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GENERATING THE CEREBRAL CORTICAL AREA MAP

Elizabeth A. Grove and Tomomi Fukuchi-Shimogori
Department of Neurobiology, Pharmacology, and Physiology, University of Chicago, 947 East 58th Street, Chicago, Illinois 60637; email: egrove@drugs.bsd.uchicago.edu, tfukuchi@drugs.bsd.uchicago.edu

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■ **Abstract** The view that the cortical primordium is initially patterned in similar ways to the rest of the embryo has been a conceptual breakthrough. We now have a new starting point for understanding how the cortical area map is established and how maps may change and evolve. Here we review findings that signaling molecules secreted from distinct cortical signaling centers establish positional information in the cortical primordium and regulate regional growth. In other embryonic systems, positional signals would regulate the patterned expression of transcription factors, leading, in a gene regulatory cascade, to the patterned differentiation of the tissue. We discuss candidate transcription factors with respect to such a model of cortical patterning. Finally, embryonic structures interact to pattern one another. We review data suggesting that the thalamus and cortex are patterned independently then interact to generate the final cortical area map.

INTRODUCTION

A spectacular advance in biology has been to uncover many of the principles and molecular mechanisms that underlie embryonic patterning of the vertebrate and invertebrate body plans (Wolpert 1996). These discoveries have been applied to understanding the morphogenesis of tissues as diverse as the fly wing and the vertebrate spinal cord (Wolpert 1996). However, not until recently has evidence emerged that the mammalian cerebral cortex is patterned, at least in part, by the same types of mechanisms (Bishop et al. 2000, Bishop et al. 2002, Ragsdale & Grove 2001, Garel et al. 2003, Mallamaci et al. 2000, Rubenstein et al. 1999). The pattern to be explained is the division of the cerebral cortex into anatomically distinct and functionally specialized areas, which form a species-specific area map (Nauta & Feirtag 1986). The mechanisms that initiate map formation in development have been elusive. Here, we review classic and recent studies that open up the problem in a new way.

Other structures are patterned in the embryo by signaling centers that lie at the boundaries of the tissue to be patterned. These centers release signaling proteins that regulate regional growth and specify regional identity in the tissue. In an influential model, signaling proteins called morphogens diffuse through the tissue and establish a gradient that directly confers positional information (Wolpert 1996). Not all secreted signaling molecules involved in patterning are morphogens, however. For example, in the developing spinal cord, Wingless-Int (WNT)1 and 3a form a concentration gradient of protein but one that coordinates tissue growth, a different element of patterning (Megason & McMahon 2002).

In some systems, such as the *Drosophila* embryo or the embryonic vertebrate spinal cord, it is well established that cells respond to different levels of a morphogen by expressing specific transcription factors (Wolpert 1996). These regionally expressed transcription factors in turn control regional expression of downstream genes that regulate local differentiation of the tissue (Briscoe & Ericson 2001). Here we review new findings that begin to fit development of the cortical area map to such a model.

Development of the area map, however, cannot be understood by focusing on the cortical primordium in isolation. In the adult, the cerebral cortex can be viewed, both by connections and function, to be at the apex of a hierarchy of brain structures (Nauta & Feirtag 1986). Because embryonic tissues interact to pattern one another, other developing brain structures would be expected to influence cortical pattern. In particular, a key feature of the cortical area map is that different areas receive distinct sets of projections from different thalamic nuclei, relaying information from the periphery and other parts of brain (Nauta & Feirtag 1986). We survey selected recent studies of whether thalamic axons and their activity initiate, maintain, or regulate select features of cortical area identity. Meanwhile, differential thalamocortical innervation across the cortex is itself a major part of cortical patterns. We summarize findings that molecular cues in the cortex guide thalamic axons but that patterning in the thalamus itself and cues along the thalamocortical pathway are also critical to initiating this component of cortical pattern. Taken as a whole, available data suggest that the cerebral cortex and thalamus are first patterned independently and then coordinate and interact to generate the mature cortical area map.

CLASSIC MODELS OF CORTICAL PATTERNING

Over the past several years, two classic models have dominated research into the development of the cortical area map (Rakic 1988, O'Leary 1989) (Figure 1).

The Protomap Model

In the protomap model of area map formation (Rakic 1988), the cortical primordium is patterned as it is generated. Although this model was proposed before recent advances in understanding molecular mechanisms of embryonic patterning, it already implied that the cortex is like other parts of the embryo, patterned as cells are dividing. Intrinsic area differences, specified by molecular determinants,

are set up in the ventricular zone (VZ), the germinal cell layer of the cortical primordium. As newborn neurons migrate out of the VZ in radial arrays they carry the area protomap with them to form the cortical plate (CP), the incipient grey matter of the cortex.

The Protocortex Model

The cytoarchitectonic features classically used to define areas appear relatively late in corticogenesis; moreover, some early cortical transplant experiments suggested prolonged plasticity of area identity (O'Leary 1989, Schlaggar & O'Leary 1991). These observations support the protocortex model (O'Leary 1989), in which the cortical primordium is essentially homogeneous as it is generated and is patterned into areas later by cues from axons growing in from the thalamus. Patterning mechanisms in cortex are thus somewhat distinct from those in the rest of the embryo. Thalamic afferents arrive after growth of the cortical primordium is well underway, whereas, in other embryonic systems, patterning is initiated before major growth (Edgar & Lehner 1996).

The protocortex model received strong support from retroviral labeling studies showing significant tangential dispersion of cells across the developing cortical primordium (Walsh & Cepko 1988, Walsh & Cepko 1992). A broad tangential dispersion of newborn cortical neurons would appear to disrupt the translation of a protomap from the VZ to the cortical plate. The area map could not be set up as neurons are born and would have to depend on later cues. This issue has been clarified by tracking the tangentially dispersing cells and, most recently, by fate-mapping progenitor cells in the cortical primordium proper (Anderson et al. 1997, Gorski et al. 2002, Tan et al. 1998). In rodent, most or all tangentially migrating cortical cells appear to be interneurons migrating from the ventral telencephalon. Pyramidal neurons, the projection neurons of the cerebral cortex, are generated in the cortical primordium proper and migrate radially to the cortical plate, consistent with a protomap model (Anderson et al. 1997, Gorski et al. 2002, Tan et al. 1998).

NEED FOR A NEW MODEL OF CORTICAL PATTERNING

Inhomogeneities in the Cortical Primordium

The emphasis of the protocortex model on viewing the cerebral cortex in the context of the rest of the developing brain remains vital. However, throughout the last decade, and accelerating in recent years, evidence has accumulated to support a version of a protomap model. First, although the cortical primordium may appear morphologically homogeneous [though see review of work by Kennedy, Dehay and colleagues (Dehay et al. 1993), below], it is, in reality, highly heterogeneous. Before thalamic innervation, the limbic system–associated membrane protein (LAMP), latexin, and the *H-2Z1* transgene are each specified to be expressed respectively in rodent limbic cortex, lateral cortex, and primary and secondary somatosensory areas (S1, S2) (Arimatsu et al. 1992, Barbe & Levitt 1991, Cohen-Tannoudji et al. 1994). Although LAMP, latexin, and the *H-2Z1* transgene are themselves upregulated only late in corticogenesis or postnatally, other gene expression patterns indicate regional differences among the proliferating cells of the VZ themselves. Genes expressed regionally in the VZ include genes encoding components of patterning signaling cascades, such as Fibroblast growth factors (FGFs), FGF receptors, (Wingless-Ints) WNTs, WNT receptors, and mediators of WNT signaling, as well as transcription factors Emx1, Emx2, Lhx2, Pax6, COUP-TFI, and COUP-TFII (Bachler & Neubuser 2001, Donoghue & Rakic 1999b, Galceran et al. 2000, Gulisano et al. 1996, Kim et al. 2001b, Lee et al. 2000b, Nakagawa et al. 1999, Ragsdale et al. 2000, Zhou et al. 2001). Some of these genes have now been implicated in early patterning of the cortical primordium. Still other gene expression patterns appear in primate and rodent cortical primordium later in corticogenesis but before cytoarchitectonic features distinguish areas and, in some cases, even before thalamic input (Donoghue & Rakic 1999a,b; Mackarehtschian et al. 1999; Nakagawa et al. 1999). Given that connectivity is a major area-specific feature, it is not surprising that several of these molecules are associated with axonal guidance and growth, including LAMP, the classic cadherins Cdh6, Cdh8, and Cdh11, ephrins, and Eph kinase receptors (Donoghue & Rakic 1999a,b; Mackarehtschian et al. 1999; Nakagawa et al. 1999).

