Mechanisms of cerebral cortical patterning in mice and humans

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All the higher mental and cognitive functions unique to humans depend on the neocortex ('new' cortex, referring to its relatively recent appearance in evolution), which is divided into discrete areas that subserve distinct functions, such as language, movement and sensation. With a few notable exceptions, all neocortical areas have six layers of neurons and a remarkably similar thickness and overall cell density, despite subtle differences in their cellular architecture. Furthermore, all neocortical areas are formed over roughly the same time period during development and provide little hint at early developmental stages of the rich functional diversity that becomes apparent as development comes to an end. How these areas are formed has long fascinated developmental neuroscientists, because the formation of new cortical areas, with the attendant appearance of new cortical functions, is what must have driven the evolution of mammalian behavior.

There are two general viewpoints about how cortical areas form which can be seen as defining the ends of a mechanistic spectrum. One school of thought suggests that cortical organization reflects the afferent input received—for example, visual cortex is visual because that is where information from the eyes ends up[1,2]. There is now a large body of literature supporting the importance of destination and electrical activity of afferent inputs in shaping cortical pattern and refining the cellular architecture of cortical areas. This literature has been reviewed recently[3] and will not be further discussed here.

The other school of thought suggests that a significant amount of patterning information exists in the cortex before and independent from the arrival of afferent inputs. Indeed, early experimental evidence for such 'intrinsic' patterning of the cortex preceded by decades our more recent insights into how these differences might be determined. Both limbic cortex, the evolutionarily 'older' neighbor of neocortex[4,5], and neocortical regions[6,7] have a molecular 'memory' of their origin when deprived of normal afferent input in transplant or explant settings during cortical neurogenesis. More recently, a striking amount of intrinsic cortical patterning has been shown in two different mouse mutants that lack thalamocortical connections, the major afferent input into the cortex[8,9]. These and other studies suggest that intrinsic cortical specification occurs by the time neurons are being generated by the dividing progenitor cells of the cortex, which lie next to the ventricles in a layer known as the ventricular zone (VZ). The possibility that positional information could be encoded by the cortical VZ progenitor cells themselves, then maintained by postmitotic cortical neurons, developed from the observation that most postmitotic neurons enter the cortex from the cortical VZ through a restricted radial migration along radially oriented glial guide fibers[10].

There is now considerable evidence supporting the cortical VZ as a repository of positional information that is critical for cortical areal patterning. The mechanisms involved in patterning the cortical VZ are the subject of this review. Although much of our insight into these mechanisms has relied on studies in mice, humans are subject to a wide variety of naturally occurring mutations that have identified cortical patterning genes through a 'forward genetics' approach. We therefore attempt to integrate mouse and human studies into a hierarchy of events that pattern the cortical VZ.

Early morphological changes in the forebrain

To define the anatomical context in which the developing cortex is patterned, we first summarize the dramatic topological transformations that accompany the earliest stages of corticogenesis (**Fig. 1**). After the initial induction of neural tissue[11], the cortex is formed at the rostralmost portion of the neural tube and is quickly subdivided into two halves—the left and right telencephalic vesicles (the future cerebral hemispheres). This subdivision depends on the dorsal midline roof plate, where low levels of proliferation and high levels of apoptosis result in its fixation and invagination relative to the rapidly expanding hemispheres[12] (**Fig. 1**). During this period, each hemisphere forms a distinct bulge in its ventral region, the ganglionic eminence (the future basal ganglia), which morphologically distinguishes ventral from dorsal telencephalon. As these events proceed, the cortex becomes exposed to several potential sources of secreted signaling molecules, often called 'organizers', which include, first, the anterior neural ridge in the rostral midline, second, the roof plate in the dorsal midline, third, the cortical hem, which lies between the dorsal midline roof and the cortical VZ, and fourth, other potential sources of signaling molecules, such as the surface ectoderm (the future skin) and mesenchymal elements that lie between the

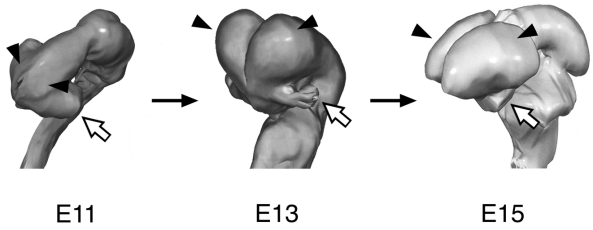

Fig. 1. Morphogenesis of the forebrain. Schematic of forebrain morphogenesis from the time of rostral neural tube closure (embryonic day (E) 11 in rat, E9 in mice, 3–4 weeks in humans) through the formation of the two telencephalic (cerebral) vesicles (E13 in mice, 7–8 weeks in humans). At the time of neural tube closure, the telencephalic vesicles (cerebral hemispheres) are not morphologically detectable. Rapid expansion of the telencephalon then begins, except in the dorsal midline roof plate, which results in fixation and relative invagination of the telencephalon at the dorsal midline and the apparent 'cleavage' of the forebrain. Arrowheads, location of telencephalic vesicles; open arrows, eyes. Three-dimensional images of rat forebrain reprinted with permission from ref. 96.

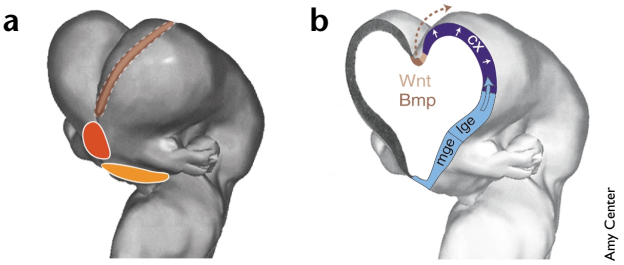

Fig. 2. Organizers of the cortical VZ. (**a**) Schematic three-dimensional view of the E13 rat forebrain, illustrating the locations of the prechordal mesoderm (orange), anterior neural ridge (red), roof plate and cortical hem (brown). Each of these organizers is associated with a particular growth factor or family of factors: the prechordal mesoderm produces Sonic hedgehog (Shh), the ANR produces Fgf8, the roof plate region produces bone morphogenetic proteins (Bmps), and the cortical hem produces Wnts. (**b**) Schematic coronal view of the E13 rat forebrain, illustrating the dorsal midline sources of Bmps (dark brown) and Wnts (light brown) that act on the adjacent cortical VZ. Information encoded by cortical VZ progenitor cells provides a 'protomap' that becomes radially translated into the cortical areas of mice and humans (blue). Neurons also migrate into the mature cortex from subcortical sites (lge and mge; light blue), which provide an additional potential source of patterning information. Modified with permission from ref. 96.

skin and brain, including the future meningeal cells that surround the adult brain (**Fig. 2a**).

