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Nature versus nurture revisited: an old idea with a new twist

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Abstract

The nature versus nurture debate has recently resurfaced with the emergence of the field of developmental molecular neurobiology. The questions associated with "nature" have crystallized into testable hypotheses regarding patterns of gene expression during development, and those associated with "nurture" have given over to activity-dependent cellular mechanisms that give rise to variable phenotypes in developing nervous systems. This review focuses on some of the features associated with complex brains and discusses the evolutionary and activity-dependent mechanisms that generate these features. These include increases in the size of the cortical sheet, changes in cortical domain and cortical field specification, and the activity-dependent intracellular mechanisms that regulate the structure and function of neurons during development. We discuss which features are likely to be genetically mediated, which features are likely to be regulated by activity, and how these two mechanisms act in concert to produce the wide variety of phenotypes observed for the mammalian neocortex. For example, the size of the cortical sheet is likely to be under genetic control, and regulation of cell-cycle kinetics through upregulation of genes such as *β-catenin* can account for increases in the size of the cortical sheet. Similarly, intrinsic signaling genes or gene products such as *Wnt*, *Shh*, *Fgf2*, *Fgf8* and BMP may set up a combinatorial coordinate system that guides thalamic afferents. Changes in peripheral morphology that regulate patterned activity are also likely to be under genetic control. Finally, the intracellular machinery that allows for activity-dependent plasticity in the developing CNS may be genetically regulated, although the specific phenotype they generate are not. On the other hand, aspects of neocortical organization such as sensory domain assignment, the size and shape of cortical fields, some aspects of connectivity, and details of functional organization are likely to be activity-dependent. Furthermore, the role of genes versus activity, and their interactions, may be different for primary fields versus non-primary fields.

Contents

1. Introduction

Many of us first encountered the work of Harry Harlow (Harlow and Zimmerman, 1959; see below) and his infant monkey studies early in our education. By rearing infant monkeys in a variety of social conditions, from partial social isolation to complete maternal separation with no access to attachment objects or surrogates, Harlow and coworkers were able to induce a variety of behavioral abnormalities from depression to full-blown psychosis (Kerr et al., 1969; Mckinney et al., 1971; Suomi et al., 1971; Suomi and Harlow, 1972; Suomi et al., 1976). These experiments

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demonstrated the dramatic effect of environmental conditions on an individual's social behavior, and had a tremendous impact on both the psychological and biological sciences.

Around this time there were major events occurring in other disciplines that ran counter to the ideas proposed by Harlow. Watson and Crick (1953) and Wilkins (see Wilkins et al., 1995) discovered the molecular structure of DNA, and its significance for information transfer in living organisms. Their discovery that life could be reduced to this very basic unit of organization, which continually replicates itself, revolutionized our thinking about biological organisms. On the heels of this discovery the ethologists Konrad Lorenz, Niko Tinbergen, and Karl von Frisch (see Lorenz, 1970, 1971; Tinbergen, 1953; Von Frisch, 1967) provided evidence from animal studies that seemingly complex, multi-faceted behaviors were intrinsically mediated or innate, and could be understood from a biological rather than psychological perspective. Wilson (1975) proposed that there is a genetic contribution to social behavior, and Dawkins (1976) took the extreme stance in his highly controversial book "The Selfish Gene" that all behavior could be understood in terms of gene function.

All of these studies contributed to the "nature versus nurture" debate. The central question of the debate was 'what is the contribution of our genes to overt and covert behavior, and what is the contribution of the environment to these same behaviors?'. While this issue is guaranteed to capture one's interest, at the time the ideas for each camp were formulated, very little was known about the genetic and experience dependent mechanisms that generate a brain and resultant behavior. As a result, the issue was relegated to the realm of psychology, and most neurobiologists never really grappled with this nebulous problem since there was nothing very solid to sink one's experimental teeth into.

The nature versus nurture debate has recently re-surfaced with the emergence of developmental molecular neurobiology as one of the leading disciplines in the field of modern neuroscience. The questions associated with "nature" have crystallized into testable hypotheses regarding the temporal and spatial patterns of gene expression during development, and those associated with "nurture" have given over to activity dependent cellular mechanisms that give rise to variable phenotypes in both developing and adult nervous systems. We became interested in these issues several years ago in a rather roundabout fashion, and only recently have we conceded that we are thoroughly entrenched in the age-old issue of nature versus nurture.

For some time we have examined the neocortex of mammals and questioned how complex cortical phenotypes arose in evolution. A complex neocortex contains a large number of functional parts or cortical fields that are specifically interconnected to produce various types of motor, perceptual and cognitive behaviors that may or may not be directly stimulus bound. Although it is not clear what a large neocortex with multiple parts endows, it seems likely that the neocortex enhances stimulus features, generates probabilities based on sensory experience, and constructs a species-specific interpretation of the environment generated from some set of physical parameters that a particular animal can actually detect. Further, most data indicate that mammals that have neocortices with many functionally heterogeneous parts that are specifically interconnected generally have more complex behaviors. These observations generated the central question posed by our laboratory: How does evolution build a complex brain with multiple, functionally distinct parts?

