5 Patterning of the Cerebral Cortex

SONIA GAREL AND JOHN L. R. RUBENSTEIN

ABSTRACT The cerebral cortex is characterized by a modular functional organization: distinct cortical areas with specific patterns of input and output projections are devoted to different functions. Whereas this precise areal organization is shaped and modified by experience, its elaboration begins during embryogenesis. For several decades, the role of nature versus nurture in cortical development has been debated. Recent experiments have shown that cortical regionalization occurs early in embryogenesis, independently of extrinsic input. These observations contribute the elaboration of a new model of cortical development where early intrinsic patterning of the cerebral cortex plays a key role in the emergence of cortical areas.

The functioning of the mammalian cerebral cortex, which regulates most aspects of perception, cognition, and behavior, relies on a precise organization that forms during embryonic and postnatal development (O'Leary, Schlaggar, and Tuttle, 1994; Monuki, Porter, and Walsh, 2001; Pallas, 2001; Ragsdale and Grove, 2001; Ruiz i Altaba, Gitton, and Dahmane, 2001; Sur and Leamey, 2001; O'Leary and Nakagawa, 2002; Lopez-Bendito and Molnar, 2003). Different regions of the cortex are dedicated to distinct functions (Brodmann, 1909). For instance, rostral regions regulate motor and executive functions, caudal regions process somatosensory, auditory, and visual inputs, and ventral regions process olfaction. These different cortical areas are defined by a specific histology, molecular identity, and connectivity pattern, particularly with the dorsal thalamus, which provides the main input to the cerebral cortex.

Over several decades, two models of cortical regionalization have been debated. The protocortex model proposes that cortical areas are defined by the input they receive from the dorsal thalamus ("extrinsic" patterning; O'Leary, 1989). An extension of this model proposes that the nature of the input, for instance visual, is the key parameter. Support for this view comes from axonal rerouting experiments showing that the auditory cortex can process a visual input (Pallas, 2001; Sur and Leamey, 2001). On the other hand, the protomap model proposes that molecular determinants intrinsic to the cortical primordium generate cortical subdivisions with histologically and functionally distinct properties (Rakic, 1988). This model is supported by numerous recent studies, described herein, that demonstrate the role of patterning signals and transcription factors in defining the map of the developing cortex. The weight of the data suggests that both a protomap and the pattern of cortical inputs contribute to the emergence of cortical areas. In this chapter, we present evidence for this unified model, focusing on recent findings that have identified embryonic events controlling regionalization of the neocortex in mice.

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Embryonic development of the cerebral cortex: A brief overview

ONTOGENIC ORIGIN OF THE CEREBRAL CORTEX To understand regionalization of the cerebral cortex, we must consider the processes that control early development of the telencephalon. The telencephalon is induced in the rostrolateral neural plate (Cobos et al., 2001). Its molecular regionalization is coupled with a series of major morphological changes, including neurulation (neural tube closure) and evagination of the telencephalic vesicles. The cerebral cortex develops from the "dorsal" part of the telencephalic vesicles, or pallium (figure 5.1). The pallium is further subdivided into medial (MP), dorsal (DP), lateral (LP), and ventral pallium (VP), which will give rise respectively to the hippocampal formation (limbic lobe), the neocortex, the olfactory/piriform cortex, and the claustrum (figure 5.1) (Puelles et al., 2000; Marin and Rubenstein, 2002; Rubenstein and Puelles, 2003). Each of these large domains is subdivided into subdomains, such as the areas of the neocortex (figure 5.1) or the hippocampal fields.

In parallel with these morphogenetic processes, cortical progenitors located in the proliferative neuroepithelium initiate the production of postmitotic neurons that radially migrate toward the pial surface (Marin and Rubenstein, 2002; Rubenstein and Puelles, 2003). In mice, these neurons differentiate into glutamatergic projection neurons, whereas GABAergic interneurons mainly derive from the subpallium proliferative epithelium (Marin and Rubenstein, 2001, 2003). In humans, the cortical proliferative zone generates some GABAergic neurons as well (Letinic, Zoncu, and Rakic, 2002). In the cortex, early-born neurons give rise to subplate neurons, layer I neurons (Cajal-Retzius cells), and deep layer neurons (layers V and VI in the neocortex) (Marin

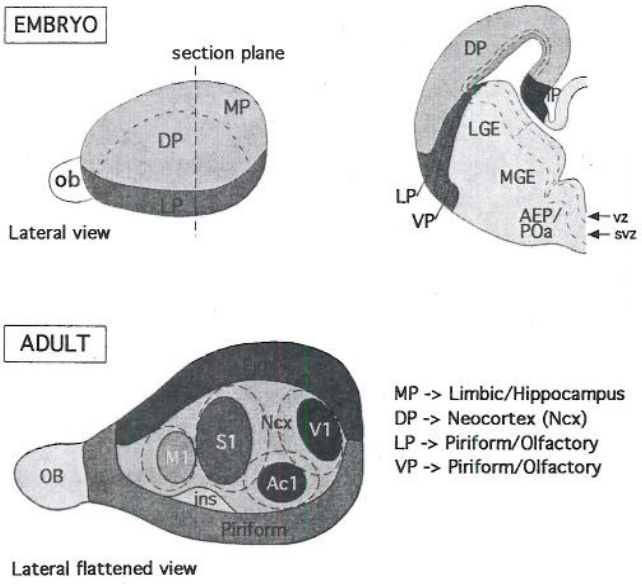

FIGURE 5.1 The development of cortical subdivisions. Schema at top left represents a lateral view of an E14 embryonic telencephalic vesicle showing the lateral (LP; intermediate gray) and dorsal pallium (DP; light gray). The medial pallium (MP) is located on the other side of the vesicle (dotted line). Top right schema represents a cross-section of the same telencephalic vesicle at the level indicated on the left. The ventral and lateral pallium (light gray), dorsal pallium (intermediate gray) and medial pallium (dark gray) subdivide the "dorsal" part of the vesicle, while the lateral and medial ganglionic eminence (LGE, MGE), the anterior entopeduncular region (AEP), and the preoptic area (POa) subdivide the "ventral" part. Schema at bottom represents a flattened lateral view of an adult telencephalic vesicle (i.e., a projection of the lateral view, which includes the normally hidden limbic or medial wall). The correspondence between embryonic and adult structures is indicated in the list. Furthermore, the relative positions of neocortical areas are represented by ovals surrounded by solid lines for primary areas or dotted lines for nonprimary areas. Nonlabeled neocortex is multimodal.

Abbreviations: Ac1, primary auditory cortex; AEP, anterior entopeduncular area; DP, dorsal pallium; ins, insular cortex; LGE, lateral ganglionic eminence; LP, lateral; M1, primary motor cortex; MGE, medial ganglionic eminence; MP, medial pallium; Ncx, neocortex; OB, olfactory bulb; POa, preoptic area; S1, primary somatosensory area; SVZ, subventricular zone; V1, primary visual area; VP, ventral pallium; VZ, ventricular zone. (Figure compiled from Marin and Rubenstein, 2002; Rubenstein and Puelles, 2003.)

and Rubenstein, 2002). Later-born neurons migrate past layers 5 and 6 to form more superficial layers of the cortical plate (layers II to IV in the neocortex).