Cortical VZ as a source of projection neurons

Direct studies of cell lineage are consistent with the idea that cortical VZ progenitor cells help to establish cortical patterning (**Fig. 3**). These studies show that cortical VZ progenitors can form large columnar 'clones' of neurons (**Fig. 3a**), most of which become glutamatergic projection neurons of the cortex[13–17]. Because projection neurons form the efferent output of the cortex and define areal-specific connectivity, these cell-lineage studies provide the cortical VZ with the potential to directly affect area-specific development. A defect that resembles these large columnar clones can be seen in certain human cortical dysplasias ('abnormal development'; **Fig. 3b**). Human cortical dysplasias can involve large expanses of the cortex, but more often involve only small cortical regions. The shape of these dysplasias—presumably clonal in origin, though this is not proven—is occasionally strikingly reminiscent of the large clones seen in cell lineage studies, with a broadening 'tornado' outline and an apparent origin in or near the VZ (**Fig. 3a** and **b**). These highly epileptic lesions commonly express glutamatergic markers, but often lack GABAergic markers, which is consistent with a clonal origin from cortical VZ progenitor cells[18–20].

In contrast to the columnar clones of cortical VZ progenitors, other cortical neurons are derived from clones that disperse widely across the cortex[14,15,21–23] (**Fig. 3c**). Remarkably, many if not all of these widespread clones are not generated by cortical VZ progenitors at all, but instead by progenitor cells in subcortical sites, including the ganglionic eminence[24,25]. Subcortically derived neurons migrate long distances to reach the cortex and seem to represent the bulk of cortical GABAergic interneurons (roughly 75% in mice)[16,26] as well as some glutamatergic neurons[4,24,25,27] (see Fishell review in this issue). Another potential source of cortical neurons that come from outside the cortical VZ is the roof plate region[28]. Whether these non-cortical VZ-derived neurons are important for area-specific patterning or are selectively deficient in any human cortical disorders remains unknown, although cortical GABAergic neuronal dysfunction may be central to the pathogenesis of both schizophrenia and bipolar disorder[29,30].

Induction of the telencephalon

The cell-lineage studies show that the cortical VZ has the potential to directly affect cortical areal development, but how does the cortical VZ get formed in the first place? Perhaps the earliest definitive step in cortical VZ induction is mediated by an organizer at the rostralmost end of the developing embryo known as the anterior neural ridge[31] (ANR; **Fig. 2a**). Removal of the ANR from explants results in a failure to express Foxg1 (previously Bf1), a transcription factor that selectively marks future cortical VZ progenitors before the telencephalon is morphologically distinguishable[31,32] and that is required for normal telencephalic and cortical morphogenesis[33]. The ability of the ANR to induce Foxg1 expression in explants can be mimicked by the exogenous application of an ANR-derived signaling molecule, fibroblast growth factor 8 (Fgf8). Because some cortical tissue remains in mice without Foxg1 function[33,34], the ANR-Fgf8-Foxg1 pathway may be complemented by other pathways that help induce the cortex.

Induction of the midline roof plate

A step in forebrain development that seems distinct from telencephalic induction is the formation of the dorsal midline roof plate, as illustrated by a mouse and human malformation known as holoprosencephaly (HPE). HPE, the most common congenital brain malformation in humans, is defined by the failure to separate the forebrain into two hemispheres, resulting in a single forebrain 'holosphere' and a continuous cerebral cortex across the midline[35] (**Fig. 4**). The cerebral cortex is always smaller than normal but present[36], whereas the lack of forebrain division and continuity of cortex across the dorsal midline indicate a failure in dorsal midline development. The pathogenesis of HPE therefore involves a fundamental defect of the roof plate, and the genes implicated in HPE are likely to belong to signaling pathways that induce and/or maintain roof plate function or confer competence in roof plate neuroepithelium to respond to such signals. At least 12 genetic loci have been implicated in human HPE[37], and the genes corresponding to four of these loci are now known.

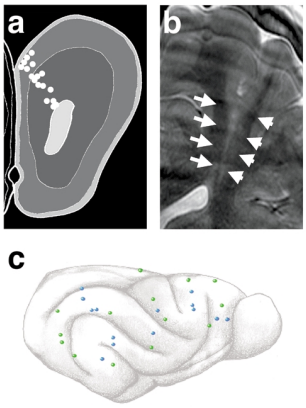

Fig. 3. Clonal patterns and human cortical dysplasia. (**a**) Cortical cluster, presumably clonal, from a ferret brain labeled by injection of a retroviral library at E28 and analyzed at P0 (before neurogenesis and neuronal migration to the cortex are complete). The cluster is ~300 μm in each tangential dimension, and includes cells stretching from the ventricle to the developing cerebral cortex (modified from ref. 15). (**b**) Human cortical dysplasia imaged from the brain of a child with severe epilepsy (courtesy of Dr. Ellen Grant[97]). Dysplastic cells have abnormal MRI signal characteristics and are found in a funnel-shaped pattern that extends from near the ventricle to the cortex, which appears focally thickened. (**c**) Two widepread clones (one in green, one in blue) from a ferret cortex labeled by injection of a retroviral library at E33 and analyzed 24 days after birth. Widespread clones, particularly in ferrets where they typically contain many neurons, characteristically cover almost the entire cortical surface (modified from ref. 27).

One of the genes implicated in human[37] and mouse[38] HPE is Sonic hedgehog (*SHH*, the HPE3 locus), a well-known secreted signaling molecule that acts as a primary inducer of ventral neural structures and fates in several organisms[35,39]. In the mouse forebrain, Shh is expressed in ventral domains within neural tissue (the hypothalamic region and ventral telencephalon) and in prechordal mesoderm, a group of mesodermal cells that underlie the ventral forebrain[35,39] (**Fig. 2a**). In mice, total loss of Shh function results in HPE[38], whereas selective loss of Shh from its ventral telencephalic domain does not cause HPE[40]. This suggests that the HPE phenotype and roof plate induction rely on Shh that is produced in prechordal mesoderm and/or the hypothalamic region.