Early Cortical Pattern Does Not Depend on Thalamic Innervation

New data indicate that area-specific molecular features are specified independent of thalamic input. Prenatal gene expression domains that prefigure area boundaries develop in mice lacking the transcription factor Gbx2, even though thalamocortical innervation is disrupted in these animals (Miyashita-Lin et al. 1999). Similar observations are reported in mice deficient in the transcription factor Mash-1 (Nakagawa et al. 1999). Cortical explants isolated near the onset of neurogenesis upregulate the *H-2Z1* transgene (Gitton et al. 1999b) or molecular markers of hippocampal fields (Tole & Grove 2001) in clear, correctly positioned domains that resemble areas in vivo. Although these gene expression patterns are nonclassical features of area identity, some persist into adulthood, making them bona fide aspects of mature area identity (Tole & Grove 2001). Finally, the topography of thalamocortical projections is shifted in mice deficient in the transcription factors Ebf1 and Dlx1/2, yet cortical regional gene expression is unaltered (Garel et al. 2003). Thus, molecular regionalization that anticipates the cytoarchitectonic area map arises in spite of disturbed or even absent thalamic input.

A proviso is the contribution of differential cell proliferation to area parcellation in primate and rodent—that is, the cortical primordium is not quite homogeneous morphologically (Dehay et al. 1993, Polleux et al. 1997). In primates, these authors report that presumptive primary visual cortex proliferates most rapidly, so that, in the adult, this area contains twice as many neurons in cross section as does neighboring cortex. Differing rates of proliferation may depend in part on a mitogen derived from early thalamic afferents (Dehay et al. 2001). This finding is nonetheless

consistent with the classic protomap model in that the effect of the thalamus occurs on the cortical proliferative layer in which the protomap is laid out.

A still more fundamental question remains to be asked, namely, how is the protomap itself set up? That is, how is positional information initially conferred upon the cortical primordium, and how is this information interpreted to generate a protomap?

CORTICAL PATTERNING CENTERS

Patterning of the head and forebrain, which incorporate cerebral cortex, depends on sources of signaling molecules including Bone Morphogenetic Proteins (BMPs), WNTs, and their antagonist proteins (Bachiller et al. 2000, Kiecker & Niehrs 2001b, Nordstrom et al. 2002). Signaling centers determine dorsal/ventral or anterior/posterior (A/P) axes in the spinal cord and at the midbrain-hindbrain boundary (Briscoe & Ericson 2001, Crossley et al. 1996, Lee et al. 2000a, Liem et al. 1997, Shamim et al. 1999, Wurst & Bally-Cuif 2001). In chick, in which a true cerebral cortex is lacking, morphogenesis of the telencephalon is coordinately regulated by sources of BMP4, Sonic hedgehog (SHH), and FGF8 (Ohkubo et al. 2002). Why should mammalian cerebral cortex be different?

Typical embryonic patterning molecules have been identified at the poles or boundaries of the cortical primordium, which are characteristic sites for embryonic signaling centers (Bachler & Neubuser 2001, Crossley & Martin 1995, Furuta et al. 1997, Grove et al. 1998, Shimamura & Rubenstein 1997) (Figure 2). Multiple *WNT* and *BMP* genes are expressed at the medial margin of the cortical primordium, a tissue termed the cortical hem (Grove et al. 1998). At the anterior pole several *FGF* family members including *FGF3*, *8*, *17*, and *18* overlap in expression (Bachler & Neubuser 2001). Other potential signaling sources, expressing SHH, retinoids, or members of the Epidermal Growth Factor (EGF) family, are also under investigation (Eagleson & Levitt 1999, Ragsdale et al. 2000, Rubenstein et al. 1999). What have these signaling centers been shown to do?

ANTERIOR/POSTERIOR PATTERNING OF THE CORTICAL PRIMORDIUM

An FGF Signal Imparts Anterior-Posterior Positional Information

The role of FGF8 in anterior/posterior (A/P) patterning of the midbrain (Crossley et al. 1996, Shamim et al. 1999) suggests that FGF8, possibly in concert with other FGFs, confers A/P positional information to the cortical primordium. This hypothesis has been difficult to test because mice lacking FGF8 die at gastrulation (Sun et al. 1999) before the cortex develops. Ideally, area patterning in the cerebral cortex should be analyzed at postnatal ages. We therefore developed a method to manipulate gene expression in mouse embryos (Fukuchi-Shimogori & Grove 2001) that allows the mouse pups to be born normally and analyzed at any age of interest.

We utilized this method, in utero microelectroporation, to test the hypothesis that FGF8 imparts A/P positional information to the cortical primordium (Fukuchi-Shimogori & Grove 2001). First, the anterior source of FGF8 was augmented by electroporating *FGF8* into the anterior cortical primordium at embryonic day (E) 11.5, shortly after cortical neurogenesis begins. Second, the endogenous source was reduced by electroporating a construct encoding a truncated, soluble form of a high-affinity FGF8 receptor, FGFR3 isoform c (sFGFR3c). The soluble receptor is presumed to sequester FGF8 and potentially other related FGFs, preventing them from binding to their endogenous receptors (Ye et al. 1998). Third, and most instructive, a new source of FGF8 was introduced into the cortical primordium at the opposite pole to the endogenous source.

Area identity was evaluated postnatally with gene expression markers, classic features of cytoarchitecture, and for primary somatosensory cortex (S1) labeling of the barrel fields, supporting the hypothesis that anterior overexpression of FGF8 enlarges anterior cortical areas and shrinks more posterior areas, shifting them back toward the posterior pole of the cortex. Conversely, expression of sFGFR3c shifts area boundaries toward the anterior pole. The barrel fields, normally centrally located in the cerebral hemisphere, move into the posterior half of the hemisphere after overexpression of FGF8 or into the anterior half after electroporation of sFGFR3c (Fukuchi-Shimogori & Grove 2001).

A new posterior FGF8 source should locally reverse the A/P axis of the map, leading to partial area duplications. Consistent with this hypothesis, posterior electroporation of *FGF8* elicits duplication of somatosensory whisker barrels (Figure 2). In some brains, extra ectopic barrels merge with the posterior boundary of native S1. Most striking, in several cases ectopic barrels form a separate, duplicate subfield. Morphologically, duplicates resemble the barrel subfield that represents the large whiskers of the main whisker pad; moreover, they seem mirror-reversed with respect to native subfields. Thus, a new source of FGF8 locally reverses A/P polarity: A posterior region of the cortical primordium is specified to take on a more anterior identity and form a new, inverted S1 subfield.

Electroporation of FGF17 has similar effects to FGF8 (Grove 2002), which suggests that FGF family members selectively expressed near the anterior pole of the cortical primordium work together to impart A/P positional information. Among these, strong candidates are FGF17 and 18. *FGF17* and *-18* are expressed at the right place (Bachler & Neubuser 2001) and are closely related to FGF8 in sequence identity, receptor binding properties, and function in other developmental systems (Ornitz & Itoh 2001, Xu et al. 2000).

