Chapter 3

The evolution of human neocortex: Is the human brain fundamentally different than that of other mammals?

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Abstract

The neocortex is composed of areas that are functionally, anatomically, and histochemically distinct. In comparison to most other mammals, humans have an expanded neocortex, with a pronounced increase in the number of cortical areas. This expansion underlies many complex behaviors associated with human capabilities, including perception, cognition, language, and volitional motor responses. We consider data from comparative studies as well as from developmental studies to gain insight into the mechanisms involved in arealization, and discuss how these mechanisms may have been modified in different lineages over time to produce the remarkable degree of organizational variability observed in the neocortex of mammals. Because any phenotype is a result of the complex interactions between genotypic influences and environmental factors, we also consider environmental, or epigenetic, contributions to the organization of the neocortex.

3.1 Introduction

How did humans evolve their remarkable cognitive abilities? What makes the human brain different from that of other animals, and the behavior it generates unique? Although these questions are fundamental to psychologists and neuroscientists alike, they are difficult to answer, for several reasons. First, evolution of the mammalian brain, and neocortical evolution in particular, is difficult to study directly. Secondly, even if we could study evolution directly, considering evolutionary contributions to phenotypic variability in isolation is too restrictive. Finally, these questions are

highly subjective and therefore their answers would provide limited information regarding cortical function and evolution. However, we can circumvent some of these problems by studying evolution indirectly and making inferences about the process. In addition, we can examine the non-evolutionary mechanisms that generate phenotypic variability. Finally, we can re-formulate our questions in a more objective fashion.

Like other mammals, our sensory receptor arrays are capable of sampling only a limited portion of the physical environment. Our nervous system enhances stimulus features, generates probabilities, and constructs the reality of our world in a highly biased manner that considers only those parameters that we can actually detect. Unfortunately, the concepts we generate regarding the organization and function of the nervous system, particularly the neocortex, reflect these same biases. So, how can we get out of our own skin?

For addressing questions of human brain evolution, we can talk about complexity rather than intelligence, cognition, or any other covert behavior generated by the human brain whose definition requires subjective human experience. We appreciate that some mammals, such as human and non-human primates and cetaceans, have a relatively large neocortex that is complexly organized. For our purposes, complexity can simply be defined as a large number of functionally distinct parts that are intricately interconnected. We also appreciate that mammals that have a relatively large, complexly organized neocortex appear to generate more complex behaviors. As with the nervous system, complex behavior refers to behaviors that have many parts, and includes motor behaviors such as reaching, grasping, locomotion, articulation of sounds, or behaviors such as stimulus detection, perception, learning and memory, components of which can be quantified. Although it is difficult to detect subtle differences in complex brains and behavior using this rather gross scheme of classification, one can feel fairly confident stating that mammals that have neocortices with many functionally heterogeneous parts that are specifically interconnected, generally have more complex behaviors. Thus, we can refine our questions regarding human brain evolution, and achieve at least some modicum of objectivity if we examine how the brain evolves more functional parts (cortical fields), how connections between these parts become specified, and ultimately how the addition of these parts are specifically related to the generation of complex behavior.

However, considering only the *evolution* of cortical fields is problematic because it is far too restrictive. Evolution requires the transmission of genes from one generation to the next. When we consider evolution in isolation, we only consider those characteristics of the brain that are heritable. Yet, studies of cortical plasticity in adult and developing mammals indicate that the nervous system is capable of remarkable change within the life of the individual which takes the form of functional map reorganization in adults (Recanzone et al. 1992, 1993; Recanzone, 2000), and in the developing nervous

system includes large sensory domain shifts, changes in functional map organization and changes in connectivity (Kahn and Krubitzer 2001, 2002).

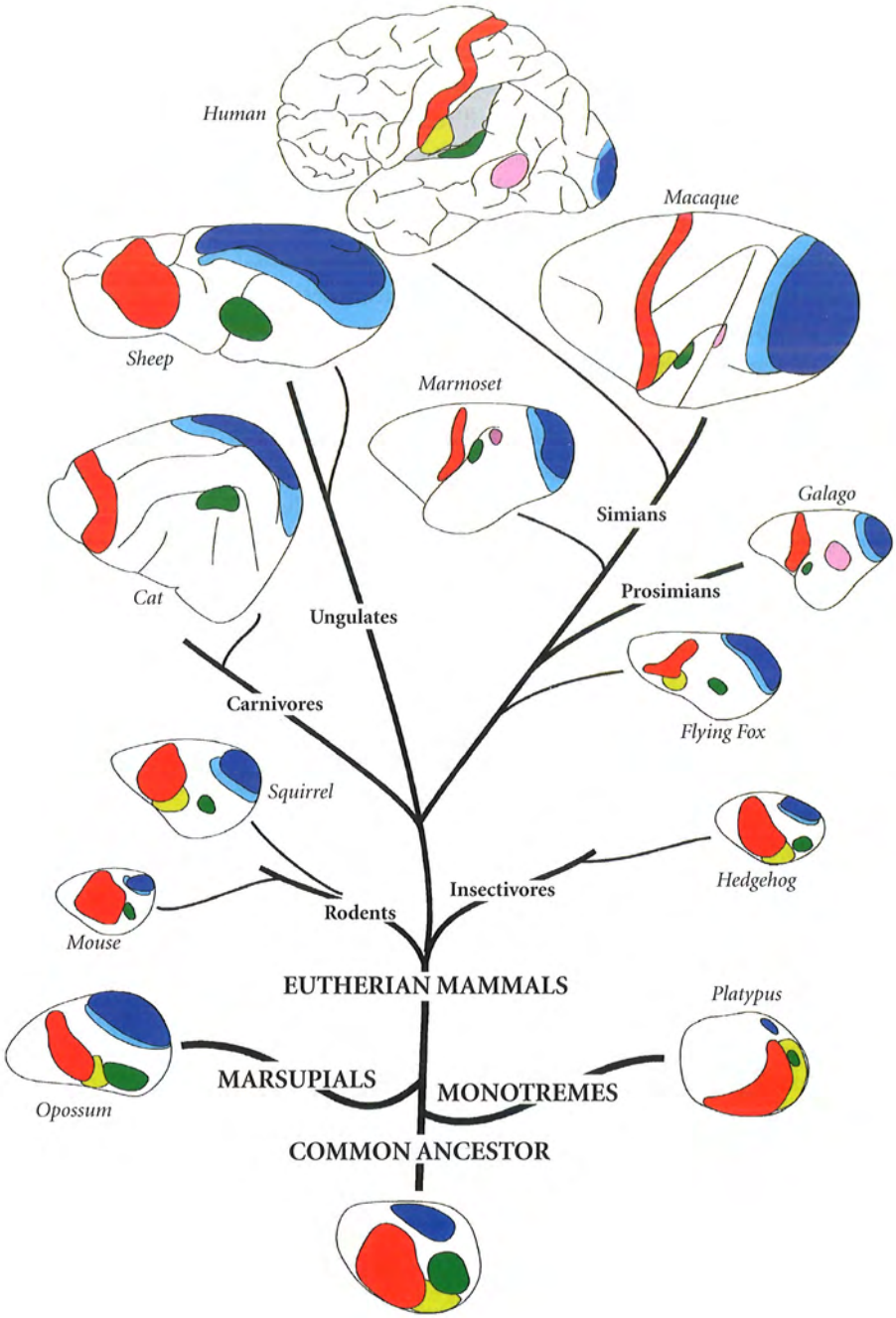
The final problem associated with the questions posed at the beginning of this chapter is that evolution is difficult to study directly because the time course of change is relatively slow by individual life-span standards, and subtle changes often occur over thousands of generations. However, there are two ways to circumvent this problem: (1) use of a comparative approach, and (2) examining the developmental mechanisms that give rise to particular characteristics of complex brains. Using the comparative approach we can study the products of the evolutionary process and make inferences about the process itself. This method allows us to deduce general characteristics of nervous systems and the types of brain changes that are actually possible. A comparative approach in combination with a developmental approach allows us to examine the constraints that direct the course of evolution.