In addition to *SHH*, heterozygous germline mutations in three other genes have been implicated in human HPE, all of which encode transcription factors: the *SIX3* homeobox gene (HPE2)[41,42], the *ZIC2* zinc-finger gene (HPE5)[43,44] and the *TGIF* homeobox gene (HPE4)[45,46]. Presumably these transcription factor genes form some kind of genetic pathway along with *SHH*, but exactly how they interrelate is still unknown. In mice, Six3 is expressed first in rostralmost neural and non-neural tissue, then later within the ventral forebrain[47], thus coinciding with SHH-expressing regions as well as the ANR. Six3 may therefore be important for anterior and/or ventral organizer function, but the *Six3* null phenotype in mice has not yet been reported.

In contrast to Shh and Six3, Zic2 is predominantly expressed in the dorsal neural tube in and around the roof plate[48]. This distinctive dorsal pattern of Zic2 expression is consistent with the lack of significant craniofacial defects in humans[43,44] and mice[49] with HPE due to *ZIC2* mutations, and suggests that Zic2 is involved in the induction and/or maintenance of the roof plate. TGIF has been genetically linked to SHH on the basis of coexisting *TGIF* and *SHH* mutations within some HPE families[45]. In mice, TGIF has also been implicated in a transforming growth factor-β (TGFβ) signaling pathway[50–52] that leads to HPE when disrupted[46], but the site of action of TGIF and its relationship to the SHH pathway remain to be determined.

In summary, a failure in roof plate development represents a central defect in HPE, but exactly how the growing number of HPE genes interrelate and lead to roof plate dysfunction remains unclear. Some HPE genes (*SHH* and *SIX3*) are expressed in or around the ANR and prechordal mesoderm (**Fig. 2a**), suggesting that these anterior and ventral organizers are somehow linked to dorsal roof plate development. On the other hand, *ZIC2* is expressed in the roof plate itself and seems to be essential for normal roof plate development.

Specification of the dorsal telencephalon

After its initial induction by extrinsic signals, the telencephalon becomes specified into dorsal (pallial) and ventral (subpallial) regions, and certain basic helix-loop-helix (bHLH) transcription factors regulate this process[53]. The bHLH proteins Neurogenin1 (Ngn1) and Ngn2 are selectively expressed by dorsal telencephalic progenitors, and *Ngn2* or *Ngn1;Ngn2* mutant cortices show a loss of dorsal markers and a gain of ventral markers, including the ventral bHLH gene *Mash1*. Misexpression of Mash1 in the cortical VZ is sufficient to drive ventral marker expression, suggesting that Ngn1 and Ngn2 promote cortical development by suppressing Mash1-dependent ventral fates[53]. Like the Neurogenins, mouse Gli3 is expressed throughout the dorsal telencephalon, and the Gli3 null cortex lacks certain dorsal cortical (hippocampal) markers and gains expression of ventral markers[54]. Mutations in the *GLI3* gene have been discovered in some human diseases, but it is uncertain if these mutations cause abnormal cortical development. The Ngn1/2 and Gli3 studies suggest that dorsal specification of the cortical VZ involves the suppression of ventral fates. The defects in Ngn1/2 and Gli3 mutants are not identical, however, and Ngn2 expression does not appear to be affected in $Gli3^{-/-}$ embryos[54]. Gli3 and Ngn2 may therefore act in parallel pathways rather than in series to regulate dorsal specification of the cortical VZ.

Selection of a cortical VZ fate

Following dorsal telencephalic specification, the dorsal telencephalon must be further subdivided into dorsal midline epithelial fates (choroid plexus epithelium and cortical hem) and the cortical VZ, a process in which the LIM homeobox gene *Lhx2* has a specific role. Lhx2 is selectively expressed in the cortical VZ but not in dorsal midline epithelia, and mice lacking *Lhx2* show a near-total loss of the cortical VZ and a massive excess of dorsal midline fates[28,55]. Residual cortical VZ progenitors in *Lhx2* mutants (as defined by expression of the mutant *Lhx2* allele[28]) continue to express Foxg1 and Ngn2, thus indicating a normal dorsal telencephalic progenitor identity, but fail to express several cortical VZ markers[28,55]. This suggests that Lhx2 is not essential for the specification of telencephalic or dorsal identity, but acts instead to 'select' a cortical VZ fate from already-specified dorsal telencephalic progenitors[28]. This Lhx2 selector function may be conserved evolutionarily, because the *Drosophila* orthologue of *Lhx2 (apterous)* is a well-known selector gene that acts in a similar fashion within the dorsal wing compartment[56–58].

Regional specification of the cortical VZ

Once the cortical VZ fate is selected, regional specification of the cortical VZ must occur. This critical step in cortical VZ patterning remains poorly understood, but some studies suggest that localized signaling molecules may be central to this process. For example, sensorimotor VZ progenitors can be reprogrammed to induce limbic cortex-specific markers (LAMP)[5] and connectivity[59,60] when transplanted at appropriate times into putative

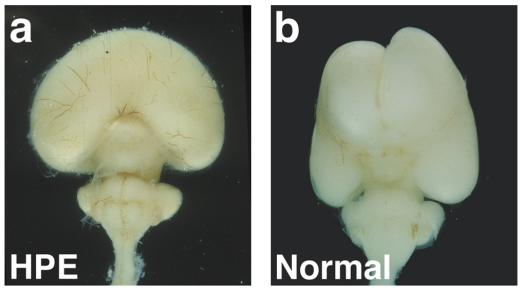

Fig. 4. Human holoprosencephaly. Anterior views of the CNS from an 18-week gestation human fetus with holoprosencephaly (**a**) and a normal 13-week fetus (**b**). The fetus with holoprosencephaly has a single forebrain vesicle ('holosphere') and a cortex that is continuous across the dorsal midline, due to the lack of relative invagination of the roof plate region.

limbic areas, suggesting the importance of location within the cortical VZ for regional specification. The reprogramming for limbic marker expression can be recapitulated *in vitro* by treating actively cycling sensorimotor progenitors with TGFα, a ligand for the erbB receptors, which are differentially expressed by cortical VZ progenitor cells[61]. Finally, the highest expression levels of TGFα in the normal brain occur in the subpallium, and the cortical regions closest to the subpallium are the ones that express LAMP[61]. Taken together, these findings lead to a model in which regional specification of the cortical VZ depends on the localized production of instructive signaling molecules as well as the location and receptor repertoire of individual progenitor cells in the cortical VZ[61].