FORMATION OF THALAMOCORTICAL INTERCONNECTIONS Distinct cortical areas have unique patterns of connectivity. In particular, they exhibit specific connections with nuclei of the dorsal thalamus, which relays information from the periphery (O'Leary, Schlaggar, and Tuttle, 1994; Monuki, Porter, and Walsh, 2001; Pallas, 2001; Ragsdale and Grove, 2001; Ruiz i Altaba, Gitton, and Dahmane, 2001; Sur and Leamey, 2001; O'Leary and Nakagawa, 2002; Lopez-Bendito and Molnar, 2003).

Dorsal thalamus neurons begin to send axonal projections during embryogenesis. These axons travel through the ventral thalamus and the basal ganglia before reaching the intermediate zone of the cortex (figure 5.2) (Catalano, Robertson, and Killackey, 1991; Miller, Chou, and Finlay, 1993; Molnar, Adams, and Blakemore, 1998; Auladell et al., 2000). Conversely, corticofugal axons from the subplate, layer V and VI, leave the cortex, enter the basal ganglia, and then split into two tracts as they approach the telencephalic-diencephalic boundary: corticothalamic axons (layer VI neurons) run through the ventral thalamus into the dorsal thalamus, and corticospinal axons (layer V neurons) join the cerebral peduncule and innervate subcortical targets such as the superior colliculus, the pons, and the spinal cord (figure 5.2) (Jones, 1984; De Carlos and O'Leary, 1992; Miller, Chou, and Finlay, 1993; Auladell et al., 2000). Corticofugal and thalamocortical axons running through the basal ganglia together form the fiber tracts of the internal capsule.

Once thalamocortical axons from a given thalamic nucleus reach the appropriate cortical region, they transiently synapse on subplate cells (Herrmann, Antonini, and Shatz, 1994). Then, after a short waiting period, thalamic axons grow branches into the cortical plate (Catalano, Robertson, and Killackey, 1991; Miller, Chou, and Finlay, 1993; Molnar, Adams, and Blakemore, 1998; Auladell et al., 2000). On reaching layer IV, they elaborate terminal arbors and synapse upon cortical neurons (Senft and Woolsey, 1991; Agmon et al., 1993; Kageyama and Robertson, 1993; Catalano, Robertson, and Killackey, 1996; Rebsam, Seif, and Gaspar, 2002).

Different thalamic nuclei project to a specific cortical areas in a domain-specific manner (figure 5.2) (Kageyama and Robertson, 1993; O'Leary, Schlaggar, and Tuttle, 1994; Schlaggar and O'Leary, 1994; Agmon et al., 1995; Sur and Leamey, 2001; Lopez-Bendito and Molnar, 2003). This feature is clearly illustrated by the sensory thalamic nuclei: the dorsal lateral geniculate nucleus (dLGN), the ventrobasal nucleus (VB), and the medial geniculate nucleus (MG). These nuclei receive peripheral input from the retina, from skin sensory receptors, and from the cochlea; thalamic nuclei, in turn, project to visual, somatosensory, and auditory cortical areas, respectively. Thus, ascending sensory projections of different modalities (e.g., vision, audition, somatosensory perception) are relayed through specific nuclei of the dorsal thalamus and ultimately to precise cortical areas. Conversely, cortical neurons reciprocally project to the same thalamic nuclei that innervate the area they are located in.

Within each area, a second level of topographic organization is observed: thalamocortical projections form a physical map representing the entire sensory field or space

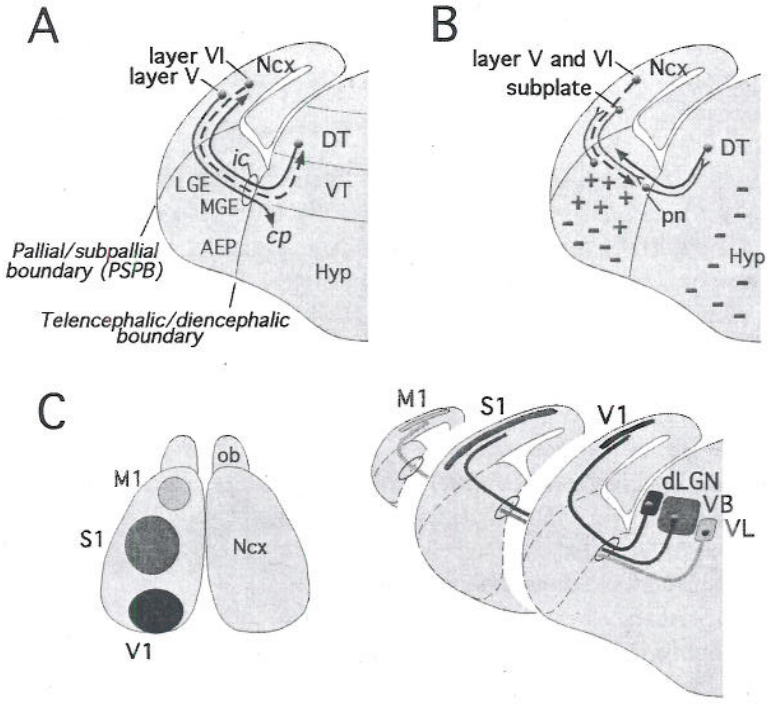

FIGURE 5.2 The formation of thalamocortical interconnections. (*A*) Schematic representation of the embryonic axon tracts formed by thalamocortical (solid dark gray), corticothalamic (dotted dark gray) and corticospinal (solid light gray). In the lateral and medial ganglionic eminences (LGE, MGE), these axons form the internal capsule (ic), and at the telencephalic/diencephalic boundary, corticothalamic and corticospinal tracts diverge; corticospinal axons join the cerebral peduncle (cp). (*B*) Schematic representation of some cues involved in the pathfinding of thalamic and cortical axons; these include attractive (+) and repulsive (–) axon guidance cues as well as transient axonal projections from the basal ganglia (including the perireticular nucleus, pn) and the cortical subplate (thin gray line). (*C*) Cortical primary areas (on the left) receive specific thalamic input (series of rostrocaudal coronal sections on the right). The primary motor cortex (M1) receives projections from the ventrolateral (VL) thalamic nucleus (light gray), the primary somatosensory cortex (S1) receives projections from the ventrobasal (VB) complex (intermediate gray), and the primary visual cortex (V1) receives projections from the dorsolateral geniculate nucleus (dLGN) (dark gray).

Abbreviations: AEP, anterior entopeduncular region; cp, cerebral peduncle; dLGN, dorsolateral geniculate nucleus; DT, dorsal thalamus; Hyp, hypothalamus; ic, internal capsule; LGE, lateral ganglionic eminence; M1, primary motor cortex; MGE, medial ganglionic eminence; Ncx, neocortex; ob, olfactory bulb; pn, perireticular nucleus; S1, primary somatosensory cortex; V1, primary visual cortex; VB, ventrobasal complex; VL, ventrolateral nucleus; VT, ventral thalamus. (Compiled from Metin and Godement, 1996; Molnar, Adams, and Blakemore, 1998; Braisted, Tuttle, and O'Leary, 1999; Bagri et al., 2002; Marin et al., 2002; Lopez-Bendito and Molnar, 2003.)