For instance, electrophysiological recording studies, architectonic analysis, and studies of cortical and subcortical connections indicate that all mammals have a constellation of specifically interconnected cortical fields (Krubitzer 1995, Krubitzer and Huffman 2000). Some of these fields include the primary and secondary sensory areas such as S1, S2, V1, V2, A1 and R (Fig. 3.1). These same types of studies indicate that the types of system changes that are possible are limited and include changes in:

- the size of the cortical sheet;
- the number of cortical fields;
- the amount of cortex devoted to a particular sensory system (sensory domains);
- the amount of a cortical field devoted to a particular portion of the sensory epithelium;
- connectivity;
- modularity of existing fields.

Within these large categories, further modifications in cell size, dendritic and axonal arborization, and laminar organization have been made to the neocortex over time. Although we propose that the types of modifications with respect to all of the possible ways in which brains could change are limited, there is still a large degree of freedom for phenotypic change within these constraints.

The limited types of modifications that are observed in extant brains, particularly those that have evolved independently, indicate that there are constrained developmental mechanisms that generate nervous systems. Thus, the second way to study the evolution of the neocortex, in particular the mechanisms that give rise to current organization and constraints imposed on evolving nervous systems, is to study the development of the neocortex. This chapter will focus on some of the modifications associated with complex brains and discuss the evolutionary (inherent, genetic contributions) and activity-dependent mechanisms that give rise to these features.


Human
Macaque
Sheep
Marmoset
Simians
Galago
Cat
Prosimians
Ungulates
Carnivores
Flying Fox
Squirrel
Hedgehog
Insectivores
Rodents
Mouse
EUTHERIAN MAMMALS
Platypus
MARSUPIALS
MONOTREMES
Opossum
COMMON ANCESTOR

3.2 Increases in the size of the cortical sheet

Probably the most salient feature associated with complexity in mammalian brains, particularly the human brain, is the disproportionate increase in the size of the cortical sheet (Fig. 3.2). This feature, described several decades ago by Stephan *et al.* (1981), and termed encephalization, has recently been indexed by Finlay and Darlington (1995) and Clark *et al.* (2001). The selective pressures that led to an enlarged cortical sheet are not clearly understood, but it has been proposed that frugivory (fruit eating), longevity, and sociality may be the driving forces behind the evolution of an enlarged brain in primates (for a review, see Allman 1999). Although these conjectures are viable, they are only correlative and not necessarily causal. Even if they were causal, it is difficult to link the expansion of one portion of cortex compared to the others, with any of these global features of social organization, foraging, or longevity. Further, it is not clear exactly what a larger cortical surface area with more functional areas confers to an individual. Therefore, it is not surprising that the selective pressures that led to an expanded cerebral cortex are elusive. However, as noted earlier, it is clear that larger brains with many cortical areas that are uniquely interconnected generate more complex behavior.

Thus, an increase in the size of the neocortex is a necessary (although, perhaps not sufficient) step in the evolution of complex mammalian nervous systems. Therefore, to appreciate human brain evolution and the factors that contribute to its current phenotype, it is necessary to understand how cortical sheet size is regulated in development, the types of genetically mediated developmental mechanisms that could give rise to an expanded cortical sheet, and how and why neocortex expands at the expense of other telencephalic structures.

Two mechanisms have been proposed to explain how the cortical sheet may increase in size. One suggestion is that more cells are generated in development. Kornack and Rakic (1998) propose that a simple change in the timing of cell division cycles of progenitor cells in the ventricular zone during neurogenesis could result in an exponential

Fig. 3.1 An evolutionary tree depicting the phylogenetic relationship of major orders of mammals and the cortical organization of some of the sensory fields that have been described in particular species. Electrophysiological, anatomical, histochemical, and molecular analyses have revealed that certain cortical regions, such as S1, S2, A1, V1, and V2, are common to all mammals and most likely are homologous areas that arose from a common ancestor. On the other hand, some regions, such as MT (pink) have been observed in only a few orders, such as primates, and likely evolved independently in these lineages. If a number of species are compared, one can be fairly confident when assigning features of cortical organization to the unknown state, such as the common ancestor or human, even in the absence of direct data. S1 = primary somatosensory area (red), S2 = secondary somatosensory area (yellow), A1 = auditory (green), V1 = primary visual area (dark blue), V2 = secondary visual area (light blue); rostral is left, medial is up.

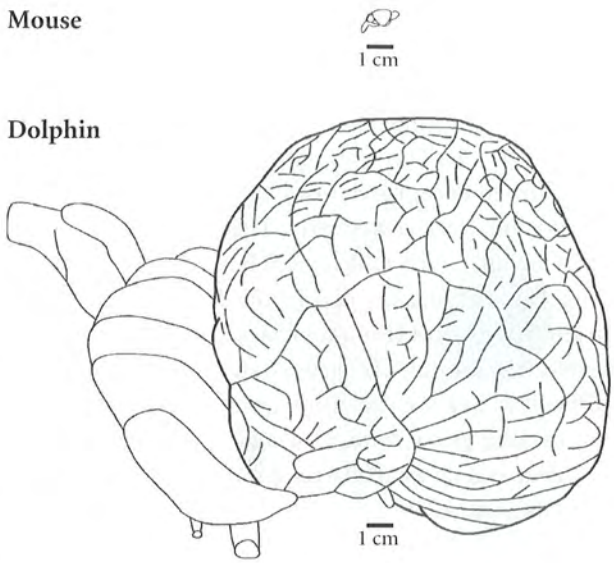


Fig. 3.2 A comparison of the mouse and dolphin brain drawn to scale illustrates the dramatic differences in the size of the neocortex (gray). The difference in size is even larger in magnitude than is illustrated here, since the dolphin brain contains a number of fissures in which the neocortex is buried. Rostral is right, medial is up.

increase in the size of the cortical sheet (Fig. 3.3). Kornack's comparative analysis on the kinetics of cell division in monkeys and rodents (Kornack and Rakic 1998; Kornack 2000) reveals that in macaque monkeys, the cell cycle duration is five times as long as in the mouse, and that there are more total cycles of cell division than in the mouse. This prolonged and accelerated cell division during cortical neurogenesis could account for the pronounced increase in the cortical sheet in some lineages, such as anthropoid primates.