Expansion of the cortical VZ

In addition to their potential roles in regional specification, signaling molecules seem to regulate the expansion of the cortical VZ. One source of these signals is the dorsal midline roof plate[28] (**Fig. 2**). Selective ablation of roof plate cells results in a cortical VZ that is signficantly reduced in size. Because cortical tissue is not directly ablated in these experiments[28], the roof plate seems to provide signals that act on the cortex at a distance. The role for roof plate signals in cortical expansion would be consistent with the holoprosencephaly phenotype, in which the roof plate deficit is associated with a cortex that is present, but always small. Roof plate-derived signals are likely to include secreted proteins known as the bone morphogenetic proteins (Bmps; **Fig. 2**), based on their central roles in roof plate-mediated signaling during spinal cord development[62]. Multiple *Bmp* genes are induced in and around the forebrain roof plate before the telencephalon can be recognized morphologically (~E8.5)[12], and the disproportionately small telencephalon in mice lacking Bmp5 and Bmp7 (ref. 63) provides genetic evidence that Bmp signals regulate expansion. In addition to regulating cortical VZ expansion, roof plate-derived Bmps may also regulate cortical VZ patterning, because roof plate signals, which probably include Bmp4 and Bmp2, regulate the graded expression of Lhx2 in the cortical VZ[28].

Signals from the cortical hem, which is located next to the dorsal midline roof (**Fig. 2b**), are also directly implicated in local cortical VZ expansion[12,64,65]. Like Bmps, the Wnts represent a large family of secreted proteins that mediate signaling functions in a number of embryonic systems. Mouse *Wnt* genes are activated in and around the cortical hem after the cortical VZ has been induced (~E11.5)[64], and loss of hem-specific Wnt3a function in mice causes defective proliferation of progenitors in the hippocampal VZ, the region of the cortical VZ immediately adjacent to the cortical hem[65]. This Wnt3a null defect appears to be phenocopied by loss-of-function mutations to mouse Lef1, a transcriptional mediator of Wnt signaling that is expressed by hippocampal VZ progenitors[66], suggesting that a cortical hem-Wnt3a-Lef1 pathway is critical for hippocampal VZ proliferation. These studies support the notion that Wnt signals have a primary role in stimulating proliferation throughout the mouse nervous system[65,67].

Regulating the relative sizes of cortical areas

Recent studies on the *Emx2* and *Pax6* homeobox genes demonstrated how patterning of the cortical VZ can affect cortical areal development. Specifically, these two transcriptional regulators expressed in the cortical VZ regulate the relative sizes of cortical areas. Emx2 and Pax6 are expressed in graded and opposing fashions within the cortical VZ: the Emx2 gradient is high posterior–low anterior, whereas the Pax6 gradient is low posterior–high anterior[68]. Loss of Emx2 function in mice results in marked size reductions to posterior cortical areas (including hippocampus and visual neocortical areas), whereas anterior neocortical regions (including motor areas) are either shifted or expanded[68,69]. Correspondingly, loss of Pax6 function (*Small eye* mice) results in a decreased anterior neocortical size[68]. Importantly, these defects correspond well to the normal expression gradients of Emx2 and Pax6, suggesting that the Emx2 and Pax6 countergradients within the cortical VZ provide an intrinsic code that directly regulates cortical area size.

The human *EMX2* and *PAX6* genes have also been implicated in human cortical malformations and may reflect the essential functions of Emx2 and Pax6 defined in mice. Human *EMX2* mutations are associated with a rare cortical malformation known as schizencephaly ('split brain'), which is characterized by a full-thickness defect or cleft in the cerebral wall (**Fig. 5**). Heterozygous germline mutations in *EMX2* have now been described in several sporadic cases and in affected siblings[70–72]. In general, *EMX2* mutations are found in severe cases of schizencephaly in which much of the cerebral cortex is absent. When less of the cortex is affected, the location of the clefts is neither stereotyped[70,72] nor predisposed to occur in the posterior cortical regions most dependent on Emx2 function in mice[68,69]. It is possible, however, that haploinsufficiency together with incomplete penetrance could account for the differences among humans carrying the same *EMX2* mutation[72] and between humans and mice with *EMX2* mutations.

Humans with *PAX6* mutations possess a complex brain malformation that compares favorably with the Pax6 mutant phenotype in mice[73,74]. In addition to eye defects, human *PAX6* heterozygotes have subtle alterations in forebrain size and shape[74]. More severe defects in the olfactory system and cerebral cortex are present in a compound heterozygote carrying two different

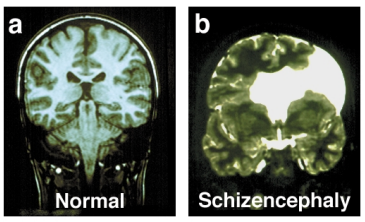

Fig. 5. Human schizencephaly. Coronal MRI scans of normal (left) and schizencephaly (right) patients (courtesy of Dr. Alma Bicknese). The schizencephaly patient has a cleft that extends through the entire cerebral wall.