(O'Leary, Schlaggar, and Tuttle, 1994; Monuki and Walsh, 2001; Ragsdale and Grove, 2001; Sur and Leamey, 2001; O'Leary and Nakagawa, 2002; Lopez-Bendito and Molnar, 2003). For instance, sensory input from individual mouse facial whiskers are processed in specific positions within the barrel field of the somatosensory cortex (Schlaggar and O'Leary, 1993; Killackey, Rhoades, and Bennett-Clarke, 1995; Erzurumlu and Kind, 2001). Topographic sensory maps form postnatally, and their organization is plastic, that is, it is modified in response to activity (Erzurumlu and Kind, 2001). On the contrary, domain-specific thalamocortical and corticothalamic projections are initiated early during embryonic development and appear to form independently of peripheral input (Godement, Saillour, and Imbert, 1979; Kaiserman-Abramof, Graybiel, and Nauta, 1980) and of evoked neuronal activity (Molnar et al., 2002).

Early "intrinsic" regionalization of the cerebral cortex

MOLECULAR PARCELLATION OF THE EMBRYONIC NEOCORTEX The initial evidence supporting the protomap model came from experimental manipulations in primate embryos (Rakic, 1988). Subsequently, the expression patterns of different genes revealed the existence of molecular boundaries within the developing cortex. These genes include the somatosensory cortex H-2Z1 transgene (Cohen-Tannoudji, Babinet, and Wassef, 1994; Gitton, Cohen-Tannoudji, and Wassef, 1999b), cell surface proteins (LAMP: Zacco et al., 1990; latexin: Hatanaka et al., 1994; cadherins: Suzuki et al., 1997; Nakagawa, Johnson, and O'Leary, 1999; Eph/Ephrin: Gao et al., 1998; Donoghue and Rakic, 1999; Mackarehtschian et al., 1999; Miyashita-Lin et al., 1999; Sestan, Rakic, and Donoghue, 2001; Takemoto et al., 2002;

Yun et al., 2003; and transcription factors [Tbr1, Id2, RZR-beta]: Bulfone et al., 1995; Nothias, Fishell, and Ruiz i Altaba, 1998; Rubenstein et al., 1999).

Notably a number of these molecules showed restricted expression patterns before the arrival of thalamic axons, supporting the existence of a "prethalamic" molecular parcellation of the neocortex (Mackarehtschian et al., 1999; Nakagawa, Johnson, and O'Leary, 1999; Rubenstein and Rakic, 1999). Analyses of *Gbx-2* and *Mash1* knockout mice, which lack thalamocortical input, have demonstrated that early steps in cortical molecular regionalization are independent of thalamic innervation (Miyashita-Lin et al., 1999; Nakagawa, Johnson, and O'Leary, 1999; Yun et al., 2003). Consistently, the expression of the H-2Z1 transgene is induced in cortical explants that do not receive thalamic inputs (Gitton, Cohen-Tannoudji, and Wassef, 1999b), and the expression of latexin is induced in isolated cortical cells (Arimatsu et al., 1999). Finally, heterotopic transplantation experiments showed that the expression patterns of H-2Z1 and LAMP are specified early in development (Barbe and Levitt, 1991; Cohen-Tannoudji, Babinet, and Wassef, 1994). Together these experiments have established that the cerebral cortex is intrinsically regionalized from very early stages of embryogenesis.

Early Patterning of the Cortex by Signaling Centers

Discrete signaling centers producing secreted molecules are implicated in early cortical regionalization. These centers are localized along and flanking the midline of the telencephalic vesicles (figure 5.3) (Rubenstein and Rakic, 1999; Wilson and Rubenstein, 2000; Monuki and Walsh, 2001; Ragsdale and Grove, 2001; Ruiz i Altaba, Gitton, and Dahmane, 2001; O'Leary and Nakagawa, 2002). Dorsally, molecules of the bone morphogenetic protein (BMP) and WNT families control patterning of the medial and dorsal pallium, such as the hippocampus, choroid plexus, and neocortex. Indeed, inactivation of *Wnt3a*, or of the Wnt signaling factor *Lef-1*, severely disrupts the formation of the hippocampus (Galceran et al., 2000; Lee et al., 2000). Ectopic expression and conditional inactivation of the BMP signaling pathway alter respectively the patterning of the dorsal pallium and the development of dorsal midline structures development of the dorsal midline structures (Hebert, Mishina, and McConnell, 2002), as well as a broader patterning of the dorsal pallium (Furuta, Piston, and Hogan, 1997; Golden et al., 1999; Panchision et al., 2001; Ohkubo, Chiang, and Rubenstein, 2002). At the rostral margin of the telencephalon, a source of FGF8 positively regulates telencephalic outgrowth and rostrocaudal regionalization within the cortex (Crossley and Martin, 1995; Shimamura and Rubenstein, 1997; Meyers, Lewandoski, and Martin, 1998; Reifers et al., 1998; Crossley et al., 2001; Fukuchi-Shimogori and Grove, 2001; Garel, Huffman, and Rubenstein, 2003; Storm, Rubenstein, and Martin, 2003). Finally, sources of

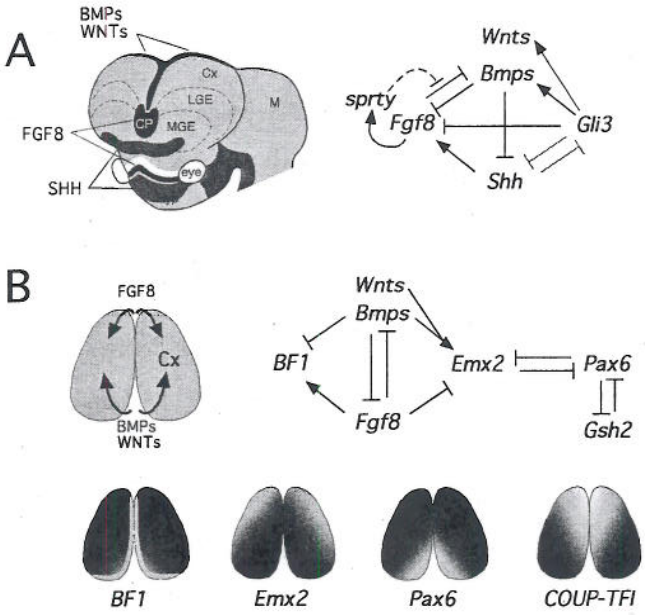

Figure 5.3 Signaling centers pattern the telencephalic vesicles. (*A*) A schematic frontal view of an E10.5 brain shows the expression domains of selected *Wnts* (e.g., Wnt3a) and *Bmps* (e.g., BMP4) (dark gray), *Fgfs* (e.g., *Fgf8*) (intermediate gray) and *Shh* (light gray). On the right is a summary of some of the epistatic relations between the different signaling molecules. Dotted lines indicate a hypothetical relationship and light gray lines between *Shh* and *Gli3* indicate a mutual repression of their signaling pathways. (*B*) Role of FGF8 and BMPs/WNTs in controlling the expression of transcription factors in the cerebral cortex. On the left is a schematic diagram of an E11.5 telencephalon and the relative action of secreted midline molecules on the patterning of the cerebral cortex. On the right are some of the known epistatic relationships between secreted molecules and transcription factors. The lower panel shows the resulting rostrocaudal and mediolateral gradients of expression of *BF-1* (*Foxg1*), *Emx2*, *Pax6*, and *COUP-TFI* in the dorsal telencephalon.