Another proposition of how the cortical sheet increases in size is that there is a decrease in naturally occurring cell death (apoptosis) during corticogenesis. Several genes and their products (proteins) have been demonstrated to decrease the rate of apoptosis. For example, in mutant mice in which a gene associated with cell death (caspase 9; *Casp9*) is deleted, a larger proliferative zone is observed in the forebrain, along with an increase in the size of the neocortex (Kuida *et al.* 1998). Additionally, there is evidence that the apoptotic process may be further regulated by certain genes in the Bcl-2 family, which function to inhibit or facilitate apoptosis by acting upon caspases (Boise *et al.* 1993; Motoyama *et al.* 1995; Roth *et al.* 2000). Like the former mechanism proposed by Kornack, a small change in the timing of the expression of a gene or genes involved in apoptosis could change the size of the cortical sheet dramatically.

While the genes responsible for the kinetics of cell division of progenitor cells and rates of apoptosis during development are not well known, there is evidence indicating that the protein β-catenin, and the genes that regulate its production, may be involved in the determination of cortical sheet size in different lineages. β-Catenin, which activates signaling molecules involved in cell growth and cell fate (Peifer and Polakis 2000), is expressed in neuroepithelial precursor cells (which will become the neocortex) in the

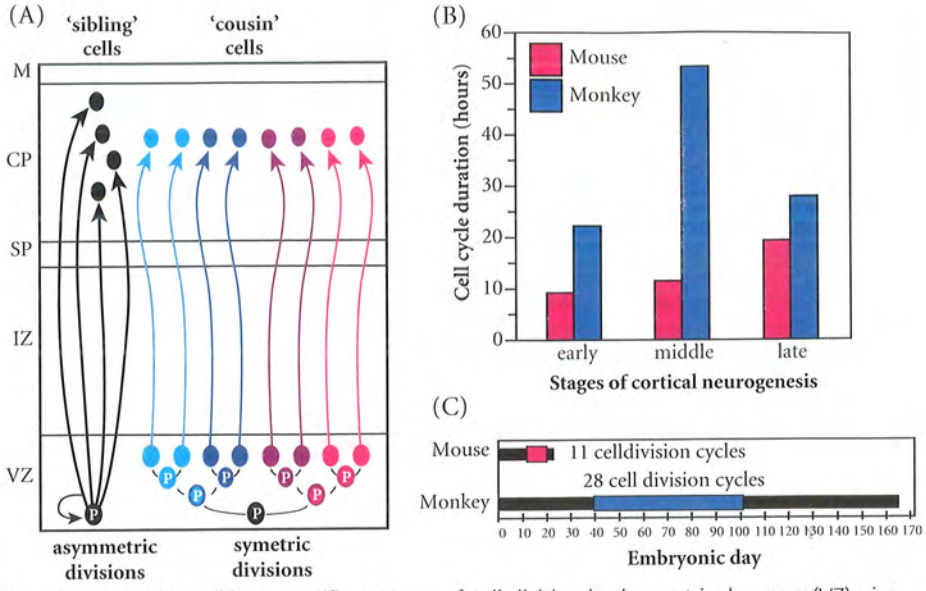


Fig. 3.3 Illustrations of how specific patterns of cell division in the ventricular zone (VZ) give rise to the patterns of clonally related neurons in the neocortex. In (A), asymmetric divisions from a single progenitor cell (P) generate 'sibling' cells that migrate sequentially to different layers of the cortical plate (CP). This type of cell division determines cortical thickness. Symmetric divisions from a single progenitor cell generate several progenitor cells that, in turn, simultaneously generate 'cousin' cells which then migrate, in parallel, to the same cortical layer. This type of division determines cortical sheet size. Duration (B) and number (C) of cell cycle divisions vary dramatically in the mouse (pink) and the rhesus monkey (blue). (B) Cell cycle duration is significantly longer in the monkey at all stages of neurogenesis. (C) Black bars represent the length of gestation in the mouse (19 days) and the monkey (165 days). In the mouse (pink rectangle) neurogenesis lasts 6 days, from embryonic day (E) 11 to E17. In the monkey, neurogenesis lasts 60 days, from E40 to E100. The expanded duration and the increased number of cell cycles could be one mechanism involved in expansion of the primate neocortex. IZ, intermediate zone (white matter); M, marginal zone (layer I); SP, subplate zone. (Modified from Kornack and Rakic 1998; Kornack 2000.)

ventricular zone during neurogenesis (Chenn and Walsh 2002). These investigators demonstrate that transgenic mice that overexpress a truncated form of β-catenin have an exaggerated horizontal growth of the cortex (without a change in cortical thickness). Indeed, the increased size of the cortical sheet was so massive that the normally lissencephalic cortex of the mouse became gyrencephalic. This enlargement of the cortical sheet was the result of a twofold increase in the proportion of progenitor cells that re-entered the cell cycle and continued mitotic division. Thus, the disproportionate increase in cortical sheet size in some mammals could be regulated in part by β-catenin or other proteins, whose temporal and spatial patterns of expression vary slightly in different lineages.

The inordinate increase in the size of the cortical sheet in some cetaceans, such as odontoceti (dolphins and toothed whales) and their distant cousins, proboscidea (elephants) rivals that of humans. Because no other extant mammal exhibits such a disproportionate increase, and because the primate and cetacean lineages are very distantly related, the most parsimonious interpretation is that this cortical expansion has been independently achieved in these separate lineages. By comparing the temporal and spatial expression patterns of genes, or gene products such as Bcl-2 and β-catenin, in the developing ventricular zone of primates, dolphins, whales, and elephants, one could determine which genes specifically regulate the process of cortical sheet expansion. In doing so, we could determine whether this process occurs via homologous genetic mechanisms, or if there is more than one way in which the neocortex can change in size.

3.3 Genetic regulation of cortical domains and cortical fields

Accumulating developmental and comparative data indicate that both genes and neuronal activity regulate the organization and connectivity of the developing neocortex. However, the extent to which the emergence of cortical domains and individual cortical fields and their connectivity is genetically specified is not clear. There is ample evidence indicating that genes play a significant role in specifying the gross geometric anatomical relationships of the cortex.

Recent work in the field of molecular neurobiology has demonstrated that patterning or signaling centers exist in particular portions of the developing brain. These signaling centers are specific portions of neural tissue which express particular genes or gene products, and serve as morphogens. In turn, these signaling centers induce the fate or specification of nearby neural tissue, and contribute to the cellular architecture, type of neurotransmitter utilized, connectivity, and ultimate function of developing neurons. The role of signaling centers in allocating large portions of the central nervous system has been recognized for some time. For instance, major subdivisions of the brain, such as the telencephalon, diencephalon, midbrain, hindbrain, and spinal chord, are specified by either graded or abrupt patterns of gene expression during development. The homeobox genes *Emx1*, *Emx2*, *Otx1*, and *Otx2* are expressed in rostral portions of developing embryonic brains, and their expression domains are contained within each other (Simeone *et al.* 1992*a*, *b*). The boundaries of expression domains or particular overlap zones coincide with the boundaries of major brain structures, such as the telencephalon and diencephalon (see Boncinelli *et al.* 1995 for a review). At a finer level of detail, expression domains of genes such as *Otx1*, *Otx2*, and *Wnt3* within a particular structure such as the diencephalon, coincide with anatomical divisions within the diencephalon such as the dorsal and ventral thalamus and pretectum, and are involved in specifying these large subdivisions of the central nervous system, as well as

smaller subdivisions therein (Marin and Rubenstein 2002). Because the neocortex is composed of multiple parts (cortical fields) with boundaries that are often abrupt, a situation analogous to the structural borders of subcortical structures and the smaller subdivisions described above, it is tempting to speculate that the same rules of specification apply to the developing neocortex. That is, genes or particular spatial and temporal combinations of gene expression strictly control cortical field emergence, organization, architecture, and connections.