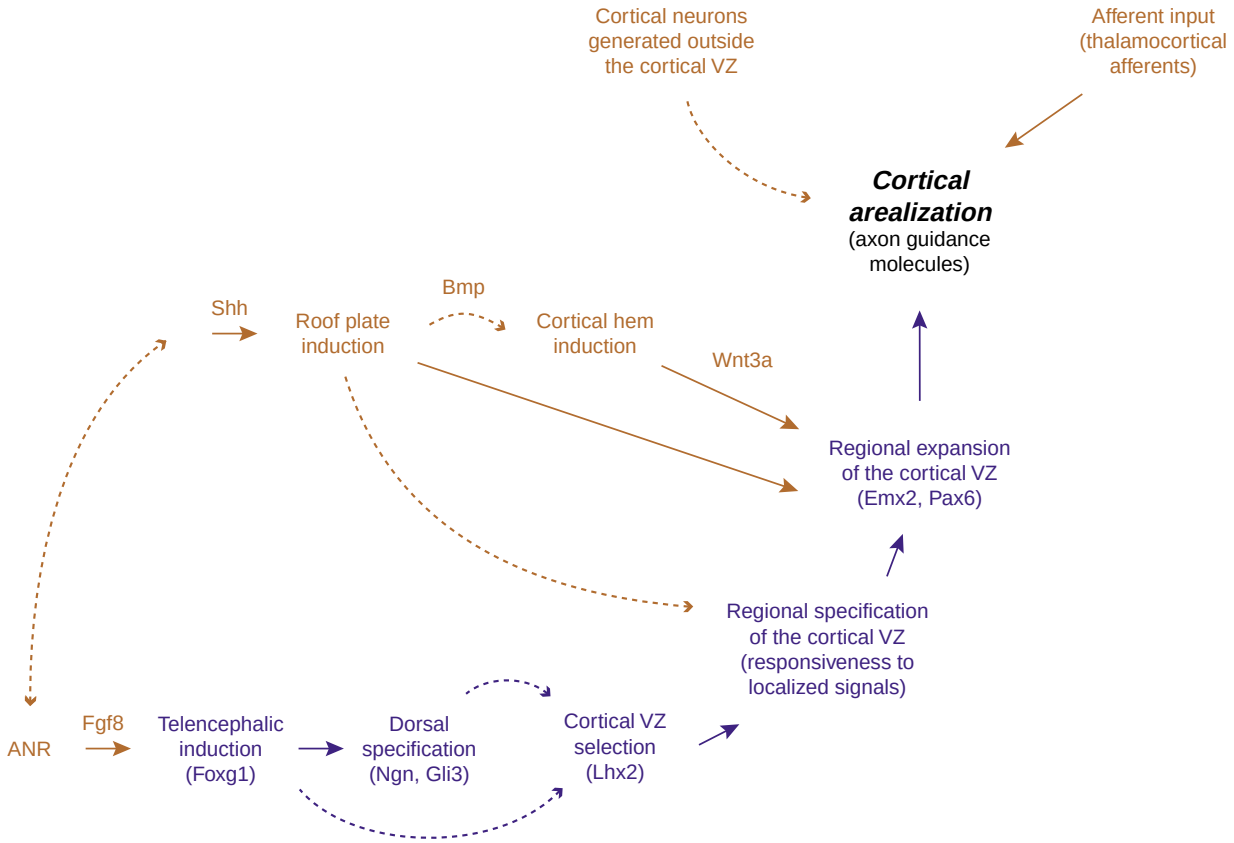

Fig. 6. Events in cortical patterning. Regulatory events that pattern the cortical VZ and cortical areas. Brown, extrinsic influences (organizers, signals, afferent inputs and cortical neurons generated outside the cortical VZ); blue, intrinsic events and factors in the cortical VZ. Dashed curved lines, hypothetical connections between parts of this hierarchy. See text for details.

PAX6 mutant alleles[75], which supports a gene dosage effect for human *PAX6* mutations. However, it remains unknown if patients with *PAX6* mutations might have shifts or changes in cortical areal size that would correspond to the essential patterning function of Pax6 in mice.

From cortical VZ to cortical areas

Ultimately, the initial blueprint laid out by organizers and intrinsic transcriptional regulators of the cortical VZ must be converted into the topographically organized and functionally diverse areas of the mature cortex. Although the Emx2 and Pax6 studies suggest that this conversion must occur, they do not reveal its underlying mechanisms. One possibility would be to transfer either the transcription factors themselves or their expression profiles in cortical VZ progenitors directly to their neuronal progeny. Although Emx2 and Pax6 may not be expressed by cortical neurons, other transcription factors such as Emx1 and Lhx2 in rodents[76,77] and primates[78] show similar patterns of expression in the cortical VZ and in cortical neurons, which is consistent with such a mechanism. The unique combinations of transcription factors in either progenitors or neurons could then direct the differential expression of target genes that allow area-specific differentiation and connectivity. Molecules that regulate axon guidance, such as those that mediate cell–cell interactions, are among the most likely of these transcriptional targets, because they are directly involved in axon target selection in multiple systems[79]. Importantly, several genes encoding cell–cell interaction proteins, such as cadherins[78,80–82], immunoglobulin superfamily members[83,84] and the ephrin/Eph receptors[82,85,86] are expressed in specific patterns across the cortex, many of which are graded and/or correspond to functional areas and their boundaries. The ephrin/Eph receptor system in particular seems to have a general role in the formation of topographic maps in the brain[82,85,87], including in the somatosensory cortex[82,88]. It is particularly tempting to speculate that the cell–cell interaction molecules that show graded expression in the cortex might be directly regulated by transcription factors that also show a graded pattern in the cortex or cortical VZ.

The hierarchy of events in cortical patterning

These studies provide insight into the sequence of events that specify the cortical VZ and ultimately pattern the cerebral cortex (**Fig. 6**). The initial pathways responsible for induction of the telencephalon (ANR-Fgf8-Foxg1) and the dorsal midline roof plate (HPE genes) are separable. Following induction of the telencephalon, dorsal telencephalic tissue is specified (Neurogenins and Gli3), and a cortical VZ fate is selected from other dorsal fates (Lhx2). The cortical VZ then becomes regionally specified by the influence of localized signaling molecules. Signaling molecules, including those from the dorsal midline region (roof plate and cortical hem), then act on the cortical VZ to regulate its expansion, with the relative sizes of cortical VZ fields and ultimately cortical areas being determined by gradients of intrinsic transcriptional regulators (Emx2 and Pax6). Regional patterning information encoded in the cortical VZ is then transferred into the overlying cortex, and afferent input

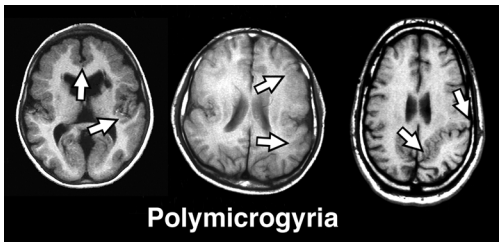

Fig. 7. Human symmetric polymicrogyria. Axial (transverse) MRI scans of patients with bifrontal (left), biparietal (middle) and biparieto–occipital (right) symmetric polymicrogyria. The abnormal cortical regions within the right hemisphere are delimited by arrows.

into the intrinsically patterned cortex acts to sculpt mature area-specific features.

This hierarchical model of cortical patterning requires much further elaboration and elucidation. During early induction, it is unclear if telencephalic and roof plate induction are linked, how anterior and ventrally derived signals lead to roof plate induction, and whether the cortical neuroepithelium is selectively induced. The roof plate is likely to have other roles in addition to regulating cortical VZ expansion, which may include induction of the cortical hem and regional specification of the cortical VZ, based on its known functions in the spinal cord. During cortical VZ specification, dorsal specification may occur in parallel or in series with cortical VZ selection, and may also be directly linked to Pax6-mediated cortical VZ expansion, because the proper spatial localization of Neurogenins depends on an interplay between the *Pax6* and *Gsh2* homeobox genes[89–91]. The involvement of particular molecules in specification versus proliferation and cell death can be a difficult problem for developmental biologists, but will need to be further clarified. Finally, the later stages of this process involving the conversion of cortical VZ pattern into the overlying cortex, the differential expression of axon guidance molecules, and the potential role of non-cortical VZ-derived neurons in providing some aspects of area specificity remain almost completely unexplored.