Abbreviations: CP, commissural plate; Cx, cerebral cortex; Hyp, hypothalamus; LGE, lateral ganglionic eminence; M, midbrain; MGE, medial ganglionic eminence. (Compiled from Chiang et al., 1996; Gulisano et al., 1996; Furuta, Piston, and Hogan, 1997; Shimamura and Rubenstein, 1997; Theil et al., 1999; Bishop, Goudreau, and O'Leary, 2000; Tole, Ragsdale, and Grove, 2000; Toresson, Potter, and Campbell, 2000; Crossley et al., 2001; Yun, Potter, and Rubenstein, 2001; Anderson et al., 2002; Aoto et al., 2002; Kobayashi et al., 2002; Marin and Rubenstein, 2002; Ohkubo, Chiang, and Rubenstein, 2002; Rallu, Machold, et al., 2002; Theil et al., 2002; Storm, Rubenstein, and Martin, 2003.)

sonic hedgehog (Shh) in the ventral forebrain are essential to the regionalization of the "ventral" telencephalon and might play a role in the patterning of the pallium as well (Chiang et al., 1996; Kohtz et al., 1998; Ohkubo, Chiang, and Rubenstein, 2002; Rallu, Corbin, and Fishell, 2002; Rallu, Machold, et al., 2002). Thus, an array of at least three midline patterning centers is involved in the establishment of regional differences in the telencephalon. The potential for other signaling centers that could be mediated by retinoids (LaMantia et al., 2000; Schneider et al.,

2001; Smith et al., 2001; Halilagic, Zile, and Studer, 2003), the WNT pathway (Kim, Lowenstein, and Pleasure, 2001), or the FGF/EGF pathway (Gimeno et al., 2002; Assimacopoulos, Grove, and Ragsdale, 2003) remains.

The activities of these patterning molecules are regulated by inhibitors and/or repressors of their transduction pathways. For instance, secreted inhibitors of BMPs and WNTs, such as Noggin, Chordin, and sFRPs, are present in the medial and dorsal pallium, and their patterns of expression are consistent with a role in restricting the extent of BMPs and WNTs signaling inside pallium subregions (Shimamura et al., 1995; McMahon et al., 1998; Klingensmith et al., 1999; Kim, Lowenstein, and Pleasure, 2001; Anderson et al., 2002). Similarly, *sprouty* genes, which encode negative regulators of the FGF pathway (Hacohen et al., 1998; Furthauer et al., 2001; Hanafusa et al., 2002), have an expression pattern that overlaps the *Fgf8* domain (Chambers and Mason, 2000; Furthauer et al., 2002; Storm, Rubenstein, and Martin, 2003). Finally, *Gli3*, a repressor of the Shh pathway, is expressed in the pallium, and its inactivation in mouse leads to ventralization of the cortex (Franz, 1994; Grove et al., 1998; Theil et al., 1999; Tole, Ragsdale, and Grove, 2000; Rallu, Corbin, and Fishell, 2002; Rallu, Machold, et al., 2002).

Another level of complexity is added by the fact that these different signaling centers regulate each other's activity (figure 5.3). For instance, ectopic BMP application (Golden et al., 1999; Ohkubo, Chiang, and Rubenstein, 2002) or a reduction in the level of BMP antagonists Chordin and Noggin (Anderson et al., 2002) both negatively regulate *Fgf8* and *Shh* expression in the telencephalon. Furthermore, a reduction in FGF8 activity leads to an increase in *Bmp4* expression and a reduction in *Shh* expression (Schneider et al., 2001; Storm, Rubenstein, and Martin, 2003). Loss of *Shh* expression leads to loss of *Fgf8* expression and an apparent expansion of BMP signaling (Ohkubo, Chiang, and Rubenstein, 2002). In addition, in *Gli3* mutant mice, *Bmps* and *Wnts* expression levels are drastically down-regulated, whereas *Fgf8* expression is up-regulated (Grove et al., 1998; Theil et al., 1999, 2002; Aoto et al., 2002). Thus, multiple levels of cross-regulation and negative feedback loops are likely to create a specific dosage of active secreted molecules and provide precise positional information during development. As such, a subtle imbalance in the *active* levels of these important signaling molecules could affect cortical regionalization.

SIGNALING CENTERS REGULATE PROLIFERATION, CELL DEATH, AND THE EXPRESSION OF TRANSCRIPTION FACTORS Members of the WNT, BMP, and FGF families or their antagonists have been shown to modulate telencephalic morphogenesis, in part by regulating cell proliferation and cell death. For instance, *Wnt3a* inactivation drastically reduces proliferation in the medial pallium, leading to a failure in hippocampus development (Lee et al., 2000). Conversely, the BMP pathway is implicated in dorsal midline cell death (Hebert, Mishina, and McConnell, 2002). Finally, *Fgf8* loss-of-function experiments have shown that this factor is a positive regulator of telencephalic outgrowth via modulation of cell death and proliferation (Meyers, Lewandoski, and Martin, 1998; Reifers et al., 1998; Shanmugalingam et al., 2000; Garel, Huffman, and Rubenstein, 2003; Storm, Rubenstein, and Martin, 2003). These effects appear to function in part through transcription factors. For instance, *BF-1* (*Foxg1*), a member of the winged helix family, has been shown to regulate telencephalic outgrowth (Xuan et al., 1995; Dou, Li, and Lai, 1999), and its expression is regulated positively by FGF8 (Shimamura and Rubenstein, 1997; Kobayashi et al., 2002; Storm, Rubenstein, and Martin, 2003) and negatively by BMP4 (Furuta, Piston, and Hogan, 1997; Ohkubo, Chiang, and Rubenstein, 2002). Similarly, *Msx* genes, which act as positive regulators of apoptosis, are induced in response to BMP4 (Furuta, Piston, and Hogan, 1997).

More generally, it has been proposed that a balanced input of these patterning signals regulates by synergy or competition the graded or localized expression of transcription factors in the cortical neuroepithelium, which in turn controls pallium development and regionalization (figure 5.3). Such a mechanism would account for the observation that a relatively normal sensory map is formed on a surgically reduced cortical sheet in marsupials (Huffman et al., 1999). In particular, two genes encoding homeodomain transcription factors, *Emx2* and *Pax6*, have been shown to play key roles in cortical regionalization and will be presented in detail in the following section. These genes are expressed in gradients along the mediolateral (dorsoventral) and rostrocaudal axes of the cerebral cortex (Simeone, Acampora, et al., 1992; Simeone, Gulisano, et al., 1992; Stoykova and Gruss, 1994; Gulisano et al., 1996; Stoykova et al., 2000; Toresson, Potter, and Campbell, 2000; Yun, Potter, and Rubenstein, 2001; Muzio et al., 2002b), and their expression is likely regulated by patterning centers. In particular, promoter analysis has shown that *Emx2* is a direct target of BMP and WNT signaling pathways in the cortical primordium (Theil et al., 2002). Furthermore, both ectopic FGF8 and a reduction in *Fgf8* levels have shown that FGF8 negatively regulates *Emx2* expression (Crossley et al., 2001; Garel, Huffman, and Rubenstein, 2003; Storm, Rubenstein, and Martin, 2003). Finally, when *Fgf8* levels are severely reduced, *Pax6* expression is down-regulated (Garel et al., 2003; E. Storm, S. Garel, and J. Rubenstein, unpublished observations). Thus, there is accumulating evidence that signaling centers regulate cell proliferation, cell death, and the expression of key transcription factors in the cortical primordium. However, the epistatic relationship between these three downstream events remains largely unexplored.