There is evidence that particular genes and proteins serve as signaling centers and mark general axes of the cortex, such as rostro-caudal and dorso-ventral, and that particular spatial and temporal combinations of their expression patterns serve as a coordinate system for incoming thalamocortical axons. For instance, mounting evidence suggests that genes such as sonic hedgehog (*Shh*; Chiang *et al.* 1996) and some genes in the *Wnt* family (Grove *et al.* 1998) mark ventral telencephalic structures and the dorsal edge of the telencephalon, respectively, and proteins such as bone morphogenic protein (BMP; Furuta *et al.* 1997) may assign the dorsal telencephalon (see Levitt *et al.* 1997; Rubenstein *et al.* 1999; Marin and Rubenstein 2002 for reviews). Recent studies by Bishop *et al.* (2000) demonstrate that regulatory genes such as *Emx2* and *Pax6* are also involved in specifying the anterior–posterior axis of the cortex, since the deletion of such genes results in a caudal or rostral shift of thalamocortical afferents, respectively, and presumably the associated cortical fields (Fig. 3.4; Bishop *et al.* 2000). Fukuchi-Shimogori and Grove (2001) demonstrated that electroporation of the molecule FGF8, which is thought to serve as a signaling marker of rostral cortex, results in a posterior shift of anterior cortical fields and an antero-posterior elongation of cortical fields. FGF8 appears to function in part through repression of *Emx2* expression (Crossley *et al.* 2001). These data indicate that particular molecules (regulated by intrinsic gene patterning) may contribute to the emergence of cortical fields, although how these abrupt and graded patterns of gene expression would be altered to produce new cortical fields is not yet clear.

Recent studies indicate that these and other signaling centers can operate independently of peripheral activity. For instance, studies in mutant mice that fail to develop thalamocortical axons (Miyashita-Lin *et al.*, 1999; Nakagawa *et al.* 1999; *Gbx2 –/–; Mash1–/–*), and thereby have no access to patterned activity from peripheral sensory arrays, still have graded and abrupt patterns of gene expression (Fig. 3.5). These expression patterns are proposed to mark boundaries of cortical areas, but there is no direct evidence to support this contention.

These data are compelling in that they clearly demonstrate that an anterior-posterior/dorsoventral coordinate system is likely to be intrinsically mediated. Thus, the general location of primary fields and some aspects of the fields themselves may be specified by intrinsic genetic patterning which operates independently of thalamocortical input. Further, these studies demonstrate that cortical domains and primary cortical fields can be shifted when genes and molecules are manipulated via mutations and electroporation.

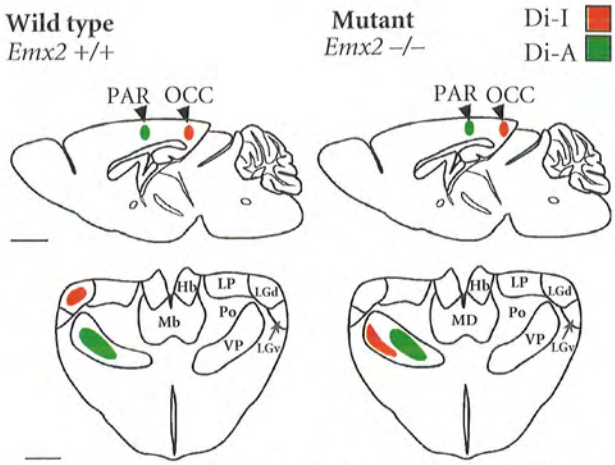


Fig. 3.4 Thalamocortical projections in *Emx2* wild-type (+/+) and mutant (–/–) mice as revealed by anatomical tracers placed into the cortex. *Emx2* is a regulatory gene which is expressed in a low rostral to high caudal gradient in mouse cortex during the late embryonic period. In both wild type (top left) and *Emx2*-deficient (mutant; top right) mice, the post-mortem tracer Di-A (green) implanted into the somatosensory cortex (PAR) retrogradely labeled cells in the ventroposterior nucleus of the somatosensory thalamus (VP, bottom). Di-I (red) implanted into visual cortex (OCC) of the mutant mice revealed retrogradely labeled cells in VP (red oval, bottom right), rather than in the normal location, in the dorsal lateral geniculate nucleus (LGd; red oval, bottom left). These differences in thalamocortical projections indicate that in the *Emx2*-deficient mice, there was a caudal shift in the thalamocortical projection patterns and presumably somatosensory cortical fields. The top row is an illustration of a lateral view of the brain, rostral is to the left. Green and red ovals in the top row represent Di-A and Di-I injection sites into the parietal (PAR) and occipital (OCC) regions of the neocortex, respectively. The bottom row depicts areas in which retrogradely labeled cells were observed in coronally sectioned thalamic tissue (data used to construct this figure is from Bishop *et al.* 2000). Scale bar = 1 mm. Top row rostral is left and medial is to the top. Bottom row, dorsal is to the top.

It should be noted that all current developmental studies examine arealization of primary sensory fields exclusively. Therefore, if indeed there are intrinsic signaling centers that specify a cortical field, this may only be true for primary fields. This notion is supported by recent comparative, embryonic, genetic, and immunohistochemical analyses indicating that all of the neocortex may not be of the same phylogenic origin. Specifically, medial portions (which contain primary sensory cortices) may have different phylogenetic precursors (Butler and Molnár 2002; Molnár and Butler 2002). It has been proposed that medial neocortex (and the sensory cortices therein) arise from the corticostriatal junction, and that these fields are homologous to the anterior portion of the dorso-ventricular ridge of sauropsids (birds and reptiles), while the more lateral portions, which contain non-primary fields, have different origins. This suggests that

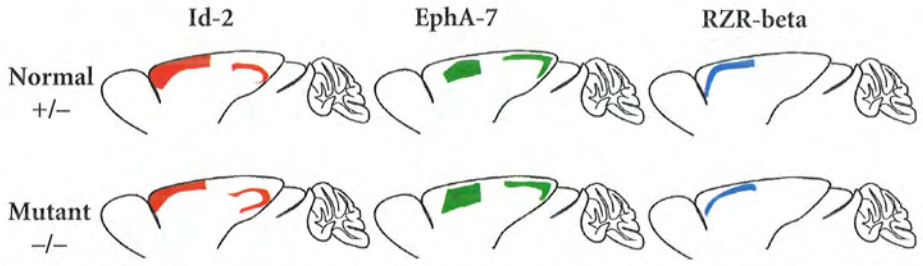


Fig. 3.5 Expression patterns of genetic markers in normal (+/–; top row) and *Gbx2* mutant (–/–; bottom row) mice at postnatal day 0. Illustrations depict a lateral view of the neocortex, rostral is to the left. In *Gbx2*-deficient (–/–) mice, thalamic axons do not form connections with the cortex. In a study by Miyashita-Lin *et al.* (1999), expression patterns of region-specific genes in the cortex were analyzed in *Gbx2* mutants. In the normal animal, *Id-2* (left column), *EphA-7* (middle column) and *RZR-beta* (right column) are expressed in discrete cortical regions and layers. Despite the lack of input from the thalamus in the mutant mice, expression patterns of these three gene markers were not different than in normal animals. These results demonstrate that thalamic input (and the patterned activity it relays to cortex) is not necessary for patterned expression of particular genes in the cortex (data used to construct this figure is from Miyashita-Lin *et al.* 1999). Rostral is left and medial is up.

the rules of arealization for primary fields and non-primary fields may be different, and each may be more or less influenced by activity versus genes.