Abnormal cortical patterning in other human disorders

Just as the human HPE genes provide a 'forward genetics' approach to define genes involved in patterning, there are other naturally occurring human mutations that could provide similar clues. One such set of diseases are the symmetric polymicrogyrias (**Fig. 7**). Several familial forms of polymicrogyria ('too many small gyri') have now been reported[92], many of which show a regional distribution within the cortex[93] (bilateral frontal, bilateral perisylvian, biparietal and bioccipital; **Fig. 7**). These genetic polymicrogyrias produce a cortex that appears relatively normal overall, but shows regional disorganization of cortical cellular architecture and lamination. Patients with regional polymicrogyrias have clinical findings that tend to correspond to the location of the most severe abnormalities. For example, bilateral frontal polymicrogyria is associated with prominent motor signs due to the involvement of motor cortex, but with only mild to moderate mental retardation[94]. In contrast, perisylvian polymicrogyria is most severe in cortical areas devoted to language and control of the mouth, thus resulting in difficulties with swallowing, articulation and language acquisition as well as frequent and severe seizures. The regional nature of these defects suggests the involvement of genes that are required for regional patterning of the cortex, but this remains unproven until the responsible genes are identified.

Neural tube defects represent a large and heterogeneous group of disorders that involve incomplete closure of the neural tube (and therefore by definition, an improperly formed roof plate), and some neural tube defects may represent primary defects of early induction and patterning. Two human malformations potentially related to neural tube defects are exencephaly (openings of the skull with protruding brain) and anencephaly (complete absence of the brain), which seem to be well-modeled by certain mouse mutants[49,64,95]. Microcephaly ('small head') is relatively common and may frequently represent primary defects in cortical VZ expansion. Mutations in human genes that regulate later events in cortical patterning may not cause gross malformations of the cortex, but instead more subtle cognitive and behavioral defects, such as the speech and language deficits caused by mutation of the FOXP2 transcription factor gene[98].

Summary

Recent evidence continues to implicate the cortical VZ as a critical source of patterning information that ultimately leads to the formation of functionally diverse cortical areas. Several intrinsic transcriptional regulators have been shown to regulate distinct steps during cortical VZ patterning. However, numerous extrinsic influences on the cortical VZ and emerging cortex are also known. In addition to the role of afferent cortical input, extrinsic influences include organizers that pattern the cortical VZ via secreted signaling proteins and progenitor cells outside the cortical VZ that produce migratory cortical neurons. Taken together, these studies provide insight into the hierarchy of events that lead from the induction of cortical neuroepithelium to the formation of cortical areas. The discovery of genes responsible for cortical malformations in humans has been a valuable complement to the basic studies on cortical patterning in mice, and the links between mice and humans should continue to provide invaluable insight into the pathogenesis of human disease and the mechanisms that pattern the mammalian cerebral cortex.

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1. Van der Loos, H. & Woolsey, T. A. Somatosensory cortex: structural alterations following early injury to sense organs. *Science* **179**, 395–398 (1973).
2. O'Leary, D. D. Do cortical areas emerge from a protocortex? *Trends Neurosci.* **12**, 400–406 (1989).
3. Sur, M. & Leamey, C. A. Development and plasticity of cortical areas and networks. *Nat. Rev. Neurosci.* **2**, 251–262 (2001).
4. Levitt, P. A monoclonal antibody to limbic system neurons. *Science* **223**, 299–301 (1984).
5. Barbe, M. F. & Levitt, P. The early commitment of fetal neurons to the limbic cortex. *J. Neurosci.* **11**, 519–533 (1991).
6. Arimatsu, Y. *et al.* Early regional specification for a molecular neuronal phenotype in the rat neocortex. *Proc. Natl. Acad. Sci. USA* **89**, 8879–8883 (1992).
7. Cohen-Tannoudji, M., Babinet, C. & Wassef, M. Early determination of a mouse somatosensory cortex marker. *Nature* **368**, 460–463 (1994).
8. Miyashita-Lin, E. M., Hevner, R., Wassarman, K. M., Martinez, S. & Rubenstein, J. L. Early neocortical regionalization in the absence of thalamic innervation. *Science* **285**, 906–909 (1999).
9. Nakagawa, Y., Johnson, J. E. & O'Leary, D. D. Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input. *J. Neurosci.* **19**, 10877–10885 (1999).
10. Rakic, P. Specification of cerebral cortical areas. *Science* **241**, 170–176 (1988).
11. Wilson, S. W. & Rubenstein, J. L. Induction and dorsoventral patterning of the telencephalon. *Neuron* **28**, 641–651 (2000).