Role of Transcription Factors in Defining Positional Information Within the Cerebral Cortex Primordium

Localized expression of transcription factors specify cortical domains
Several transcription factors with restricted or graded expression within the pallial neuroepithelium have been shown to control the specification of cortical domains. For instance, cortical progenitors in mutants of the LIM transcription factor Lhx2 acquire the molecular fate of the dorsalmost region of the telencephalic vesicle (Bulchand et al., 2001; Monuki, Porter, and Walsh, 2001). Furthermore, the opposing activities of the *Pax6* and *Gsh2* homeobox genes regulate the formation of the ventral pallium (VP) and its boundaries (Corbin et al., 2000; Toresson, Potter, and Camphell, 2000; Yun, Potter, and Rubenstein, 2001). The domains of strong expression of these two genes meet at the boundary between the VP and the adjacent dorsal lateral ganglionic eminence (dLGE). In *Gsh2–/–* embryos, the dLGE is respecified into a VP-like territory, whereas in *Pax6* mutant embryos, the VP is transformed into a dLGE-like structure.

In addition to these genes, some factors are implicated in controlling the size of cortical domains. For instance, inactivation of *Emx2* severely impairs medial pallium growth, leading to an absence or severe reduction of the hippocampus at birth (Pellegrini et al., 1996; Yoshida et al., 1997; Tole, Goudreau, et al., 2000). Overall, these analyses suggest that cortical patterning is generated via combinations of transcription factors that establish the identity, boundaries, and sizes of cortical domains.

Gradients of transcription factors regulate neocortical regionalization
Emx2 and *Pax6* are expressed in gradients along the mediolateral and rostrocaudal axis of the cerebral cortex. *Emx2* is expressed in a high mediocaudal to low laterorostral gradient, whereas *Pax6* is expressed in an opposite gradient within the proliferating cortical neuroepithelium (Simeone, Acampora, et al., 1992; Simeone, Gulisano, et al., 1992; Stoykova and Gruss, 1994; Gulisano et al., 1996; Stoykova et al., 2000; Toresson, Potter, and Campbell, 2000; Yun, Potter, and Rubenstein, 2001; Muzio et al., 2002b). In *Emx2–/–* mice, molecularly defined caudal cortical areas are severely reduced and more rostral areas expand caudally (figure 5.4) (Bishop, Goudreau, and O'Leary, 2000; Mallamaci et al., 2000; Bishop et al., 2002). In particular, the occipital cortex (presumptive visual cortex) in *Emx2–/–* mutant newborns adopts a molecular fate characteristic of the parietal neocortex (figure 5.4) (presumptive somatosensory cortex). This molecular shift correlates with a corresponding shift in thalamic projections (figure 5.5), suggesting that the relative size of functional cortical areas is modified in these mutants. Contrary to the phenotype observed in *Emx2–/–* mice, rostral cortical areas acquire a molecular

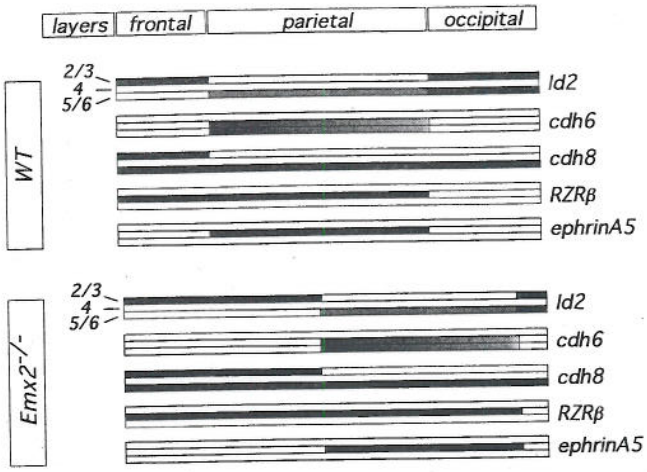

Figure 5.4 Regional and layer-specific expression of molecular markers define cortical molecular subdomains at birth. Expression of *Id2*, *cadherin 6* (*cdh6*), *cdh8*, *RZRβ*, and *ephrinA5* in different cortical layers and frontal, parietal, or occipital cortical domains is presented in wild type and *Emx2–/–* newborns. A coherent caudal shift in the boundaries of their expression is detected in *Emx2–/–* mutant newborns. (Compiled from Mackarehtschian et al., 1999; Nakagawa, Johnson, and O'Leary, 1999; Rubenstein et al., 1999; Bishop, Goudreau, and O'Leary, 2000; Mallamaci et al., 2000; Bishop et al., 2002.)

identity characteristic of more caudal ones in *Pax6* mutant mice (Bishop, Goudreau, and O'Leary, 2000; Bishop, Rubenstein, and O'Leary, 2002). However, since thalamic axons do not reach the cortex in *Pax6* mutant embryos, the pattern of connectivity of these molecularly modified cortical areas cannot be determined (Kawano et al., 1999; Pratt et al., 2000, 2002; Hevner, Miyashita-Lin, and Rubenstein, 2002; Jones et al., 2002). Finally, another gene encoding an orphan nuclear receptor, *COUP-TFI*, is expressed in a strong to weak, laterocaudal to mediorostral gradient (Wang et al., 1991; Jonk et al., 1994; Qiu et al., 1994; Liu, Dwyer, and O'Leary, 2000). In *COUP-TFI–/–* mice, molecular aspects of cortical regionalization are modified (Zhou, Tsai, and Tsai, 2001). In particular, caudal neocortical areas acquire the molecular identity of more rostral ones, and this molecular shift correlates with a change in the organization of thalamic projections.

Do these factors interact? *Emx2* expression expands in *Pax6* mutants, and vice versa (Muzio et al., 2002b), suggesting that EMX2 and PAX6 repress each other's activity. Furthermore, EMX2 and PAX6 have been implicated in early patterning of the cortex (Muzio and Mallamaci, 2003), as well as in cortical proliferation and outgrowth (Heins et al., 2001; Estivill-Torrus et al., 2002; Muzio et al., 2002a; Shinozaki et al., 2002; Bishop et al., 2003; Muzio and Mallamaci, 2003). Thus, it is possible that counteracting gradients of *Emx2* and *Pax6* regulate the relative size and/or specification of caudomedial and rostrolateral cortical domains, respectively.