Character of Ectopic Barrels

FGF manipulations alter a range of area-specific features in tandem, suggesting a true repatterning of the cortex and not merely a shifting or duplication of some area features. For example, ectopic barrels appear to be innervated by functional

thalamic afferents. They stain immunohistochemically for the 5HT transporter (5HTT) and for GAP43 (Grove 2002), both transiently expressed in the axons of the somatosensory ventrobasal nucleus (VB) of the thalamus (Maier et al. 1999). At early postnatal ages, 5-HTT immunostaining can be compared with bulk anterograde tracing of VB afferents to S1 (Rebsam et al. 2002). Classic studies describe dramatic barrel field plasticity in the first postnatal week (Jeanmonod et al. 1981, Van der Loos & Woolsey 1973). If sensory input to a barrel is disrupted, the barrel is lost or fuses with neighbors. Preliminary evidence suggests that whisker follicle cautery results in duplicate barrel losses in native and ectopic barrel fields (T. Fukuchi-Shimogori and E.A. Grove, unpublished results). Further, as in native barrels, in ectopic barrels, a cell-dense wall in layer 4 of S1 surrounds a cell-poor hollow (Grove 2002). Because barrel cytoarchitecture is disrupted in mice deficient in cortical *N*-methyl-D-aspartate (NMDA) receptor function (Iwasato et al. 2000), mGluR5, or PLC-beta1 (Hannan et al. 2001), these observations suggest that aspects of thalamocortical glutamatergic transmission are functional in ectopic barrels.

The tandem shift of multiple S1 features by manipulations of FGF signaling within the cortical primordium implies that these experiments disrupt a master cortical patterning signal. This in turn results in the disruption of a cascade of normal developmental events that include the positioning of protoareas within a protomap, regional distribution of axon guidance cues, and the area-specific receptivity of the developing cortex to molecular or activity-based cues from thalamocortical afferents.

Significantly, the ability to duplicate barrels at will opens up a new way to study development of cortical functional modularity in this classic model system. How are the ectopic barrels innervated from the thalamus, which appears to retain the normal number of barreloids? Which axon guidance cues for thalamocortical afferents are altered, where, and how? Are there behavioral consequences of duplicate barrels on the whisking behavior of the mouse?

FGF8 Hypomorphic Mice

An alternative approach to testing the role of FGF8 in neocortical patterning is to analyze mice hypomorphic for FGF8 (Garel et al. 2003). A hypomorphic allele was generated that reduces *FGF8* transcripts by 80% (Meyers et al. 1998), and mice live until birth. Consistent with findings described above, reduction of FGF8 shifts area-related gene expression patterns toward the anterior pole of the cortex, and, in some severe hypomorphs, a frontal cortical domain disappears entirely.

Surprisingly, FGF8 hypomorphs show a near-normal topography of thalamocortical connections. In the *Ebf1* and *Dlx1/2* mutant mice described above, the converse occurs: Innervation is shifted without an alteration in regionally expressed genes (Garel et al. 2002). In each mouse mutant, there is therefore a mismatch between patterns of thalamic innervation and cortical gene expression. These observations appear inconsistent with the ability of electroporation-induced FGF manipulations to shift area features in tandem, including gene expression and thalamic innervation.

A likely explanation is that more than one set of cues guide thalamic axons to the cortex, discussed further below. The apparent inconsistency here is resolved if cues outside the cortex are responsible for early guidance of thalamocortical axons and that cues within the cortex take over later. Thus, when FGF8 hypomorphs and *Ebf1* and *Dlx1/2* mutants are examined at birth, extracortical cues are dominant—and normal in the FGF8 hypomorph but abnormal in *Ebf1* and *Dlx1/2* mutants. In contrast, by the time mice electroporated with FGF constructs are analyzed postnatally, cortical cues have guided the axons to their final target (Figure 3).

Additional Involvement of FGF Signaling with Cortical Patterning

In mice deficient in FGF receptor 1 in the telencephalon, the most anterior neurons of the cortical hemisphere, those of the olfactory bulb, are misplaced posteriorly (Hebert et al. 2003). Neocortex is marked by subtle shifts in transcription factor gradients, including Emx2. The subtlety of these shifts may reflect functional redundancy of FGF receptors, also suggested by the absence of neocortical patterning defects in mice lacking FGFR3 (S. Assimacopoulos, C.W. Ragsdale, and E.A. Grove, unpublished results). Experiments to test this hypothesis will disrupt function of combinations of FGFR1, 2, and 3, each expressed in the cortical primordium VZ (Ragsdale et al. 2000).

Another FGF family member, FGF2, is not expressed exclusively at the anterior pole of the cortical primordium but appears to play a role in regulating regional growth, a critical element of patterning (Korada et al. 2002). Specifically, mice deficient in FGF2 show a decrease in the number and size of glutamatergic neurons in frontal and parietal, but not occipital, cortex (Korada et al. 2002).

How Does an Anterior FGF Signal Work to Initiate Cortical Patterning?

Classic morphogens act by diffusing through the tissue to be patterned and establishing a signaling gradient that confers positional information (Wolpert 1996). Altering the anterior FGF8 signal produces widespread effects in neocortex, and, as a morphogen should (Wolpert 1996), FGF8 repolarizes the tissue when placed in a new position (Fukuchi-Shimogori & Grove 2001). FGFs are implicated in patterning in several systems (Crossley et al. 1996, Neubuser et al. 1997, Shamim et al. 1999, Shimamura & Rubenstein 1997, Tucker et al. 1999, Ye et al. 1998); however, these proteins might not be expected to act as classic morphogens (Ornitz & Itoh 2001, Szebenyi & Fallon 1999). Heparin or heparan sulfate proteoglycans stabilize FGF proteins and allow them to activate their receptors effectively, but they are also thought to limit FGF diffusion (Ornitz & Itoh 2001).

A question for future experiments therefore is whether FGF8 forms a protein gradient that acts directly to specify different A/P positional fates. Pertinent to this, it has been difficult to establish the long-range action of other candidate

morphogens, even in simpler embryonic systems. Long-range diffusion of the BMP homolog, Decapentaplegic (Dpp), has been inferred in *Drosophila* by the patterning effects of mutated Dpp receptors expressed in cells distant from the Dpp source. It now appears that the Dpp binding protein, Short gastrulation (Sog), which can inhibit Dpp, also greatly increases Dpp diffusion (Eldar et al. 2002), potentially explaining the actions of Dpp at a distance. Other recent experiments demonstrate that the signaling protein SHH specifies position directly and at a distance in the vertebrate CNS (Agarwala et al. 2001, Briscoe et al. 2001, Hynes et al. 2000). Analogous mutant receptor misexpression studies will be useful in determining if FGF8 can be detected at a distance from its source and if different levels of FGF8 confer different A/P fates.

Whether the anterior FGF signal sets up A/P pattern directly or indirectly, the final positional gradient read off by cortical cells appears to be high resolution. Using the barrel fields as an example, when extra FGF8 is electroporated anteriorly, individual barrels are foreshortened along the A/P axis; whereas, when the anterior source is diminished by sFGFR3c, barrels are elongated (Fukuchi-Shimogori & Grove 2001, Grove 2002). These effects suggest that extra FGF8 increases the A/P slope of a fine positional gradient, leading to a shorter range of A/P positional values suitable for the development of each barrel; whereas, reducing the FGF signal decreases the slope of the gradient, leading to a longer appropriate territory.

MEDIAL/LATERAL PATTERNING OF THE CORTICAL PRIMORDIUM

A WNT Signal from the Cortical Hem Required for Hippocampal Development

A second candidate signaling center, the cortical hem, lies along the medial edge of the cortex, next to the hippocampus, and expresses multiple *WNT* and *BMP* genes (Furuta et al. 1997, Grove et al. 1998). Mice deficient in *WNT3a*, expressed early at the hem, show a near total loss of the hippocampus (Lee et al. 2000b). A similar defect appears in mice with reduced function of Lef1/Tcf transcription factors, downstream nuclear mediators of the canonical WNT signaling pathway (Galceran et al. 2000). These findings are therefore complementary, which suggests that the hem regulates hippocampal development via a WNT3a signal transduced by the canonical WNT pathway (Ragsdale & Grove 2001).