12. Furuta, Y., Piston, D. W. & Hogan, B. L. Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. *Development* **124**, 2203–2212 (1997).
13. Mione, M. C., Danevic, C., Boardman, P., Harris, B. & Parnavelas, J. G. Lineage analysis reveals neurotransmitter (GABA or glutamate) but not calcium-binding protein homogeneity in clonally related cortical neurons. *J. Neurosci.* **14**, 107–123 (1994).
14. Tan, S. S. *et al.* Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. *Neuron* **21**, 295–304 (1998).
15. Ware, M. L., Tavazoie, S. F., Reid, C. B. & Walsh, C. A. Coexistence of widespread clones and large radial clones in early embryonic ferret cortex. *Cereb. Cortex* **9**, 636–645 (1999).
16. Parnavelas, J. G. The origin and migration of cortical neurones: new vistas. *Trends Neurosci.* **23**, 126–131 (2000).
17. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. & Kriegstein, A. R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714–720 (2001).
18. Spreafico, R. *et al.* Cortical dysplasia: an immunocytochemical study of three patients. *Neurology* **50**, 27–36 (1998).
19. Najm, I. M. *et al.* Epileptogenicity correlated with increased N-methyl-D-aspartate receptor subunit NR2A/B in human focal cortical dysplasia. *Epilepsia* **41**, 971–976 (2000).
20. Crino, P. B., Duhaime, A. C., Baltuch, G. & White, R. Differential expression of glutamate and GABA-A receptor subunit mRNA in cortical dysplasia. *Neurology* **56**, 906–913 (2001).
21. Walsh, C. & Cepko, C. L. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. *Science* **255**, 434–440 (1992).
22. Reid, C. B., Liang, I. & Walsh, C. Systematic widespread clonal organization in cerebral cortex. *Neuron* **15**, 299–310 (1995).
23. McCarthy, M., Turnbull, D. H., Walsh, C. A. & Fishell, G. Telencephalic neural progenitors appear to be restricted to regional and glial fates before the onset of neurogenesis. *J. Neurosci.* **21**, 6772–6781 (2001).
24. Anderson, S. A., Eisenstat, D. D., Shi, L. & Rubenstein, J. L. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474–476 (1997).
25. Lavdas, A. A., Grigoriou, M., Pachnis, V. & Parnavelas, J. G. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* **19**, 7881–7888 (1999).
26. Anderson, S., Mione, M., Yun, K. & Rubenstein, J. L. Differential origins of neocortical projection and local circuit neurons: role of Dlx genes in neocortical interneuronogenesis. *Cereb. Cortex* **9**, 646–654 (1999).
27. Reid, C. B., Tavazoie, S. F. & Walsh, C. A. Clonal dispersion and evidence for asymmetric cell division in ferret cortex. *Development* **124**, 2441–2450 (1997).
28. Monuki, E. S., Porter, F. D. & Walsh, C. A. Patterning of the dorsal telencephalon and cerebral cortex by a roof plate-Lhx2 pathway. *Neuron* (in press).
29. Keverne, E. B. GABA-ergic neurons and the neurobiology of schizophrenia and other psychoses. *Brain Res. Bull.* **48**, 467–473 (1999).
30. Benes, F. M. & Berretta, S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* **25**, 1–27 (2001).
31. Shimamura, K. & Rubenstein, J. L. Inductive interactions direct early regionalization of the mouse forebrain. *Development* **124**, 2709–2718 (1997).
32. Tao, W. & Lai, E. Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. *Neuron* **8**, 957–966 (1992).
33. Xuan, S. *et al.* Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. *Neuron* **14**, 1141–1152 (1995).
34. Dou, C. L., Li, S. & Lai, E. Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cereb. Cortex* **9**, 543–550 (1999).
35. Golden, J. A. Holoprosencephaly: a defect in brain patterning. *J. Neuropathol. Exp. Neurol.* **57**, 991–999 (1998).
36. Yakovlev, P. I. Pathoarchitectonic studies of cerebral malformations: III. Arrhinencephalies (Holoprosencephaly). *J. Neuropathol. Exp. Neurol.* **18**, 22–55 (1959).
37. Muenke, M. & Beachy, P. A. Genetics of ventral forebrain development and holoprosencephaly. *Curr. Opin. Genet. Dev.* **10**, 262–269 (2000).
38. Chiang, C. et al. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* **383**, 407–413 (1996).
39. Jessell, T. M. & Lumsden, A. in *Molecular and Cellular Approaches to Neural Development* (eds. Cowan, W. M., Jessell, T. M. & Zipursky, S. L.) 290–333 (Oxford Univ. Press, New York, 1997).
40. Sussel, L., Marin, O., Kimura, S. & Rubenstein, J. L. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359–3370 (1999).
41. Wallis, D. E. *et al.* Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. *Nat. Genet.* **22**, 196–198 (1999).
42. Pasquier, L. *et al.* A new mutation in the six-domain of SIX3 gene causes holoprosencephaly. *Eur. J. Hum. Genet.* **8**, 797–800 (2000).
43. Brown, S. A. *et al.* Holoprosencephaly due to mutations in ZIC2, a homologue of *Drosophila* odd-paired. *Nat. Genet.* **20**, 180–183 (1998).
44. Brown, L. Y. *et al.* Holoprosencephaly due to mutations in ZIC2: alanine tract expansion mutations may be caused by parental somatic recombination. *Hum. Mol. Genet.* **10**, 791–796 (2001).
45. Nanni, L. *et al.* The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly. *Hum. Mol. Genet.* **8**, 2479–2488 (1999).
46. Gripp, K. W. *et al.* Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nat. Genet.* **25**, 205–208 (2000).
47. Oliver, G. *et al.* Six3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. *Development* **121**, 4045–4055 (1995).
48. Nagai, T. *et al.* The expression of the mouse Zic1, Zic2, and Zic3 gene suggests an essential role for Zic genes in body pattern formation. *Dev. Biol.* **182**, 299–313 (1997).
49. Nagai, T. *et al.* Zic2 regulates the kinetics of neurulation. *Proc. Natl. Acad. Sci. USA* **97**, 1618–1623 (2000).
50. Heldin, C. H., Miyazono, K. & ten Dijke, P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* **390**, 465–471 (1997).
51. Baker, J. C. & Harland, R. M. From receptor to nucleus: the Smad pathway. *Curr. Opin. Genet. Dev.* **7**, 467–473 (1997).
52. Wotton, D., Lo, R. S., Lee, S. & Massague, J. A Smad transcriptional corepressor. *Cell* **97**, 29–39 (1999).
53. Fode, C. *et al.* A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* **14**, 67–80 (2000).
54. Tole, S., Ragsdale, C. W. & Grove, E. A. Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J). *Dev. Biol.* **217**, 254–265 (2000).
55. Bulchand, S., Grove, E. A., Porter, F. D. & Tole, S. LIM-homeodomain gene Lhx2 regulates the formation of the cortical hem. *Mech. Dev.* **100**, 165–175 (2001).
56. Diaz-Benjumea, F. J. & Cohen, S. M. Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* **75**, 741–752 (1993).
57. Williams, J. A., Paddock, S. W. & Carroll, S. B. Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete subregions. *Development* **117**, 571–584 (1993).
58. Blair, S. S., Brower, D. L., Thomas, J. B. & Zavortink, M. The role of apterous in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of *Drosophila*. *Development* **120**, 1805–1815 (1994).
59. Barbe, M. F. & Levitt, P. Attraction of specific thalamic input by cerebral grafts depends on the molecular identity of the implant. *Proc. Natl. Acad. Sci. USA* **89**, 3706–3710 (1992).
60. Barbe, M. F. & Levitt, P. Age-dependent specification of the corticocortical connections of cerebral grafts. *J. Neurosci.* **15**, 1819–1834 (1995).
61. Levitt, P. & Eagleson, K. L. Regionalization of the cerebral cortex: developmental mechanisms and models. *Novartis Found. Symp.* **228**, 173–181 (2000).
62. Lee, K. J. & Jessell, T. M. The specification of dorsal cell fates in the vertebrate central nervous system. *Annu. Rev. Neurosci.* **22**, 261–294 (1999).
63. Solloway, M. J. & Robertson, E. J. Early embryonic lethality in Bmp5;Bmp7 double mutant mice suggests functional redundancy within the 60A subgroup. *Development* **126**, 1753–1768 (1999).
64. Grove, E. A., Tole, S., Limon, J., Yip, L. & Ragsdale, C. W. 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 (1998).
65. Lee, S. M., Tole, S., Grove, E. & McMahon, A. P. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* **127**, 457–467 (2000).
66. Galceran, J., Miyashita-Lin, E. M., Devaney, E., Rubenstein, J. L. & Grosschedl, R. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* **127**, 469–482 (2000).
67. Ikeya, M., Lee, S. M., Johnson, J. E., McMahon, A. P. & Takada, S. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* **389**, 966–970 (1997).
68. Bishop, K. M., Goudreau, G. & O'Leary, D. D. Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. *Science* **288**, 344–349 (2000).
69. Mallamaci, A., Muzio, L., Chan, C. H., Parnavelas, J. & Boncinelli, E. Area identity shifts in the early cerebral cortex of Emx2$^{-/-}$ mutant mice. *Nat. Neurosci.* **3**, 679–686 (2000).
70. Brunelli, S. *et al.* Germline mutations in the homeobox gene EMX2 in patients with severe schizencephaly. *Nat. Genet.* **12**, 94–96 (1996).
71. Capra, V. *et al.* Schizencephaly: surgical features and new molecular genetic results. *Eur. J. Pediatr. Surg. 6 Suppl* **1**, 27–29 (1996).
72. Granata, T. *et al.* Familial schizencephaly associated with EMX2 mutation. *Neurology* **48**, 1403–1406 (1997).
73. Warren, N. *et al.* The transcription factor, Pax6, is required for cell proliferation and differentiation in the developing cerebral cortex. *Cereb. Cortex* **9**, 627–635 (1999).
74. Sisodiya, S. M. *et al.* PAX6 haploinsufficiency causes cerebral malformation and olfactory dysfunction in humans. *Nat. Genet.* **28**, 214–216 (2001).
75. Glaser, T. *et al.* PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. *Nat. Genet.* **7**, 463–471 (1994).