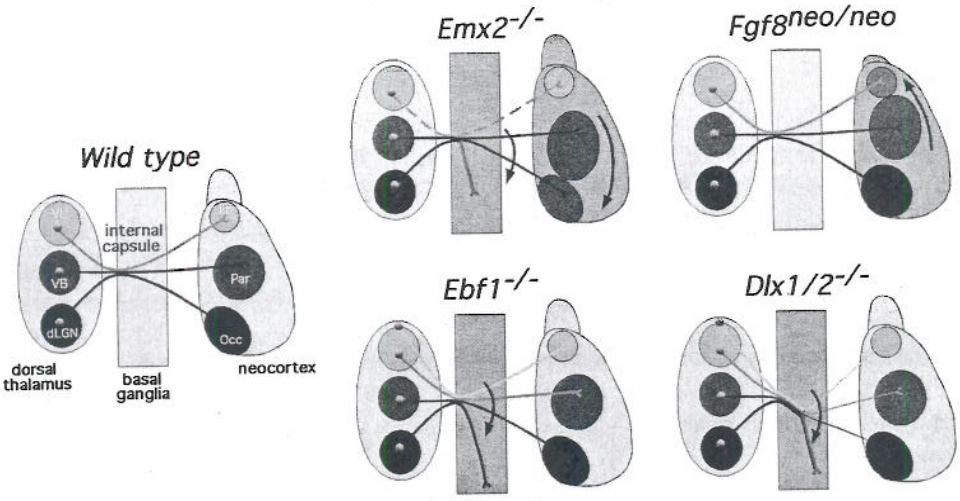

FIGURE 5.5 Cortical regionalization and thalamocortical targeting in *Emx2−/−*, *Fgf8neo/neo*, *Ebf1−/−* and *Dlx1/2−/−* mutant embryos. The initial targeting of VL, VB, and dLGN axons to the frontal, parietal, and occipital cortex is schematically represented by solid lines of light, intermediate, and dark gray, respectively. The connected thalamic nuclei and cortical subdomains are indicated by the same color code. Changes in the molecular properties of a structure are indicated by gray shading. In *Emx2−/−* mutant embryos, defects in cortical regionalization (black arrow) and axonal pathfinding in the basal ganglia lead to a caudal shift in thalamocortical projections (black arrow over the axons). In *Fgf8neo/neo* mutant embryos, a rostral shift of molecular properties (black arrow) is observed, whereas thalamocortical projections appear normal. On the contrary, basal ganglia defects in *Ebf1−/−* and *Dlx1/2−/−* mutant embryos affect thalamic axons pathfinding in this region and induce a caudal shift in the organization of thalamocortical projections (black arrows).

Abbreviations: dLGN, dorsolateral geniculate nucleus; Fr, frontal cortex; Occ, occipital cortex; Par, parietal cortex; VB, ventrobasal complex; VL, ventrolateral nucleus. (Compiled from Bishop, Goudreau, and O'Leary, 2000; Mallamaci et al., 2000; Bishop, Rubenstein, and O'Leary, 2002; Garel et al., 2002; Lopez-Bendito et al., 2002; Garel, Huffman, and Rubenstein, 2003.)

Initial targeting of thalamocortical projections: Role of intermediate structures

THALAMOCORTICAL PROJECTIONS: REACHING THEIR TARGET STRUCTURE Cortical areas are in part defined by their pattern of connection with the dorsal thalamus. Thalamocortical and reciprocal corticothalamic projections begin to form during embryogenesis (see earlier discussion under "Ontogenetic Origin of the Cerebral Cortex"), raising the question of what mechanisms control the specificity of this early innervation.

Before reaching their final target, thalamic and corticofugal axons grow through intermediate structures, where they undergo abrupt changes of trajectory and growth cone morphology (Miller, Chou, and Finlay, 1993; Metin and Godement, 1996; Molnar, Adams, and Blakemore, 1998; Braisted, Tuttle, and O'Leary, 1999; Auladell et al., 2000; Skaliora, Adams, and Blakemore, 2000). In vitro explant experiments and analyses of mutant mice have shown that guidance molecules and transient cell populations or axons located along axon pathways play a key role in regulating axon outgrowth and pathfinding (see figure 5.2). For instance, attractive guidance cues, including the secreted molecule netrin-1, regulate the turning of thalamic axons into basal ganglia at the diencephalic-telelecephalic boundary (Metin et al., 1997; Richards et al., 1997; Braisted, Tuttle, and O'Leary, 1999; Braisted et al., 2000). Conversely, the secreted factors slit-1 and slit-2 repel axons from the hypothalamus and prevent them from growing toward the midline and ventral territories of the basal ganglia (Bagri et al., 2002). Furthermore, the inactivation of *sema6A*, a transmembrane molecule of the semaphorin family, perturbs the navigation of thalamic axons at the diencephalic-telencephalic boundary (Leighton et al., 2001). Finally, patterning defects of the preoptic area due to inactivation of the transcription factor gene *Nkx2.1* affect pathfinding of corticofugal axons at the telencephalic-diencephalic boundary, whereas corticothalamic axons behave normally (Marin et al., 2002). These observations point to a key role of the embryonic basal ganglia in organizing afferent and efferent cortical projections.

Independently, transient cell populations located in the basal ganglia mantle, which extend projections into the thalamus or the neocortex, have been proposed to guide thalamocortical and corticofugal axons into the basal ganglia (see figure 5.2) (Mitrofanis and Baker, 1993; Mitrofanis and Guillery, 1993; Metin and Godement, 1996). Supporting this hypothesis, *Mash1* or *Pax6* mutant mice, which lack some of these transient cell populations, exhibit abnormal thalamocortical axonal pathfinding (Tuttle et al., 1999; Jones et al., 2002). Cortical subplate neurons also represent a transient cell population that regulates early aspects of

corticothalamic connectivity. Subplate axons are the first cortical projections to grow toward the thalamus, and they interact closely with thalamic axons (McConnell, Ghosh, and Shatz, 1989; Ghosh et al., 1990; Shatz et al., 1990; Ghosh and Shatz, 1992; Molnar, Adams, and Blakemore, 1998). Ablation of subplate cells in a cortical subregion induces pathfinding mistakes in the corresponding cortical projection and prevents thalamic innervation of the lesioned region (Ghosh et al., 1990; Ghosh and Shatz, 1993; McConnell, Ghosh, and Shatz, 1994). These observations provided a basis for the "handshake hypothesis" which proposes that converging cortical and thalamic axons meet in the basal ganglia and use each other to reach their target (Molnar and Blakemore, 1991, 1995; Molnar, Adams, and Blakemore, 1998). Consistently, thalamic and cortical axons grow in close contact within the internal capsule (Molnar, Adams, and Blakemore, 1998) and thalamic axons project to a displaced "subplate" in *reeler* mutant mice (Molnar, Adams, Goffinet, et al., 1998). Analyses of mutants with major subplate axonal defects, such as *Tbr1−/−*, *COUP-TFI−/−*, or *Emx1−/−*; *Emx2−/−* mice (Zhou et al., 1999; Hevner et al., 2001; Hevner, Miyashita-Lin, and Rubenstein, 2002; Bishop et al., 2003), or with an impaired thalamic projection, such as *Gbx2−/−* mice (Miyashita-Lin et al., 1999; Hevner, Miyashita-Lin, and Rubenstein, 2002), have shown that defects in one projection have a corresponding effect on the reciprocal thalamic or cortical projection. Similarly, in *p75−/−* mice, in which subplate axons of the occipital cortex show an abnormal outgrowth, the thalamic innervation of the occipital cortex is specifically reduced (McQuillen et al., 2002). Thus, although some experiments have suggested that thalamic and cortical axons do not fasciculate in vitro (Bagnard et al., 2001), there is accumulating evidence that these axons grow in close vicinity in vivo and require each other to reach their respective targets. These interactions could play a key role in the regulating reciprocity of thalamocortical projections in vivo.