A noted above, both conferring positional information and regulating regional growth are components of patterning. Does WNT3a instruct neighboring cells to adopt a hippocampal fate or regulate their proliferation? Existing evidence suggests the latter. Cell proliferation is decreased in the region of the presumptive hippocampus in both WNT signaling mutants (Galceran et al. 2000, Lee et al. 2000b); meanwhile, overexpressing *WNT3a* early in corticogenesis expands the cortical hemisphere but does not induce ectopic hippocampal cell fates (T. Fukuchi-Shimogori and E.A. Grove, unpublished observations). Whether the multiple

WNTs expressed at the hem additionally play a role in positional specification remains to be fully investigated. Suggesting they may, WNT signaling is involved in positional and cell-type specification throughout neural development (Kiecker & Niehrs 2001a, Nordstrom et al. 2002), and medial/lateral (M/L) cortical expression patterns of genes encoding WNTs, Frizzled receptors, and secreted Frizzled-related proteins indicate a complex function in cortical development along the M/L axis (Kim et al. 2001b, Ragsdale et al. 2000).

BMP Signals from the Cortical Hem

Hem BMP signals are implicated in development of the most medial cortical neuroepithelial derivative: the telencephalic choroid plexus epithelium (CPe). In mice engineered to lack BMP receptor 1a (BMPR1a) in the telencephalon, CPe cells are sparse (Hebert et al. 2002). Conversely, when cortical primordium expresses constitutively activated BMPR1a, CPe develops at the expense of cerebral cortex (Panchision et al. 2001). These findings, together with the established role for TGFβ family ligands in patterning the dorsal spinal cord (Lee et al. 2000a, Liem et al. 1997), encourage further studies of BMP signaling in patterning the cortical sheet.

The Anti-Hem

EGFs have attracted relatively little attention as morphogens in vertebrate nervous system patterning, but two lines of research prompted a search for a cortical EGF signaling center. First, classic in vitro experiments implicate EGF family members in the development of the cortical limbic system. LAMP expression is upregulated in cortical cells from nonlimbic domains in response to an EGF ligand, TGFα (Ferri & Levitt 1995). Second, EGF-receptor-mediated signaling controls specification of dorsal-ventral cell-type in the development of the *Drosophila* ventral nerve cord (Skeath 1998). Two EGF ligands are involved: spitz, a TGFα-like molecule, and vein, similar in structure to the Neuregulin proteins, a subfamily of vertebrate EGFs (Golembo et al. 1999). Screening expression patterns of several *EGF* genes reveals a signaling source positioned as a mirror image of the WNT- and BMP-rich cortical hem (S. Assimacopoulos, E.A. Grove, and C.W. Ragsdale, unpublished observations). This cortical "anti-hem," situated along the lateral margin of the embryonic cerebral cortex, is identified by gene expression for multiple EGF family members, *TGFα*, *Neuregulin 1*, and *Neuregulin 3* (S. Assimacopoulos, E.A. Grove, and C.W. Ragsdale, unpublished observations), as well as the secreted WNT antagonist *sFrp2* (Kim et al. 2001a, Ragsdale et al. 2000) (S. Assimacopoulos, E.A. Grove, and C.W. Ragsdale, unpublished observations). The anti-hem, or cortical/subcortical boundary, has previously been suggested as a potential signaling center (Muzio et al. 2002b). It is lost in *Pax6* mutant mice (S. Assimacopoulos, E.A. Grove, and C.W. Ragsdale, unpublished observations), suggesting that defects in lateral cortical patterning seen in *Pax6* mutants are due in part to disruption of this cortical signaling center (Muzio et al. 2002b) (see below).

POSITIONING AND INTERPRETING SIGNALING CENTERS

Identifying Molecular Determinants

How do cells interpret positional information, allowing a protomap to be set up? That is, what are the molecular determinants that respond to positional signals and define protoareas? As noted above, in other parts of the embryo, signaling centers regulate the regional expression of transcription factors. In one well-studied system, the ventral spinal cord, pairs of transcription factors subsequently repress one another's expression, creating increasingly precisely bounded domains (Briscoe & Ericson 2001). If patterning were to proceed in cerebral cortex as in the leading spinal cord model, sharpened transcription factor domains would control local expression of still other genes that would in turn regulate area-specific differentiation.

Pax6 and Emx2

The transcription factors Pax6 and Emx2, a vertebrate homolog of *Drosophila*, empty spiracles, are expressed in opposing gradients along the A/P axis of the cortical primordium. In mice deficient in either Pax6 or Emx2, cortical gene expression patterns shift along the A/P axis, analyzed just before birth, and the shifts are complementary in the two mutants. Based on these findings, it has been proposed that Emx2 and Pax6 cooperate to set up area pattern (Bishop et al. 2002). Consistent with the above hypothesis, expression levels of *Emx2* or *Pax6* are altered in mice mutant for the other gene (Muzio et al. 2002b), but there is no biochemical evidence for a direct interaction between the two proteins. More significantly, gradients of *Emx2* and *Pax6* expression do not increase in steepness as development proceeds to generate a common sharp boundary. Nor does expression of the genes appear to be controlled by the same signaling molecule. In *Emx2* mutant mice, cerebral cortex is smaller than normal but otherwise shows patterning defects similar to those that follow anterior overexpression of FGF8 (Bishop et al. 2002). The similarity in cortical gene expression shifts in *Emx2* mutants and mice in which the anterior FGF8 source is augmented suggests that FGF8 controls the graded expression (low anterior, high posterior) of *Emx2* in the cortical primordium. Findings from mice and chick bear this out (Grove 2002, Ohkubo et al. 2002, Garel et al. 2003). Augmenting FGF8 downregulates *Emx2* expression, and depleting FGF8 upregulates *Emx2*. By contrast, no robust interactions have been identified between Pax6 and FGF8 (Grove 2002, Muzio et al. 2002b, Garel et al. 2003) despite the resemblance in cortical patterning shifts between the Pax6 mutant and mice with reduced anterior FGF signaling. Thus, if Emx2 and Pax6 do establish area pattern together, their functional interplay differs from the transcription factor interactions that direct cell identity in spinal cord (Briscoe & Ericson 2001).

A plausible conclusion remains that the anterior FGF signal controls A/P patterning in part via regulation of Emx2. Emx2 is one of the protomap molecular determinants, although it is distributed in an expression gradient rather than a clear domain. An alternative conclusion is prompted however by evidence for abnormal FGF signaling in the *Emx2* mutant mouse. *FGFR3* is normally expressed in a high-posterior, low-anterior gradient, and this gradient is shifted posteriorly either by excess anterior FGF8 (Grove 2002) or by the *Emx2* mutation (Muzio et al. 2002b). Further, in mice homozygous for the *Emx2* mutation, the endogenous anterior FGF8 source is expanded (M. Yoshida, personal communication), reminiscent of the expansion produced by anterior FGF8 electroporation. Perhaps a reverse conclusion is warranted: Emx2 exerts its patterning effects by regulating the extent of the anterior FGF source. Experiments are needed to determine the effects of Emx2 overexpression on area patterning, and on the FGF8 source, and, further, to test if depleting FGF8 signaling can rescue the cortical phenotype of Emx2-deficient mice. The results of these experiments should clarify the order of these molecules in the patterning pathway or, more satisfying, clarify a patterning interaction between the two.

Muzio et al. 2002b argue that differences in VZ gene expression indicate an early impairment of the protomap in both Emx2 and Pax6 mutants (Muzio et al. 2002b) but concede that the area phenotypes in both mutants might be worsened by impairment of WNT signaling at the cortical hem and that of the Pax6 mutant by a defect at the cortical/subcortical boundary, the anti-hem. In the Emx2 mutant, the anterior FGF signaling source appears to be affected. Thus, when considering the patterning role of any transcription factor, it is critical to distinguish the action of the transcription factor itself from that of the signaling centers it may regulate.