3.4 Comparative studies of the neocortex: Peripheral morphology and activity-dependent regulation of cortical domains and cortical fields

While the evidence for genetic specification of cortical areas is strong, the concept of a strict genetic specification of cortical fields is at odds with an enormous amount of comparative data. Studies in a variety of mammals indicate that the assignment of cortical domains, the number of cortical fields within a domain, and the internal organization of a particular cortical field are dependent on peripheral morphology and the activity generated by particular sensory receptor arrays. This is best illustrated in mammals with an exaggerated or specialized morphological feature or sensory receptor array. There are three striking features of cortical organization in these animals. The first is the relationship between cortical domains and peripheral receptors; the second is a cortical magnification within a cortical field of the specialized receptor arrays; and the third is the generation of isomorphic substructures within a magnified representation which is directly related to peripheral receptor type, number, density of innervation, and use.

The first feature of organization that appears to be dictated by peripheral inputs and activity is the cortical domain territories assigned to a particular sensory system.

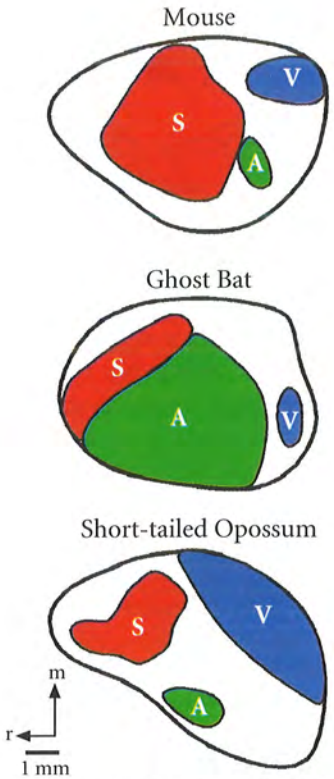


Fig. 3.6 Primary cortical areas in three species of mammals that have approximately the same size cortical sheet, but different amounts of cortex allotted to different sensory systems. These cortical differences are related to differences in peripheral sensory systems and use of particular sensory receptor arrays. For example, in the mouse, which relies heavily on tactile inputs from the whiskers for survival, the primary somatosensory cortex (red) and the rest of somatosensory cortex is enlarged, and the portion of cortex representing the whiskers is magnified, compared with that of the ghost bat and short-tailed opossum. Similarly, the primary auditory cortex and surrounding fields in the cortex of the echolocating ghost bat (green) is expanded, while the primary visual area (blue) and somatosensory area is relatively small. Finally, the cortex of the highly visual short-tailed opossum is dominated by V1 (blue) and other visual areas. Although the size, shape, and the details of internal organization of particular cortical fields vary, certain aspects of organization are conserved in these brains, such as the relative location of cortical domains and fields therein, and the general pattern of thalamocortical projections. Medial is up and rostral is to the left, scale bar = 1 mm.

Figure 3.6 illustrates three mammalian neocortices in which sensory domain assignment is remarkably different, despite the fact that the size of the neocortical sheet is approximately the same in each animal. For example, in the mouse, most of the cortical sheet is devoted to processing somatic inputs, particularly from the whiskers. In the echolocating ghost bat, most of the cortical sheet is devoted to processing auditory

inputs, and in the highly visual short-tailed opossum, most of the cortex is devoted to processing inputs from the retina. In all of these mammals, there is an enlargement in the cortical territory occupied by the dominant sensory system, and this occurs at the expense of the remaining sensory domains.

At a more detailed level, peripheral innervation is reflected in the cortical magnification of specialized body parts and the organization within a cortical field. For instance, the duck-billed platypus has a large, highly innervated bill with interdigitating parallel rows of mechanosensory and electrosensory receptors. This striking morphological specialization, accompanied by the evolution of an electrosensory receptor, manifests in cortex as an enormous representation of the bill (Fig. 3.7A). This type of peripheral modification, coincident with the enlargement of sensory domains and cortical representations of the specialized body part, can be observed in all sensory and motor systems in a variety of mammals. The star-nosed mole, for example, has a large amount of cortical territory devoted to processing inputs from its specialized nose (Fig. 3.7B; Catania and Kaas 1995). In human and non-human primates, the somatosensory cortex is largely devoted to processing inputs from the remarkably specialized forepaw or hand (Kaas *et al.* 1979), the primary visual area contains an enlarged representation of the fovea (which has a higher density of retinal ganglion cells), and in humans the motor and premotor cortex contains an exaggerated motor representation of the lips, tongue, oral structures, larynx, and associated musculature (commonly referred to as Broca's area).

Finally, within a cortical field, anatomical and functional isomorphic representations of very specific peripheral morphologies can be identified, including barrel fields in some rodents, digit subdivisions in several primates, ray or follicle patterns in star-nosed moles, and electrosensory/mechanosensory stripes in the duck-billed platypus (Figs 3.7 and 3.8). The relationship between such detailed anatomical and functional subdivisions within a cortical field and its peripheral counterpart has been clearly demonstrated by Welker and Van der Loos (1986). In mice selectively bred to have an extra whisker or row of whiskers, extra barrels or rows developed within the barrel fields in the neocortex (Fig. 3.8A). The authors noted that the relationship between peripheral innervation density and cortical isomorph was not linear, and suggested that other factors, such as patterned activity, contribute to some aspects, such as size of the isomorphic representation.

More recent studies in the star-nosed mole by Catania and Kaas (1997*a*, *b*) support the findings of Welker and Van der Loos (1986). In star-nosed mole that naturally posses an additional nose appendage or ray, there is an extra isomorph of this appendage in the neocortex. These authors extend these initial observations by demonstrating a clear use-dependent construction of some aspects of cortical isomorphs by documenting a differential magnification of some of the nose rays compared to others (Fig. 3.7B). The eleventh, ventromedial ray is preferentially used in tactile exploration. Although it is the smallest ray, with the fewest number of sensory