Thus, long-distance attractive and repulsive cues, as well as contact-mediated interactions with cell populations and perhaps their axons, guide thalamic and cortical axons to their target structures. However, so far these activities cannot fully account for the domain-specific organization of thalamocortical projections.

THE FORMATION OF DOMAIN-SPECIFIC PROJECTIONS A prevailing model for the formation of topographically organized projections stipulates that these projections are generated through the restricted or graded expression of complementary cues within the projecting and targeted structure. Evidence for this model, known as the chemoaffinity model, comes from the mapping of retinotectal system (Sperry, 1963; Drescher, Bonhoeffer, and Muller, 1997; Goodhill and Richards, 1999; Feldheim et al., 2000). Support for this mechanism in the formation of thalamocortical maps comes from the cortical expression of Eph/Ephrins (Gao et al., 1998; Donoghue and Rakic, 1999; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000; Sestan, Rakic, and Donoghue, 2001; Takemoto et al., 2002; Uziel et al., 2002; Yun et al., 2003) and from the phenotypes of *Emx2−/−* and *COUP-TFI−/−* mice, where early changes in cortical molecular regionalization correlate with a shift in thalamic projections (figure 5.5) (Bishop, Goudreau, and O'Leary, 2000; Mallamaci et al., 2000; Zhou, Tsai, and Tsai, 2001). These results suggested that *Emx2* and *COUP-TFI* regulate the restricted neocortical expression of guidance molecules that would control the targeting of thalamic axons.

However, localized attractive or repulsive cortical cues, which could account for the formation of domain-specific thalamocortical projections, have not been identified in vitro yet. Indeed, while localized factors have been shown to regulate the differential innervation of the limbic cortex versus neocortex (Barbe and Levitt, 1991, 1992; Levitt, Barbe, and Eagleson, 1997; Mann et al., 1998), thalamic axons can grow in vitro into any neocortical region without showing a preference (Molnar and Blakemore, 1991). Furthermore, the study of *Fgf8* hypomorphic mice suggests that early cortical regionalization might not strictly control the initial targeting of thalamic axons (figure 5.5), that is, the positioning of thalamic axons within the cortical intermediate zone (Garel, Huffman, and Rubenstein, 2003). Indeed, in these mutants, early gradients of *Emx2* and *COUP-TFI* expression are shifted rostrally and, rostral cortical domains adopt the molecular identity of more caudal cortical regions. However, the initial targeting of thalamic axons in *Fgf8* hypomorphic mutants was indistinguishable from the one observed in wild-type newborns.

Furthermore, the analysis of *Ebf1−/−* and *Dlx1/2−/−* embryos has revealed that the initial domain-specific organization of thalamocortical projections can be shifted along the rostrocaudal axis in the absence of cortical or thalamic regionalization defects (figure 5.5) (Garel et al., 2002). *Ebf1* and *Dlx1/2* inactivation impair different aspects of basal ganglia mantle formation. In both mutants, the shift in thalamocortical targeting was preceded by a shift in the rostrocaudal trajectory of thalamic axons within the basal ganglia. Indeed, in both *Ebf1* and *Dlx1/2* mutants, the trajectory of thalamic axons is shifted as soon as they cross the diencephalic-telencephalic boundary and enter the basal ganglia. Thus, these observations indicate that the trajectory of thalamic axons within the basal ganglia participates in the initial targeting of thalamic axons to different rotrocaudal cortical domains. This idea is supported by the observations that in *Emx2* and *COUP-TFI* mutant mice, the first defects in thalamic axonal navigation are also observed at the telencephalic-diencephalic boundary and within the

basal ganglia (figure 5.5) (Zhou et al., 1999; Zhou, Tsai, and Tsai, 2001; Lopez-Bendito et al., 2002). Overall, these results indicate a role for intermediate targets in the guidance and initial targeting of thalamocortical projections. However, these experiments do not investigate the mechanisms regulating the later steps of thalamocortical outgrowth, such as the invasion of the cortical plate, the targeting of axons to layer IV, or the survival of these projections.

Arealization of the cerebral cortex

INTRINSIC DETERMINANTS AND THE GUIDANCE OF THALAMOCORTICAL AXONS WITHIN THE NEOCORTEX Although the initial domain-specific targeting of thalamic axons might not be strictly controlled by the cortex, there is a large body of evidence that cortical cues control the guidance and behavior of thalamic axons once they reach the cerebral cortex. For instance, coculture experiments have shown that thalamic axons recognize the layer IV of the neocortex by stopping and branching (Yamamoto, Kurotani, and Toyama, 1989; Molnar and Blakemore, 1991; Bolz, Novak, and Staiger, 1992; Yamamoto et al., 1992; Yamamoto, Higashi, and Toyama, 1997; Yamamoto, Inui et al., 2000; Yamamoto, Matsuyama, et al., 2000; Yamamoto, 2002). Furthermore, extracellular matrix and adhesion molecules are implicated in regulating the outgrowth of thalamic axons into the intermediate zone and cortical plate (Bicknese et al., 1994; Emerling and Lander, 1994, 1996; Miller et al., 1995; Mann et al., 1998).

Recently, members of the ephrin/Eph family, which show a localized expression in the cerebral cortex and dorsal thalamus, have been implicated in regulating the patterns of thalamic innervation (Flanagan and Vanderhaeghen, 1998; Gao et al., 1998; Donoghue and Rakic, 1999; Mackarehtschian et al., 1999; Vanderhaeghen et al., 2000; Sestan, Rakic, and Donoghue, 2001; Takemoto et al., 2002; Uziel et al., 2002; Yun et al., 2003). In vitro experiments have suggested that the expression of *ephrinB3* in the amygdala and limbic cortex (Takemoto et al., 2002) and of *ephrinA5* in the limbic cortex (Gao et al., 1998) might prevent neurite outgrowth of nonlimbic axons into these areas. Analysis of *ephrinA5* mutant mice has confirmed the role of this factor in preventing the outgrowth of thalamic somatosensory axons into the limbic cortex (Uziel et al., 2002). Furthermore, *ephrinA5* mutants have a distorted somatosensory map, suggesting a role for this factor in cortical map formation (Vanderhaeghen et al., 2000). Finally, in vitro coculture experiments also support that these molecules are important in the targeting of thalamic axons to layer IV (Mann et al., 2002). Thus, the restricted expression and layer-specific expression of Eph and ephrins in the neocortex may, as in the retinotectal system, regulate the formation of collaterals and the final innervation of the cortical plate by thalamic axons.