COUP-TFI

Mice deficient in the orphan nuclear receptor COUP-TFI live until birth and lose specificity in the patterning of several gene-expression patterns used to analyze *Emx2* and *Pax6* mutant mice (Zhou et al. 2001). No direct links have been yet established among COUP-TF1, Emx2, or Pax6. Gradients of *Emx2* and *Pax6* are markedly normal in the *COUP-TFI* mutant, and, surprisingly, *COUP-TF1* expression has not been described in either *Emx2* or *Pax6* mutants. Nonetheless, FGF8 manipulations suggest FGF8 controls the cortical graded expression of *COUP-TF1* (Grove 2002, Rubenstein et al. 2002). Moreover, the sharpness of both *COUP-TF1* and *COUP-TFII* expression borders would be expected of genes involved in boundary specification. The loss of area specificity in mice mutant for *COUP-TFI* suggests that this transcription factor works in combination with other proteins to establish area boundaries. Its absence leads to a breakdown of boundaries rather than to a clear shift of areas.

A Continued Search for Molecular Determinants

Cortical domains marked by LAMP, latexin, or the H-2Z1 transgene are specified (i.e., are present in the protomap) and are presumably distinct in their transcriptional state early in neurogenesis. Most likely, so are other cortical areas for which markers have not yet been found. These indications prompt continued searches

for transcription factors whose abutting or overlapping expression domains define emerging area boundaries. However, the question of which genes define protoareas remains open. The relationships among *Emx2*, *Pax6*, *COUP-TF1*, and *FGF* and *WNT* family members need to be clarified, and a major step forward would be to identify fresh transcription factors involved in cortical area patterning. In this regard, *LIM homeodomain* (*LIM-HD*), *Clim*, and *Lim*-only (*Lmo*) genes show highly regionalized expression in the cortical primordium (Bulchand et al. 2003) and seem good candidates for future investigation.

MAKING A CORTICAL EMBRYONIC FIELD: THE CORTICAL SELECTOR GENE

FGF patterning cues do not operate on a naïve tissue but on one already specified to be cerebral cortex. Thus, when a new source of FGF8 is generated in posterior cortical primordium it generates duplicate cortical structures, but not new midbrain structures, as would FGF8 misexpressed in the caudal diencephalon. How does this earliest step of cortical specification occur? The selector gene that specifies cerebral cortex has not yet been definitively identified, but candidates have been suggested. One proposed candidate is Lhx2, encoding a LIM-HD transcription factor (Bulchand et al. 2001, Monuki et al. 2001), expressed throughout cortical neuroepithelium but not in the cortical hem (Porter et al. 1997). In the absence of Lhx2, the cortical primordium expresses gene markers of the cortical hem (Bulchand et al. 2001a, Monuki et al. 2001). A signaling center is an atypical structure, however, needing careful control to avoid defective patterning. Possibly Lhx2 restrains the rest of the cortical primordium from becoming hem rather than positively specifying cortical identity (Bulchand et al. 2001). A missing piece of the puzzle is whether ectopic Lhx2 is able to transform presumptive hem tissue into cortical primordium.

Emx2 and *Pax6* have also been proposed to work together in a cortical selector role. Muzio and colleagues conclude that at least one functional allele of *Emx2* or *Pax6* is necessary and sufficient to activate cortical fate and suppress ventral telencephalic fate (Muzio et al. 2002a). However, the double homozygote mutants do not show a complete failure of cortical specification. Cortical tissue disappears as the double mutants develop and is replaced with tissue showing typical ventral telencephalic gene expression. Loss of function of a selector gene (or genes) would be expected to show a more powerful effect: the absence of the specified tissue from the outset. One explanation is that still another gene is required for cortical selector function in addition to *Emx2* and *Pax6* (Muzio et al. 2002a). It is interesting, given the studies cited above, that the authors suggest *Lhx2*. An alternative explanation is that the cortical/subcortical boundary (anti-hem region) is more severely affected in double mutants than in mice lacking Pax6 alone and that this leads to a progressive breakdown of dorsal telencephalic identity (Muzio et al. 2002a). In summary, therefore, studies of the initial genetic specification of the cortical embryonic field have yielded striking but not yet conclusive results.

CONTROL OF THALAMIC INNERVATION

Implications of the New Model for Patterning Thalamic Innervation of the Cortex

Findings from FGF8 manipulations by electroporation in the embryonic cortex bring us full circle from the early protocortex model. Rather than thalamic afferents patterning the cortex, changing an FGF signal intrinsic to the developing cortex alters the postnatal pattern of thalamic innervation. These observations extend previous studies indicating an intrinsic prepattern in the cortex that regulates thalamic innervation. Grafting embryonic limbic cortex into a nonlimbic region draws in appropriate limbic system–related thalamic input (Barbe & Levitt 1992). Innervation from the lateral geniculate nucleus (LGN) is guided to visual cortex that has been transplanted into parietal cortex (Gaillard & Roger 2000). Transplants grafted at even greater distances from their normal A/P positions induce graft-appropriate thalamic innervation (Frappe et al. 1999). When a frontal cortex graft is placed in occipital cortex, axons from the ventrolateral and ventromedial thalamus grow first to the native frontal cortex and then turn within the cortex itself and travel significant distances posteriorly to innervate the graft (Frappe et al. 1999). The latter axon behavior supports the idea of a spatial and temporal series of thalamocortical guidance cues, suggested above. Here, the axons that will innervate the graft appear to ascend appropriately within the IC and turn toward the graft only when they reach the cortex itself (Frappe et al. 1999).

The activity of thalamic axon growth cones further suggests guidance cues in the cortex. Growth cones pause and test the environment as they reach the ventral intermediate zone of the cortical primordium before selecting a trajectory (Skaliora et al. 2000). Finally, as noted above, axon guidance molecules are among those expressed in regional patterns early in corticogenesis (Ragsdale & Grove 2001). Together, these findings indicate that a protomap sets up a regional distribution of axon guidance cues within the cortex, some of which can act at a distance and relatively late in corticogenesis.

Other Controls on Thalamocortical Patterning

Fundamental to correct thalamocortical innervation is the independent patterning of the thalamus into individual nuclei. This issue—as yet little explored—should be a fruitful one for future studies. Combinatorial expression patterns of *LIM-HD* genes parcel the thalamus early in development (Nakagawa & O'Leary 2001), and, intriguingly, this gene family is implicated in axonal trajectory decisions (Sharma et al. 2000b). Further, in the absence of the bHLH transcription factor, Ngn2, also expressed regionally in thalamus, anterior thalamocortical axons alter their receptivity to cues in the ventral telencephalon and are misrouted (Seibt et al. 2002).

Because the thalamus and cortex are far apart, guidance cues are needed at a variety of intermediate sites between the two, including the diencephalic/telecephalic

border and the IC, the orderly massing of axons traveling to and from the cerebral cortex (Hevner et al. 2002, Kawano et al. 1999, Lopez-Bendito et al. 2002, Molnar et al. 1998, Pratt et al. 2002, Rubenstein et al. 2002, Seibt et al. 2002). Analyses of several mutant mice disclose the locations of these sites. For example, the GAP-43 heterozygote mutant, which appears to have abnormal growth cone function, shows thalamocortical axon guidance defects in the IC as well as in the lower layers of cortex (J. McCasland, personal communication). In *Ebf1*- and *Dlx1/2*- deficient mice, axons from the LGN stall in the region of the amygdala and do not reach visual cortex. The result is a shift in the topography of thalamocortical projections in the IC and a shift of thalamocortical innervation when axons exit the IC. A prediction of this review would be that axons in the *Ebf1* and *Dlx1/2* mutant mice would shift back to their true cortical targets postnatally, except that the mutants die at birth. In normal development, guidance mechanisms, including those in the cortical subplate, extensively reviewed elsewhere (Allendoerfer & Shatz 1994), will match and coordinate thalamocortical innervation together.