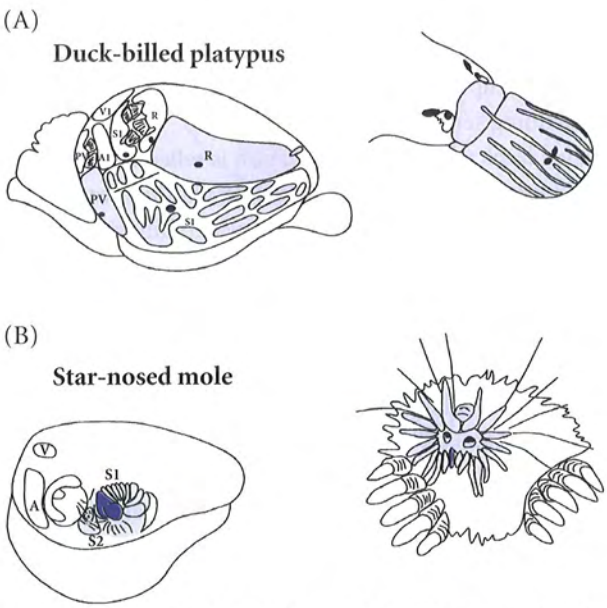


Fig. 3.7 Cortical representation of the bill of the duck-billed platypus (A) and the nose of the star-nosed mole (B). Both mammals have evolved specializations in peripheral morphology and use of specialized body parts, which are accompanied by changes in cortical organization. The large bill of the platypus has an enormous representation in the cortex that spans several cortical fields (blue). Within S1, both electrosensory and mechanosensory inputs are arranged in bands that form isomorphic representations of the striped arrangement of receptors on the bill. The star-nosed mole has a structure that consists of an array of 22 appendages (rays), 11 on each side that are arranged around the nostrils. These rays are used to explore food items and the immediate surroundings, and have been likened to a fovea. In the cortex, these rays form isomorphic representations that appear band-like in both S1 and S2 (blue). One of the rays of the star-nosed mole (number 11—dark blue) is utilized preferentially compared to the other rays, and has an even larger representation in the cortex (dark blue) than its counterparts. The unusual morphological specializations in these mammals and the cortical magnification of the regions devoted to processing inputs from these appendages are striking demonstrations of the impact of peripheral morphology on organization of the neocortex. (A, based on Krubitzer 1998; B, modified from Catania and Kaas 1997*a*.) Medial is up and rostral is to the right.

end organs, it has the largest sensory representation in S1 of the neocortex, and the greatest area of cortical innervation relative to size of any of the other rays (Fig. 3.7B).

This clear relationship between peripheral morphology and use and cortical domain assignment, cortical field magnification, and generation of isomorphic representations as observed in comparative studies is difficult to reconcile with proposed intrinsic mechanisms of cortical arealization described earlier. Indeed, some of the results from both groups appear to be in direct conflict. For instance, studies in which FGF8 was

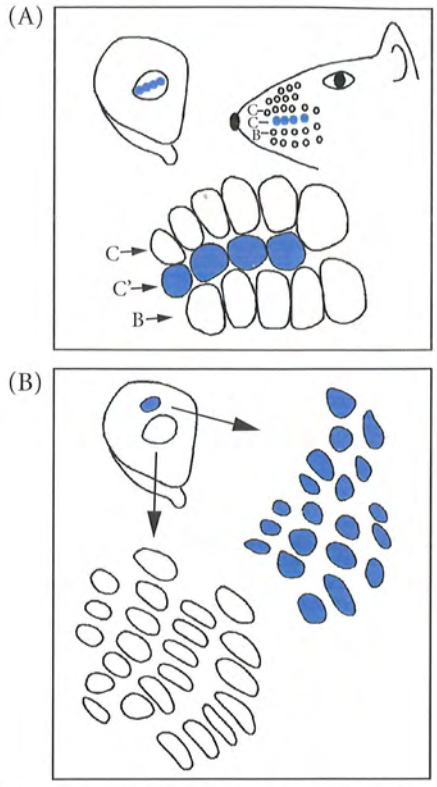


Fig. 3.8 Two potential ways in which extra representations of whiskers (barrels) may be generated in cortex. The first method (A) was demonstrated over a decade ago by Welker and Van der Loos. Mice that were selectively bred to have and extra row of whiskers (top right) had an extra row of whisker representations in the cortex (top left and bottom of A). A second method of inducing barrel formation in the neocortex is by artificially changing the pattern of intrinsic signaling centers (such as the molecule FGF8) early in development (B). In this study this FGF8 was electroporesed into a selected location caudal to its normal location of expression. An ectopic barrel field formed caudal to the barrel field in S1. (Data used to construct this figure is from Welker and Van der Loos 1986; and Fukuchi-Shimogori and Grove 2001.)

electroporesed into a caudal region of cortex clearly demonstrate the emergence of a new, ectopic barrel field (Fig. 3.8A; Fukuchi-Shimogori and Grove 2001), while other studies in mice that possess an extra row of whiskers (Welker and Van der Loos 1986), demonstrate additional rows of barrels in the cortex (Fig. 3.8B). The former study suggests a strict genetic specification of cortical fields, while the latter study indicates that peripheral innervation and use play a direct role in specification of cortical fields in development.

The issue, of course, is not how the cortex can be manipulated to produce alterations in cortical fields, but how specification of cortical fields naturally occurs in evolution, and how intrinsic and activity-dependent mechanisms operate together under normal

conditions to produce a particular cortical phenotype. If one considers both the genetic manipulation studies and the comparative studies, a clearer picture of the genetic and activity-dependent contributions to the phenotype begin to emerge. For instance, both sets of data indicate that there are some features of cortical organization that are genetically mediated and highly constrained in evolution. The first is the gross geographic relationship of primary cortical areas to each other. Indeed, the relative position of fields is invariant across mammals. The second is thalamocortical connectivity, particularly the connections between major sensory nuclei such as the lateral geniculate nucleus (LGN), medial geniculate nucleus (MGN), and the ventro-posterior nucleus of the somatosensory thalamus (VP), and primary sensory areas such as V1, A1, and S1, respectively. Finally, some aspects of cortical architecture, such as the presence of a koniocellular layer and myelin density of primary sensory fields, are likely to be genetically regulated, and certainly appear to be constrained in evolution. On the other hand, comparative data indicate that several features of cortical organization are not genetically constrained and vary with changes in peripheral morphology and with the patterned activity associated with such morphology. These features include the total extent of a particular sensory domain (not its general location), the size and shape of a cortical field, the details of the internal organization of a cortical field, and some aspects of thalamocortical and cortical connectivity. These types of changes are driven by modifications to peripheral morphology, including changes in the size of an appendage or structure and the receptor type, number, and density within the structure. These peripheral modifications may be, but are not necessarily, genetically mediated.

3.5 Testing theories of cortical domain specification: Studies of bilaterally enucleated opossums

We can test the extent to which peripheral input and associated activity contribute to specifying cortical domains by making changes to the peripheral receptors, similar to the types of changes that occur naturally in evolution. As noted in Section 3.4, important features of cortical organization are associated with distinct peripheral morphologies and behaviors. The obvious conclusion from these comparative studies is that peripheral morphology and patterned use play a large role in cortical field specification in development. One way to test the total extent to which peripheral receptors can alter cortical domain territories is to increase or decrease the size of the sensory receptor array of a specific sensory system and examine the resulting neocortex using electrophysiological recording and anatomical techniques.

In a recent set of experiments in the South American short-tailed opossum (*Monodelphis domestica*), we eliminated visual input very early in development, prior to the formation of the retino-geniculo-cortico pathway (Dunn *et al.* 2001; Kahn and Krubitzer 2002). Electrophysiological recordings in these enucleated opossums after they reached adulthood revealed that 'visual cortex' was substantially reduced (Fig. 3.9).