76. Gulisano, M., Broccoli, V., Pardini, C. & Boncinelli, E. Emx1 and Emx2 show different patterns of expression during proliferation and differentiation of the developing cerebral cortex in the mouse. *Eur. J. Neurosci.* **8**, 1037–1050 (1996).
77. Xu, Y. *et al.* LH-2: a LIM/homeodomain gene expressed in developing lymphocytes and neural cells. *Proc. Natl. Acad. Sci. USA* **90**, 227–231 (1993).
78. Donoghue, M. J. & Rakic, P. Molecular gradients and compartments in the embryonic primate cerebral cortex. *Cereb. Cortex* **9**, 586–600 (1999).
79. Goodman, C. S. Mechanisms and molecules that control growth cone guidance. *Annu. Rev. Neurosci.* **19**, 341–377 (1996).
80. Suzuki, S. C., Inoue, T., Kimura, Y., Tanaka, T. & Takeichi, M. Neuronal circuits are subdivided by differential expression of type-II classic cadherins in postnatal mouse brains. *Mol. Cell. Neurosci.* **9**, 433–447 (1997).
81. Rubenstein, J. L. *et al.* Genetic control of cortical regionalization and connectivity. *Cereb. Cortex* **9**, 524–532 (1999).
82. Vanderhaeghen, P. *et al.* A mapping label required for normal scale of body representation in the cortex. *Nat. Neurosci.* **3**, 358–365 (2000).
83. Pimenta, A. F. *et al.* The limbic system-associated membrane protein is an Ig superfamily member that mediates selective neuronal growth and axon targeting. *Neuron* **15**, 287–297 (1995).
84. Mann, F., Zhukareva, V., Pimenta, A., Levitt, P. & Bolz, J. Membrane-associated molecules guide limbic and nonlimbic thalamocortical projections. *J. Neurosci.* **18**, 9409–9419 (1998).
85. O'Leary, D. D. & Wilkinson, D. G. Eph receptors and ephrins in neural development. *Curr. Opin. Neurobiol.* **9**, 65–73 (1999).
86. Donoghue, M. J. & Rakic, P. Molecular evidence for the early specification of presumptive functional domains in the embryonic primate cerebral cortex. *J. Neurosci.* **19**, 5967–5979 (1999).
87. Feng, G. *et al.* Roles for ephrins in positionally selective synaptogenesis between motor neurons and muscle fibers. *Neuron* **25**, 295–306 (2000).
88. Prakash, N. *et al.* Malformation of the functional organization of somatosensory cortex in adult ephrin-A5 knock-out mice revealed by *in vivo* functional imaging. *J. Neurosci.* **20**, 5841–5847 (2000).
89. Toresson, H., Potter, S. S. & Campbell, K. Genetic control of dorsal–ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* **127**, 4361–4371 (2000).
90. Corbin, J. G., Gaiano, N., Machold, R. P., Langston, A. & Fishell, G. The Gsh2 homeodomain gene controls multiple aspects of telencephalic development. *Development* **127**, 5007–5020 (2000).
91. Yun, K., Potter, S. & Rubenstein, J. L. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* **128**, 193–205 (2001).
92. Guerreiro, M. M. *et al.* Familial perisylvian polymicrogyria: a new familial syndrome of cortical maldevelopment. *Ann. Neurol.* **48**, 39–48 (2000).
93. Barkovich, A. J., Hevner, R. & Guerrini, R. Syndromes of bilateral symmetrical polymicrogyria. *Am. J. Neuroradiol.* **20**, 1814–1821 (1999).
94. Straussberg, R., Gross, S., Amir, J. & Gadoth, N. A new autosomal recessive syndrome of pachygyria. *Clin. Genet.* **50**, 498–501 (1996).
95. Zhao, Q., Behringer, R. R. & de Crombrugghe, B. Prenatal folic acid treatment suppresses acrania and meroanencephaly in mice mutant for the Cart1 homeobox gene. *Nat. Genet.* **13**, 275–283 (1996).
96. Altman, J. & Bayer, S. A. *Atlas of Prenatal Rat Brain Development* 589 (CRC Press, Boca Raton, Florida, 1995).
97. Barkovich, A. J., Kuzniecky, R. I., Bollen, A. W. & Grant, P. E. Focal transmantle dysplasia: a specific malformation of cortical development. *Neurology* **49**, 1148–1152 (1997).
98. Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F. & Monaco, A. P. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* **413**, 519–523 (2001).