At present, we do not know the full, detailed topography of connections between the thalamus and cortex in the adult of any species. We do know however that no simple patterning mechanism is likely to explain thalamocortical topography. First, the thalamus and cortex are distant from one another. Second, although there is a rough mapping of the thalamus onto the cortex along the A/P and M/L [or dorsal/ventral (D/V)] axes of each structure, there are many exceptions (Nauta & Feirtag 1986). For example, the medial dorsal nucleus of the thalamus projects both to lateral cortex and to medial prefrontal cortex. Anterior thalamic nuclei innervate limbic system cortical areas as far anterior as limbic prefrontal cortex and as far posterior as entorhinal cortex; further, some midline nonspecific thalamic nuclei project widely over the cortical sheet (Nauta & Feirtag 1986). Thus, the anatomy of thalamocortical innervation itself supports the hypothesis of a complex set of guidance cues and intermediate targets for thalamic axons.

ROLES OF THALAMIC INNERVATION AND ACTIVITY IN AREA IDENTITY

Function of Thalamic Innervation in Sharpening Cortical Area Boundaries

Protoareas could be merely rough regions with variable transitions between them. Refining mechanisms, such as thalamic innervation, could then establish sharp boundaries (Pallas 2001). Some data suggest, however, that the mouse protomap contains precise positional information that leads to clear-cut boundaries. The transgene H2Z1, a marker of S1 and S2, or gene expression markers of hippocampal fields upregulate in well-delineated, spatially accurate patterns in explants isolated from all extrinsic input (Gitton et al. 1999b, Tole & Grove 2001). Moreover, there is little or no direct evidence as yet that thalamic innervation sharpens area

boundaries in normal development. When thalamic innervation is manipulated, different area-specific features do not necessarily shift in tandem, which would be the case if thalamic innervation were normally required to sharpen boundaries. If somatosensory cortex is partially ablated, for example, VB axons grow into neighboring cortex, but expression of the H2Z1 transgene does not expand accordingly (Gitton et al. 1999a). This contrasts with manipulations of FGF signaling in the cortical primordium, which shift S1 area features in tandem. The effects of altered thalamic innervation may be more consistent with cortical plasticity, in which some features of an area are altered without complete transformation into a different area (see below).

Maintaining Features of Area Identity by Thalamocortical Innervation and Activity

Thalamic innervation maintains a range of area-specific features from early in development. For example, although H2Z1 expression is specified before thalamic axons reach the cortex, thalamic innervation is transiently needed around birth to maintain it (Gitton et al. 1999a). Later in development, thalamocortical afferents and, more specifically, stimulus-driven activity in these afferents maintain the organization of area-specific functional modules that are a key part of area identity. The best-studied examples are ocular dominance or orientation columns in visual cortex and somatosensory whisker barrels. In each species studied, a critical period exists in which changed sensory activity alters functional modular organization. Extensive discussion of the critical period is outside the scope of this review. However, the phenomena and mechanisms of the critical period have become entangled with our understanding of how cortical modules initially develop (Crowley & Katz 1999). Because of the vast literature on this topic, we confine this review to a few key, recent papers.

Roles of Thalamocortical Innervation and Activity in Establishing Modular Organization

Katz and colleagues argue for a radical separation of the development of cortical modules and critical period plasticity, at least with respect to ocular dominance columns (Crowley & Katz 2002, Katz & Crowley 2002). Until recently, ocular dominance column formation has been closely linked with the critical period in which closure of one eye, in cats, ferrets, or primates, decreases the size of columns activated by the closed eye and enlarges columns devoted to the open eye. Early tracing experiments in cat indicated that before the onset of the critical period, both eyes are represented in a homogeneous band of axonal labeling in layer 4 of primary visual cortex (V1) (LeVay et al. 1978). It seemed to follow that visual activity in the critical period is required to refine profuse axonal arbors and allow ocular dominance columns to emerge. However, as noted in the initial reports, in the small developing brain, tracer from the labeled eye could spill over in the LGN to label LGN layers innervating the other eye. Consistent with these reports, further

findings in monkeys, cats, and ferrets, including studies involving precise laminar tracer injections into the ferret LGN, show that ocular dominance columns develop before the critical period and before visual activity (Crair et al. 2001, Crowley & Katz 1999, Rakic 1976).

Spontaneous activity generated independently in each eye is not yet discounted as a mechanism of ocular dominance column segregation. Katz & Crowley suggest, however, that column formation could depend on molecular interactions among axons or, in keeping with a protomap model, axon guidance molecules expressed by axons and their cortical targets (Crowley & Katz 2002, Katz & Crowley 2002). Given that activity regulates the polarity of axon guidance cues (Ming et al. 2001), this does not discount activity as a modulator of thalamocortical axon guidance (Crowley & Katz 2002). The important conclusion is that there is a distinction between two forms of activity—spontaneous and stimulus-driven—and between development and later plasticity. Visual activity is needed in the critical period for ocular dominance column plasticity but not for the initial segregation of the columns (Crair et al. 2001, Crowley & Katz 1999, Rakic 1976).

In contrast, Sur and his colleagues support an instructive role for visual activity in generating orientation columns in V1. When visual input is rerouted into auditory thalamus, and thence to auditory cortex (A1), at a very early stage of cortical development in the ferret, robust visual responses can be recorded from adult A1. Both direction selectivity and orientation tuning in single-unit recording is astonishingly similar in V1 and rewired A1 (Sharma et al. 2000a). Rewired cortex responds anatomically by altering the pattern of long-range horizontal connections, thereby linking artificially generated iso-orientation columns. Reflecting the less-than-perfect appearance of imaged orientation maps in rewired A1, these connections are less patchy and periodic than are horizontal connections in V1 but more so than in normal A1 (Pallas 2001, Sharma et al. 2000a). Thus, A1 has not been transformed into V1, but the sensory modality carried by thalamic innervation has resulted in intrinsic wiring changes in the cortex that support new responsiveness to visual stimuli.

Sur and his colleagues carefully interpret the data presented above to suggest that, within limits, input modality plays an instructive role in initiating circuitry that underlies the orientation map. In rewired A1, the orientation map is not identical to that in V1. If, as suggested by data reviewed above, cortical areas are specified from a very early age, then visual cortex is specified long before it receives visual input. Once the appropriate activity-based signal arrives, V1 is optimally prepared to create precise visual cortex circuitry. This genetic prespecification of areas would represent the limits of the instructive role of activity.

The ability of a particular mechanism to drive development of a cortical feature means that the mechanism can perform this function but not that it does so in normal development. A possibility remains, therefore, that stimulus-driven activity can generate remarkable plasticity in a sensory cortical area but that it is no more needed for the normal initial segregation of orientation columns in V1 than for ocular dominance column formation.

For rodent somatosensory barrels, axons from the thalamic barreloids have been described as conferring a blueprint of the sensory periphery onto the cortex (Erzurumlu & Jhaveri 1990), entering deep cortical layers in an orderly array from the start (Agmon et al. 1995). In contrast, a more recent study following development of individual VB axon arbors found that VB afferents have an initially broad distribution in layer 4 and coalesce into barrel domains over two days (Rebsam et al. 2002). In both views, at least some remodeling of VB axonal arbors is proposed.

Remodeling could be achieved, once again, by activity-mediated competition among axons, axon-axon recognition, or cues intrinsic to the cortex. Barrel patterns of VB thalamic afferents appear in a mouse that lacks NMDAR1 receptor function in cerebral cortical pyramidal cells (Iwasato et al. 2000). Thus, we may discount competition between VB axons based on NMDA-mediated Hebbian synaptic strengthening and retention, at least with respect to interactions among VB afferents and pyramidal cells. These observations extend previous findings that activity is not needed for segregation of VB afferents into whisker-related clusters (O'Leary et al. 1994). The onus therefore appears to be on proponents of activity-driven remodeling to provide strong supporting evidence. The issue is far from closed: In each of the experiments cited above, inactivation may be incomplete. For example, the mice studied by Iwasato et al. (2000) retain NMDA receptor function in interneurons, as well as other forms of glutamatergic activation in all neuron types. Further, glutamate is not the only neurotransmitter to be considered. Gaspar and colleagues suggest that normal 5-HT1B receptor activity is needed for VB barrel axonal patterning, either by enhancing correlated activity patterns of VB afferents or by permitting normal responses of thalamic axons to molecular cues intrinsic to the cortex (Rebsam et al. 2002).