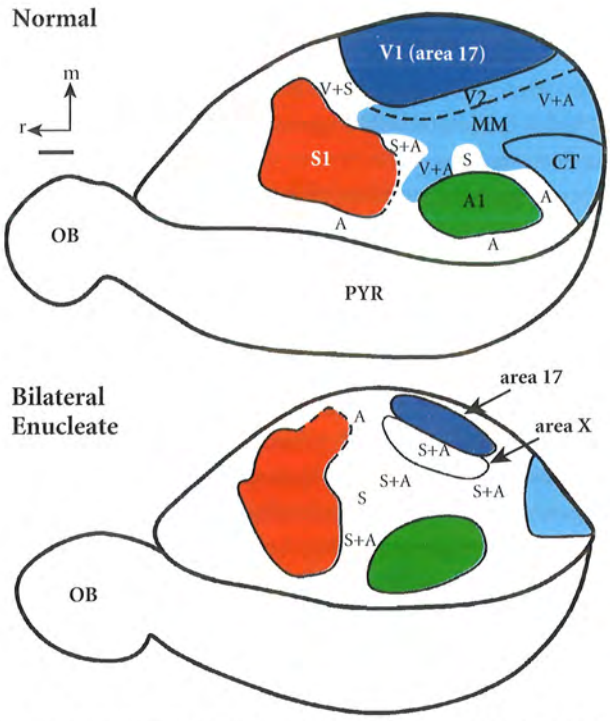


Fig. 3.9 Illustration of a dorsal view of the neocortex of a normal (top) and bilaterally enucleated (bottom) adult short-tailed opossum. Bilateral enucleations were performed early in development, prior to the formation of the retino-geniculo-cortical pathway. Despite the absence of input from retinal receptors in the enucleated animal, there was still an area in the caudomedial portion of the cortex that was anatomically similar to V1 (area 17) in the normal animals, although substantially smaller. The geographic relationship of S1 (red), A1 (green) and area 17 (blue; V1 in normal animals) was maintained despite the complete lack of activity from visual receptors. In the bilateral enucleate, area 17, which contains neurons that normally respond to visual stimulation, contained neurons responsive to somatosensory (S) and auditory (A) stimulation. Thus, when activity from visual receptors was experimentally eliminated, visual cortex was captured by the somatosensory and auditory systems. These changes in the configuration of cortical domains, and size of cortical fields indicate that some features of cortical organization are mediated by activity from peripheral receptors. Dark blue in the top figure (normal) represents the primary visual area, and in the bottom the primary visual area as defined architectonically. Light blue (top) represents other visually responsive cortical regions in the normal animal. Red = somatosensory, green = auditory, MM = multimodal area, CT = caudotemporal area, OB = olfactory bulb, PYR = pyriform cortex. Rostral is to the left and medial is up; scale bar = 1 mm. (Modified from Kahn and Krubitzer 2002.)

Further, cortical regions normally involved with visual processing, including area 17 or V1, now contained neurons that responded to a different sensory modality compared to normal animals. Thus, there were dramatic shifts in cortical domain territories, as large, or larger, than those produced by genetically modifying intrinsic signaling centers (discussed in Section 3.3).

However, there were also a number of features of the neocortex that remained unchanged, despite this massive loss of sensory input. For instance, examination of the bilateral enucleate brains using neuroanatomical tracing techniques indicated that cortico-cortical and thalamocortical connections of area 17 (V1 in normal animals) were largely preserved (Kahn and Krubitzer 2001). In addition, gross positional organization in terms of medio-lateral and rostro-caudal organization of the cortex was maintained. Finally, although V1 appeared to be substantially reduced in size, its cortical architecture was similar to that of normal animals. These results indicate that peripheral input plays a large role in assigning cortical domains, and that dramatic changes in the organization and the size of cortical fields can be determined by peripheral input. On the other hand, position, shape, architecture, and some aspects of connectivity of at least primary fields are likely to be mediated by intrinsic genetic signals (for review of related literature see Kahn and Krubitzer 2002). These observations are similar to those made in mammals that naturally have a reduced or absent visual system due to miniaturization or loss of the eye. For instance in the blind mole rat, the eyes are microophthalmic and covered with skin. In these animals, as in the bilateral enucleated animals, a geniculo-cortical pathway is still present (Bronchti *et al.* 1991; Cooper *et al.*, 1993), and neurons in the lateral geniculate nucleus (LGN) and 'visual' cortex respond to auditory stimulation (Bronchti *et al.* 1989).

These studies support observations from comparative studies in that the global geographic relationships of primary sensory fields are maintained, the architectonic features of primary cortical fields can be identified, and some aspects of connectivity are maintained even in the absence of use or loss of a sensory system. The preservation of global relationships of sensory cortical fields and of some aspects of connectivity in animals that have extreme specializations, like the platypus, or loss or reduction of a sensory system, like the blind mole rat and bilateral enucleate, fit well with data from developmental studies described earlier in this chapter. All of these studies are consistent with the view that intrinsic signaling centers (e.g. *Wnt*, *Shh*, BMP) provide positional information for incoming thalamocortical afferents, and relative location of cortical fields with respect to other cortical fields. These genes (and likely others) arose early in evolution, and certainly constrain the evolution of the mammalian neocortex.

Despite these consistencies across data sets, there are still several outstanding questions generated by comparative, molecular and developmental manipulation studies that need to be addressed. For example, how do intrinsic cortical mechanisms act in concert with activity-dependent mechanisms to allocate cortical domains and cortical fields that faithfully represent sensory receptor arrays? A second question is how are

the dynamics of particular developmental mechanisms altered over larger time scales to produce variable phenotypes? The large-scale dynamics of evolution are rarely considered in the smaller context of individual developmental cascades. In particular, how are developmental regimes altered to produce a new cortical field? A third question is related to the co-evolution of the motor system with particular sensory system morphologies. As noted above, specialized peripheral morphologies are associated with specialized use; the receptor array is never stationary but is very specifically interfaced with the environment. Particular motor sequences, such as reaching and grasping, saccadic and smooth pursuit eye movements, whisking, and head orientation, have co-evolved with these receptor arrays. Thus, the motor system is an integral part of sensory reception. How are sensory and motor systems interfaced in development? How does the evolution of one affect the evolution of the other? Finally, at the cellular level, what are the changes in the pre-and postsynaptic elements that allow for the types of activity-dependent modifications observed in extant mammals? Are these cellular changes heritable? Are they only expressed in particular environmental contexts? Some of these questions can be addressed by considering specific cellular mechanisms that are influenced by activity. As discussed in the following section, accumulating evidence indicates that several features of synaptic architecture and function may indeed be context dependent, and thus highly variable across species.

3.6 Neurotrophins and activity-dependent changes to the nervous system

There have been several activity-dependent, molecular mechanisms proposed to account for the structural and functional changes that occur in the developing nervous system. One of the best candidates for such changes involves a class of proteins called neurotrophins. Neurotrophins are likely mediators for activity-dependent changes that occur during development, for several reasons. Activity regulates their levels and secretion and is in turn regulated by them, they are expressed in portions of the neuron that undergo changes (e.g. synapses), they regulate morphological changes in both the pre- and postsynaptic element (for reviews, see McAllister *et al.* 1995, 1999; McAllister 2001), and they trigger local protein synthesis at the dendrite (Aakalu *et al.* 2001; see Zhang and Poo 2001 for a review).

