O,T,Y) coronal sections for *Sp8*, *Erm*, *Pea3*, *Er81*, *COUP-TF1*, and *Emx2*. Dashed boxes draw attention to the area in each panel with the largest change in expression, as described in Results. Asterisks in S and T indicate ectopic cortical plate *Er81* expression, as described in Results. A single plane of section is shown for each genotype/gene expression; see Figures

S7–S11 for full data sets. Schemas are drawn above the ISH results; they summarize our interpretation of the observed changes in ventricular zone gene expression. Note that frontrodorsomedial expressions of *Sp8*, *Erm*, and *Er81* are more reduced in *Fgf17*^{−/−} than in *Fgf8*^{neo/neo}, whereas *COUP-TFI* expression is increased more in the *Fgf8*^{neo/neo} than in the *Fgf17*^{−/−} mutant. *Emx2* expression does not appear to change in *Fgf17*^{−/−}, whereas it is increased in *Fgf8*^{neo/neo} (not shown in schema because of space constraints). n.c., No change. Asterisks indicate rescue of gene expression in the double mutant. Scale bar = 0.5 mm.

Fig. 6.

Model of *FGF-Emx2* genetic interactions in FC patterning. **A:** Dorsal schematic views of the cortical progenitor domain showing spatial relationships of rostral patterning center FGFs and transcription factors gradients. Rostral/anterior is to the top. FGF ligands (*Fgf8*, *Fgf18*, *Fgf17*, and *Fgf15*) are expressed in a nested pattern in the rostral patterning center; the core domain is *Fgf8/17/18*⁺ and is surrounded by a "penumbra" that dorsally is *Fgf17*⁺ and ventrally is *Fgf15*⁺. The core has higher levels of FGF signaling based on *Srpy1/2* expression. Transcription factor gradients of *Sp8*, *Erm*, *Pea3*, *Er81*, *COUP-TF1*, and *Emx2*

in the cortical primordium are approximated. **B:** Schematic of proposed genetic interactions between rostral patterning center FGFs and transcription factors relevant for global cortical patterning and local FC patterning. Acting more globally, *Fgf8* has a repressive function (lines with bars) on “caudalizing” transcription factors *Emx2* and *COUP-TF1* that may regulate the allocation of anterior and posterior cortical regions. As a subset of its functions, *Fgf8* positively regulates *Fgf17* expression (arrows). Locally within the FC primordium, *Fgf17* promotes *Er81*, *Erm*, *Pea3*, and *Sp8* expression (arrows) and represses *COUP-TF1*, which together may contribute to specifying frontal cortex regional properties.

TABLE 1**Frontal Cortex Subdivision Definitions**

Gene-defined region	Anatomical areas	
	Zilles and Wree, 1995	Krettek and Price, 1977
Dorsolateral (dL)	Fr1, Fr3	PrCl
Dorsal (D)	Fr1, Fr2	PrCl, PrCm
Dorsomedial (dM)	Cg3	PL
Dorsomedial caudal (dMc)	Cg1, Cg2	ACd, ACv
Infralimbic (IL)	IL	IL
Medial orbital (MO)	MO	MO
Ventral orbital (VO)	VO	VO
Lateral orbital (LO)	LO	LO
Dorsolateral orbital (dLO)	DLO	DLO
Agranular insular (AI)	AID/AIV	AId/AIv
Parietal (Par)	Par1	S1