Evidence for cortical cues is provided by the effects on cortical spacing of barrels in mice lacking ephrin-A5, an axon guidance molecule expressed in the early somatosensory cortex (Prakash et al. 2000). In addition, intracortical FGF manipulations change the shape of barrel subfields and individual barrels along the A/P axis, with respect to thalamic innervation as well as intrinsic cytological and cytochemical features (Fukuchi-Shimogori & Grove 2001). These observations suggest that the final organization of the barrels and the subfields they compose is regulated by interactions—possibly activity based, but also molecular—between the incoming axons and cues intrinsic to the cortex.

Plasticity following a relatively simple early manipulation of sensory input can be widespread. In a recent study in opossum, animals are bilaterally enucleated before the retino-geniculo-cortical pathway is established (Kahn & Krubitzer 2002). In adults, a cytoarchectonic area 17 is identifiable but smaller than normal. A cytoarchitectonically novel area appears anterior to 17, similar to area X described after enuculeation in monkeys (Rakic et al. 1991). Multiunit recordings indicate auditory and somatosensory responses both in area 17 (V1) and area X, as well as in other areas that would normally respond to visual input. Other areas are also affected, with somatosensory responses appearing in A1 and some sites in S1 responding to both somatosensory and auditory stimulation.

These findings, together with those of Sur and his colleagues, indicate strongly the versatility of developing sensory cortex. Sensory areas of cortex can be coopted by different sensory modalities. Moreover, alteration of the modality of input can change cytoarchitectural features of the cortex. Thus, rewiring cortex is sufficient to induce new and different features of area identity. The cortex is plastic, with reassuring implications for human disease and disability (Pallas 2001), but plasticity in development is limited. Cells take on properties more suited to another area, or unlike any area, but areas are not transformed, suggesting that these manipulations are imposed on a prespecified cortical map.

COMPARATIVE ANATOMY OF SPECIES-SPECIFIC MAPS

Comparative studies suggest that features of overall design of the neocortical map are conserved in divergent mammalian species (Krubitzer 1995). For example, primary visual cortex lies at the posterior pole in both mouse and human, primary somatosensory cortex toward the center, and primary motor cortex anterior to that. This similarity of broad outline is reminiscent of the body plan of vertebrates, which retains common features and embryological patterning mechanisms, despite species-specific modifications. These observations give confidence that the mechanisms that establish the area map in one species will generalize to other species, including our own. Consistent with this, a cortical hem has been identified by *WNT* and *BMP* gene expression in humans (Fu et al. 2000).

In the course of evolution, new areas may be added to a species map. For example, in primates, multiple visual areas have been added, distinguished by separate retinotopic maps that are mirror-reversed at area boundaries (Nauta & Feirtag 1986). Allman and Kaas suggest that new areas are created when genetic mutations cause an existing cortical field to duplicate (Krubitzer 1995). Thus, it is intriguing that a duplicate somatosensory barrel field is generated in mouse cortex by manipulating a single growth factor, FGF8, and that the duplicate appears to be a mirror image of the native subfield. In principle, then, subtle alterations in cortical positional signaling and its interpretation could lead to the evolution of new cortical areas.

Despite broad similarities, species-specific modifications of the cortical map can be spectacular. For example, about two thirds of the cerebral cortex of the platypus is covered by a sensory representation of the bill (Krubitzer 1995). In the star-nosed mole, a large cortical area processes inputs from the nose, whose star shape is elegantly represented on the cortical sheet (Krubitzer 1995). How could a set of positional coordinates create so much variety in shape, size, and modular composition? This attribute of a positional information model, however, is precisely what makes it attractive (Wolpert 1996). The same coordinate system and signals can be used repeatedly to generate different final patterns because positional information is interpreted differently even in different parts of the same tissue (Wolpert 1996). For example, Dpp is a morphogen whose source is the boundary between anterior and posterior compartments of the fly wing. The Dpp gradient is similar in the two

compartments, but interpretation of the gradient—the pattern of wing veins—is different. The new model therefore proposes that signaling molecules set up positional gradients across the cortical sheet that have different, species-specific interpretations (different protomaps). A challenge will be to determine how these interpretations are mediated at the molecular level, first in mouse and then in other mammals.

CONCLUSIONS

The main conclusion of this review is that signaling molecules establish positional information and regulate regional growth in the cortical primordium. Interpretation of these signals by the cortical primordium gives rise to a species-specific protomap. A strong version of this model would assert that the protomap is required to direct or prepare the ground for development of most of the accepted features of an area map, primarily the cytoarchitectonic boundaries of the map, but also the organization of area-specific functional circuitry and cortical modules. Thus, the "new" embryonic field model of cortical area map generation is simply a version of the classic protomap model of Rakic (1988), adding in how the protomap may be initially established (Figure 4).

Many questions and issues remain. These include whether FGF8 is a true morphogen, what other signaling molecules are involved in establishing position, and

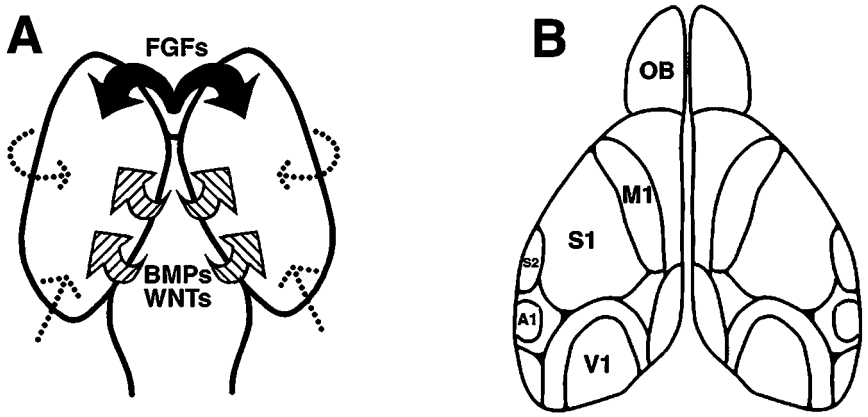

Figure 4 The embryonic field model of cerebral cortical area patterning. (*A*, *B*) Mouse cerebral cortex in dorsal view, anterior to the *top*. (*A*) The early cortical primordium is an embryonic field (Wolpert 1996), homogeneous with respect to its later area patterning. Signaling molecules, including FGFs derived from the anterior pole, and possibly WNT and BMP proteins from the cortical hem, confer positional information on the cortical primordium and regulate regional growth. These activities, mediated by regional transcriptional regulation, generate a protomap. (*B*) The protomap coordinates development of the mature area map.

what the precise roles may be of Emx2, Pax6, COUP-TF genes, and other cortical patterning genes yet to be identified. Less studied than thalamocortical innervation, and excluded from this review, is the issue of how corticocortical connections are set up; yet these connections form the bulk of cortical afferents. More study is also needed of stimulus-driven or spontaneous activity in forming the cortical map and individual area features. This potential patterning mechanism, which held theoretical sway for so many years, now needs further examination in the face of increasing understanding of cortical patterning at the molecular level.

The idea that general embryonic patterning mechanisms initiate the cortical area map represents an exciting advance. In particular, data on the role of FGF signaling in anterior/posterior patterning of the map and on the roles of certain transcription factors have supported this view. We now have a new starting point for investigating how the area map is generated and modified in a single individual and how maps may change over evolution.