One way in which activity can ultimately affect the structural configuration and function of neurons via neurotrophin release, in particular by the release of brain-derived neurotrophic factor (BDNF), is through calcium channels. Neural activity increases intracellular calcium and, through a cascade of intracellular molecular events, induces activation of the cyclic AMP pathway that phosphorylates a transcription factor, cyclic AMP response element (CRE)-binding protein or CREB (for reviews, see Finkbeiner and Greenburg 1998; West *et al.* 2001). Phosphorylated CREB can bind to the regulatory region of a gene and induce the initiation, elongation, and translation

of RNA transcripts. An RNA transcript is a complementary strand of DNA that is used as a template to translate the DNA code into a protein or peptide. In this way, activity can alter gene expression, by transcribing the code for neurotrophins such as BDNF.

Neurotrophins, such as BDNF, nerve growth factor (NGF), NT3, and NT4/5 play a critical role in the development of the nervous system and carry out a range of functions. At a very gross level, neurotrophins such as BDNF and NGF mediate both positive and negative rates of neuronal survival during development (for reviews, see Levi-Montalcini 1987; Miller and Kaplan 2001), and stimulate cell migration of neurons out of proliferative zones (Borghesani *et al.* 2002). Neurotrophins also influence the growth of axons and dendrites (Segal *et al.* 1995; Carter *et al.* 2002), exerting very specific effects on neuronal differentiation. Further, recent studies in hippocampal slices of adult animals indicate that BDNF stimulates protein synthesis in dendrites of hippocampal neurons (Aakalu *et al.* 2001). Thus, local production of particular proteins may be involved in determining dendritic and spine morphology as well as synaptic function.

The entire process by which activity promotes structural and functional changes in a neuron is intricate, and much of the evidence for exactly how activity alters structure is indirect, often correlational, and in some instances unknown. However, the important point for our discussion of phenotypic variability is that activity can induce cellular and systems level changes in the developing nervous system via calcium-induced alterations in gene expression. Such alterations in gene expression promote peptide and protein synthesis (of neurotrophins and many other proteins), which in turn generates structural and functional modifications throughout the cell. Thus, one can have changes in gene expression, alterations in connectivity, and ultimately large phenotypic changes that are not heritable. However, these modifications can masquerade as evolution as long as the physical and social environment that led to the generation of the particular patterned activity, which induced changes in gene expression and the resulting phenotype, is static. As discussed below, some phenotypic characteristics, including some features of cortical organization and connectivity, exist only within specific environmental contexts.

3.7 What are the genetic and activity-dependent mechanisms that give rise to features associated with complex brains?

We have discussed some of the features of complex brains that are likely to be under genetic control, and in some instances, the specific genes or proteins associated with a particular feature. First, the size of the cortical sheet is likely to be under genetic control, and simple regulation of cell-cycle kinetics in the ventricular zone can account for an exponential increase in the size of the cortical sheet. Proteins such as β-catenin appear to regulate some aspects of the cell cycle, particularly the fraction of cells that remain in the progenitor pool. Another feature of mammalian brains that appears to

be genetically regulated is the anterior-posterior and dorso-ventral coordinate system of the neocortex. Intrinsic signaling genes and molecules such as *Wnt*, *Shh*, BMP, and fibroblast growth factor-8 (FGF8) may set up a combinatorial coordinate system that serves as a scaffold for incoming thalamocortical axons. Although not discussed in detail, changes in peripheral morphology that ultimately control the types of patterned activity that the CNS can access are likely to be under genetic control. Features such as the size and shape of an appendage, and the sensory receptor type, number, density, and location may also be genetically regulated. Finally, the intracellular machinery that allows for activity-dependent changes in the developing nervous system are likely to be genetically regulated and heritable, although the specific phenotype they generate is not.

The contribution of patterned activity to the construction of a complex phenotype is also critical. Although not discussed in this chapter, it is certainly worth mentioning that both passive environmental influences as well as active influences play a large role in nervous system construction. Passive influences can have resounding effects on the development of both the somatic and nervous system phenotype. Some types of passive influences include diet, toxins, pH, and temperature. As an extreme example, the phenotype of a nervous system that develops in the presence of alcohol is dramatically different from a normal phenotype, yet still viable. In these cases, gross morphological structure, organization and, we suspect, even connections are significantly modified. This change is not adaptive (but analogous modifications may well be) and is not heritable.

Active influences include changes in the relative activity patterns across sensory receptor arrays, and patterned activity associated with specialized morphology. Activity can alter indirectly the temporal and spatial patterns of gene expression, possibly via neurotrophins which, in turn, can alter the structure and function of neurons and their connections. These types of alterations can masquerade as evolution, because they are genetically mediated and the resulting phenotype can be dramatically altered. However, they are not heritable, but, rather, situation dependent. The examples we have provided are easily related to peripheral morphology and use, and include the bill of a platypus, the hand of a primate, and the lips, tongue, oral structures, and larynx of humans. However, one can also consider active influences that are not strictly tied to a particular sensory receptor array or associated behavior, such as language and skill acquisition, and social and cultural learning. These types of active influences can fundamentally alter the phenotype by changing patterns of synaptic efficacy, connectivity, and ultimately the organization and function of the neocortex. It is likely that much of the human neocortex that does not include the primary and secondary sensory and motor areas is largely shaped by such active influences, and the organization of these cortical fields is only expressed in a particular environmental context. This makes defining such fields across species difficult, since the stimuli that ultimately shape the field are complex, multifaceted, often multimodal, and are variable for different species.

It may seem that the extended discussion of genes, activity, and peripheral input somehow strays from the question that serves as a title for this paper: Is the human brain fundamentally different than that of other mammals?

The answer, of course, is no. The human brain is enslaved by the same genetic constraints and shaped by the same activity-dependent mechanisms as the brain of other mammals. Consequently, its future evolution will follow predictable paths. Although the precise specializations that may emerge cannot be known, one can speculate with a fair degree of accuracy the types of change possible, and the mechanisms by which changes will be achieved. If we consider the human brain and its evolution in this light, then our current ideas regarding derived or specialized areas, which we believe endow us with our uniqueness, need to be reconsidered. Morphology and use alone do not specify brain structure and therefore do not solely constitute the differences observed in the brains of humans and other primates. On the other hand, human brains are not chimpanzee brains with a few new parts added on (e.g. Broca's area, more prefrontal cortex, Wernicke's area, fusiform face area) via very specific genetic changes. Rather, these derivations are much like those observed in non-human mammals, in that several are tied to changes in peripheral morphology and use (such as the language areas), and the cortex in which they reside is likely to be an expanded and connectional specialized version of homologous cortical areas in other primates. While most current work in the human brain focuses on primary and secondary sensory and motor fields, these fields may be more genetically constrained, less variant, and their functional and anatomical attributes more predictable. However, that portion of cortex that we (as a species) are particularly interested in—the regions traditionally referred to as association cortex, including inferotemporal, posterior parietal, and prefrontal cortex—may be less genetically constrained than primary and secondary sensory fields, and the ultimate phenotype of cortical fields within these regions may only occur within a particular environmental context.

Acknowledgements

We wish to thank Deborah Hunt, Jeff Padberg, Marie Burns, Bruno Olshausen, and Elizabeth Disbrow for helpful comments on this manuscript.

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