Additional factors, such as cadherins and neurotrophins, are implicated in the formation and refinement of thalamocortical projections. For instance, N-cadherin-blocking antibodies inhibit the ability of thalamic axons to stop in the layer IV of cortical explants (Poskanzer et al., 2003). Furthermore, genetic ablation of neurotrophin-3 in the cerebral cortex results in a decrease of thalamic innervation of the retrosplenial and visual cortex, which are two sites of neurotrophin-3 expression (Ma et al., 2002). Since neurotrophin-3 regulates axonal survival and sprouting, these results support a role for local neurotrophin activity in the establishment of thalamic innervation.

In addition to these local activities that apparently control thalamic outgrowth, branching, and potentially survival, there is evidence that a guidance activity can regulate the directionality of thalamic axons within the neocortex. Heterotopic transplantation experiments in the cortex of newborn rats have shown that, in some cases, the grafted tissue receives thalamic inputs characteristic of their cortical region of origin (Levitt, Barbe, and Eagleson, 1997; Frappe, Roger, and Gaillard, 1999; Gaillard and Roger, 2000). The factors responsible for this activity remain to be determined.

Thus, restricted cortical cues regulate the establishment of an initial thalamocortical innervation pattern, which is further refined in response to activity.

A MAJOR ROLE OF CORTICAL "INTRINSIC" REGIONALIZATION IN AREA MATURATION While the approximate locations of presumptive cortical areas can be identified at birth, functional cortical areas can only be defined postnatally. Key experiments have demonstrated the role of thalamic input in the maturation and plasticity of the cortical areal map (Rakic, Suner, and Williams, 1991; Catalano and Shatz, 1998). These experiments have recently been reviewed and will not be presented here (Pallas, 2001; Ptito et al., 2001; Sur and Leamey, 2001). However, recent experiments have shown that early patterning molecules are also essential to the functional organization of a neocortical area (Fukuchi-Shimogori and Grove, 2001).

In utero electroporation has been used to alter the levels and spatial distribution of FGF8 signaling while not seriously affecting the postnatal viability of the mice (Fukuchi-Shimogori and Grove, 2001). In these experiments, rostral electroporations of *Fgf8* or of a dominant-negative *Fgf8* receptor at early stages of embryonic development modify the rostrocaudal position of the somatosensory cortex, similar to what is observed in *Fgf8* mutant mice (Garel, Huffman, and Rubenstein, 2003). Unlike the *Fgf8* mutants, the electroporated mice survive and show a rostral displacement of the somatosensory barrel cortex, whose development depends both on intrinsic properties of the cortex and

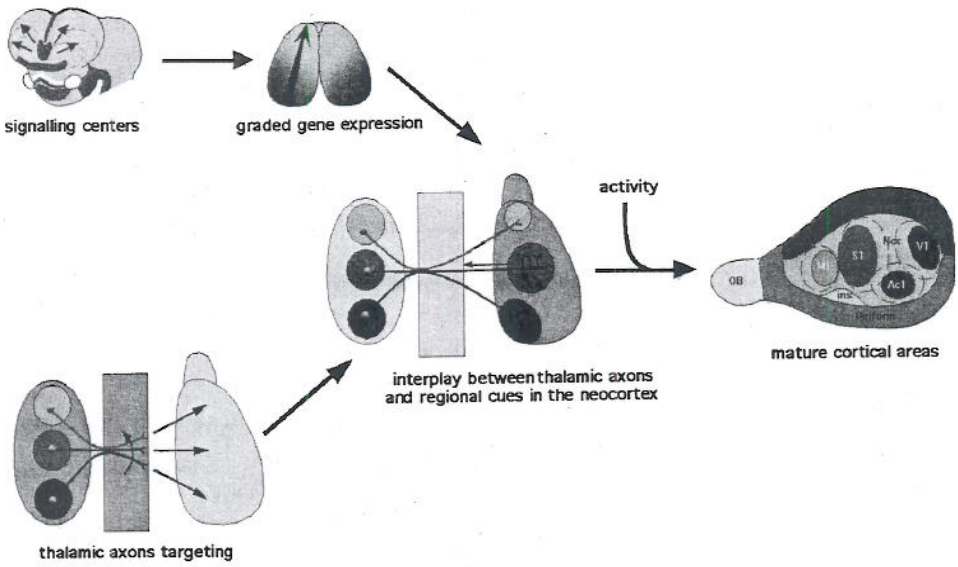

FIGURE 5.6 A model for the formation of cortical areas. Sequential steps in convergent pathways involved in the development of cortical areas are shown. The top pathway controls the regionalization of the cortical neuroepithelium via the effects of secreted molecules from patterning centers that control the graded expression of transcription factors. The bottom pathway controls thalamic development and the subsequent growth of thalamic axons through the diencephalon and basal ganglia to distinct regions of the cerebral cortex. The pathways converge when thalamic axons encounter regional cues within the cortex that direct their growth and branching into appropriate cortical areas. Finally, activity drives the further maturation of cortical areas.

on thalamic activity. Strikingly, an ectopic caudal source of FGF8 induced a partial mirror-image duplication of the barrel field (Fukuchi-Shimogori and Grove, 2001). This remarkable duplication reproduces the phenomenon by which new cortical areas have emerged during evolution: duplication of central representation and reverse symmetry (mirror image). Together these experiments point out a key role of localized secreted factors in the emergence of functional cortical areas. The mechanisms involved, however, remain to be fully elucidated.

THE PATTERN OF THALAMIC INNERVATION IN EARLY CORTICAL AREALIZATION While activity and input can modify cortical areas properties (Pallas, 2001; Ptito et al., 2001; Sur and Leamey, 2001), some observations conversely suggest that thalamic input may regulate or refine some early aspects of cortical molecular regionalization (O'Leary, Schlaggar, and Tuttle, 1994; Paysan et al., 1997; Gitton, Cohen-Tannoudji, and Wassef, 1999a; Dehay et al., 2001; Gurevich, Robertson, and Joyce, 2001; Polleux et al., 2001). For instance, whereas the activation of the H-2Z1 transgene in the somatosensory cortex is independent of thalamic input, it is not independently expressed once thalamic axons have reached the neocortex (Gitton, Cohen-Tannoudji, and Wassef, 1999a,b). Similarly, in the cortex of *Mash1* mutants, which lack thalamic input, *ephrinA5* expression is modified, although this could be due to the expression of *Mash1* in cortical progenitors (Yun et al., 2003).

A model of cortical arealization

The data reviewed herein suggest an integrated model of cortical arealization (figure 5.6). During embryogenesis, the cerebral cortex primordium is regionalized by discrete sources of secreted molecules that regulate the localized or graded expression of transcription factors. Interactions between these secreted molecules or transcription factors control the relative growth of cortical domains and delimit the boundaries between cortical territories. In a subsequent step, the positional information in the cortical progenitors is imprinted in radially migrating postmitotic neurons. In parallel, thalamic axons grow through the diencephalon and then basal ganglia, where their relative positioning is regulated; from there they enter distinct parts of the cortex. Once in the cortex, thalamic axons sense local cues that control their outgrowth and branching into appropriate regions. Determining the respective roles of intrinsic and extrinsic factors in the emergence of mature cortical areas remains an essential step in our understanding of cortical patterning and development.

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