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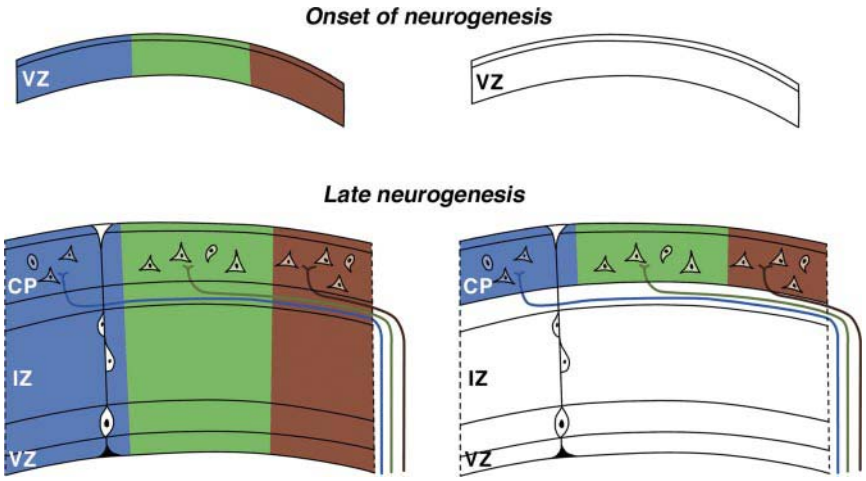

Figure 1 Classic models of cortical patterning. Cross sections through the cortical primordium at an early stage of corticogenesis (*top*), and a later stage when thalamocortical axons have begun to enter the cortical plate (CP) (*bottom*). (*Left*) Simplified protomap model (Rakic 1988). The cortical primordium is patterned as cells are dividing in the ventricular zone (VZ). Intrinsic area differences are specified by molecular determinants—boundaries are represented as sharp for simplicity. As pyramidal cells migrate radially out of the VZ, they transfer the protomap to the CP. Axon guidance cues set up by the protomap (not in the original model, but see text) help to guide thalamocortical axons to the correct area (indicated by color coding of axons and protoareas). (*Right*) Simplified protocortex model (O'Leary 1989). The cortical primordium is essentially homogeneous as it is generated and is patterned into areas later by cues from axons growing in from the thalamus. Thalamic axons thus impart their positional information to the CP (indicated by color coding of axons and areas).

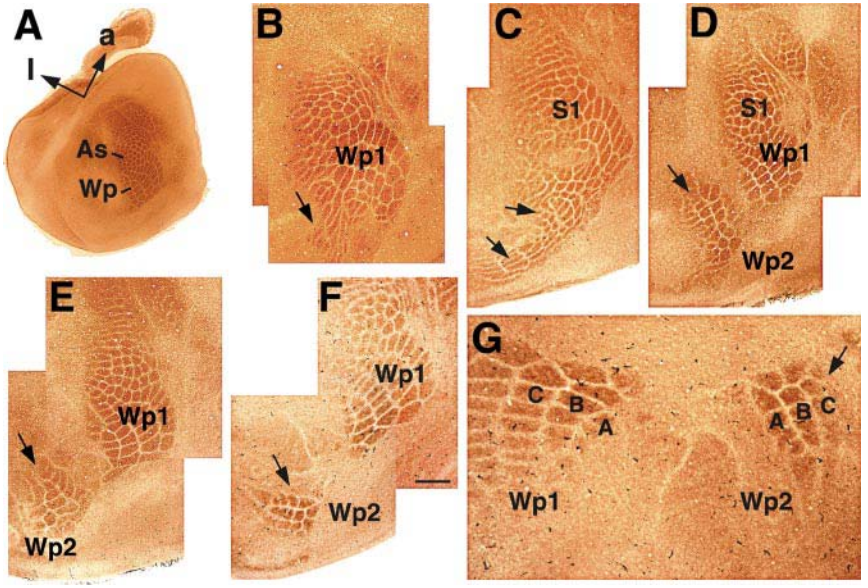

Figure 2 A new posterior source of Fgf8 generates ectopic somatosensory barrels. (*A–G*) Tangential sections through the cortices of P6 embryos electroporated at E11.5 with *AP* (*A*), a control construct, or *Fgf8* (*B–G*) to produce a second, posterior source of Fgf8 signal. Sections are stained with cytochrome oxidase histochemistry. (*A*) Central position of the barrel fields in a control mouse. Abbreviations: As, anterior snout subfield; Wp, main whisker pad subfield; a is anterior; l is lateral. (*B–G*) Cortices with ectopic barrels (arrows). In some brains (*B*, *C*) ectopic barrels merge with the posterior boundary of native S1. In others (*D–G*; *G* is a higher magnification of *F*), a secondary Wp subfield (Wp2) forms in mirror image (*G*) to the native field (Wp1). Scale bar in (*F*) is 1.5 mm for A; 0.6 mm for (*B–F*); and 0.25 mm for (*G*). Reprinted and adapted with permission from T. Fukuchi-Shimogori, E.A. Grove, Patterning of the neocortex by the secreted signaling molecule FGF8. *Science* 294:1071–74. Copyright 2001 American Association for the Advancement of Science.

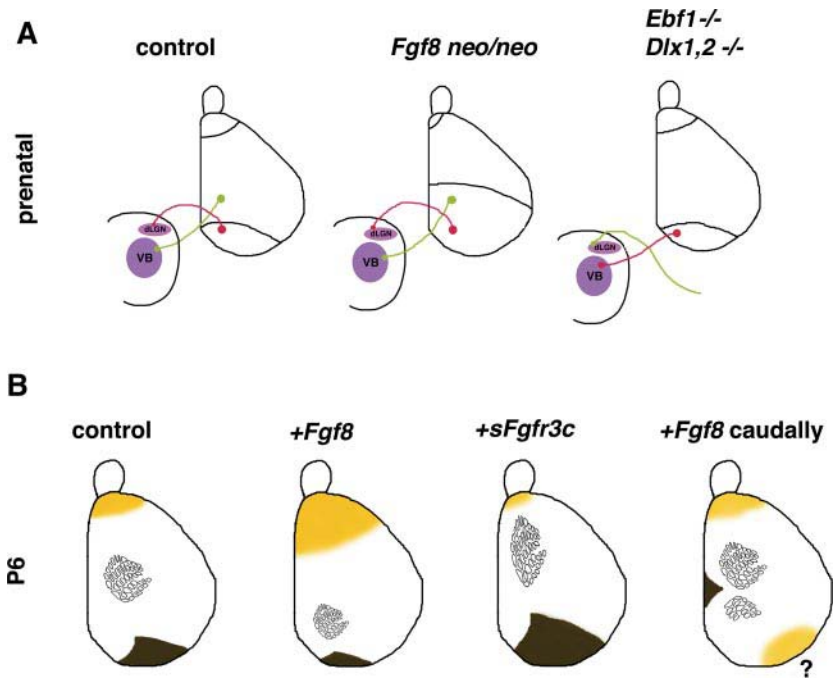

Figure 3 Evidence for sequential guidance cues for thalamocortical axons. (*A*) Thalamocortical projections in control mice, Fgf8 hypomorphs, and *Ebf1* or *Dlx1/2* mutant mice, analyzed before birth. Cortex is viewed from the dorsal side, anterior is up; thalamus is viewed in coronal section, medial to left. In a control mouse, the LGN projects to area 17 and VB innervates S1. In Fgf8 hypomorphs, cortical gene expression boundaries are shifted anteriorly but thalamocortical axons do not shift coordinately. In *Ebf1* or *Dlx1/2* mutants, LGN axons do not reach the IC, shifting the position within the IC of other thalamic axons, which then innervate inappropriate areas. Cortical gene expression patterns are unchanged in the latter mutants. (*B*) After Fgf8 manipulations (see text), the somatosensory barrel fields examined at P6 are shifted or duplicated. By contrast, with animals analyzed at earlier ages, gene expression patterns and VB innervation are shifted in tandem (see text). Together these observations suggest that subcortical axon guidance cues guide thalamic axons at prenatal ages; subsequently, guidance cues intrinsic to the cortex are active, matching the correct thalamic axons to the correct area.

In each of the Fgf8-manipulated animals, the position, size, and shape of area 17 (green) is also altered, consistent with a role for Fgf8 in A/P positional patterning. When somatosensory subfields are duplicated, the duplicates appear to share a single, reduced area 17 (T. Fukuchi-Shimogori and E.A. Grove, unpublished observations). Yellow marks the presumed position and extent of Fgf8 sources.