Unfortunately, evolution of any structure is difficult to study directly in mammals. Although fossil records can be useful for speculating about changes in gross morphological features of the brain, such as size and shape, to a large extent interpreting the fossil record relies on assumptions regarding phylogenetic relationships, and as such is confounded. Probably the main reason why brain evolution, or the evolution of any structure, organ, individual or species of mammal, is difficult to study directly is 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) examine the developmental mechanisms that give rise to particular characteristics of complex brains. By 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, the types of brain changes that are actually possible, and in combination with a developmental approach, the genetic 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 second sensory areas such as S1, S2, V1, V2, A1, and R (Fig. 1). Even in the absence of use of a particular sensory system, these cortical fields and some aspects of their connectivity are still present. These same types of studies indicate that the number of systems level 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 pre- and post-synaptic morphologies, for example, have also occurred in the neocortex over time. Although we propose that the

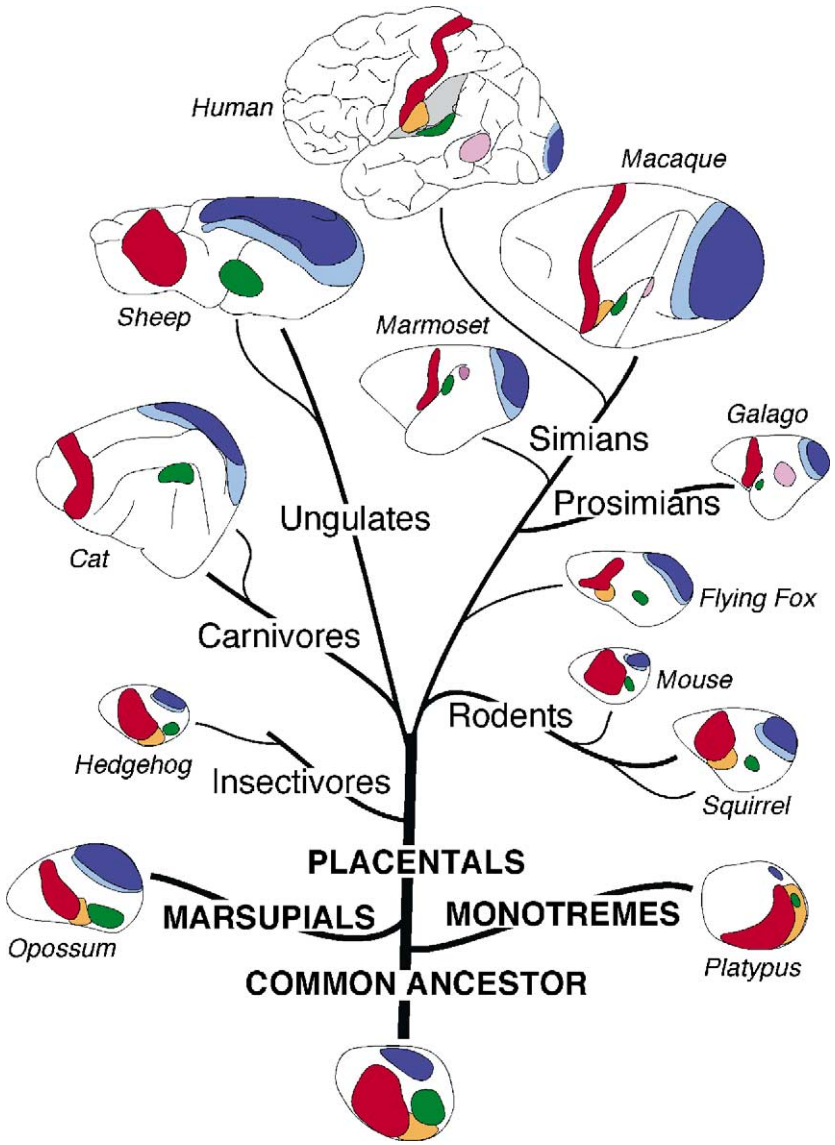

Fig. 1. An evolutionary tree depicting the phylogenetic relationship of major orders of mammals and the cortical organization of some of the sensory fields that have been described in particular species. Electrophysiological, anatomical, histochemical and molecular analyses have revealed that certain 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. A comparative analysis of the neocortex, using the criteria described above, allows one to infer the organization of an unknown mammal, such as the common ancestor or human. If a number of species are compared, one can be fairly confident when assigning features of cortical organization to the unknown state, even in the absence of direct data. S1: primary somatosensory area (red), S2: second somatosensory area (orange), A1: auditory (green), V1: primary visual area (dark blue), V2: second visual area (light blue), rostral is left, medial is up.

types of modifications with respect to all of the possible ways in which brains could change are limited, there are still large degrees of freedom for phenotypic change within these broad categories.

The limited number of systems level modifications that are observed in extant brains, particularly those that have independently evolved, indicate that there are constrained, developmental mechanisms that generate nervous systems. Thus, the second approach to studying 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. To determine if a developmental mechanism in question were indeed responsible for one of the modifications listed above, one could alter some aspect of development thought to be responsible for the particular modification and see if the cortical phenotype generated is consistent with the types that occur naturally in evolution. This can be done by physically manipulating the developing nervous system, or making a genetic change via mutations, over-expression, or inducing ectopic expression of specific molecules in a manner that mimics that of evolution. These manipulations

can induce a change in the trajectory of the developmental cascade and will ultimately generate an altered phenotype.

But what about nurture? What about the role of the environment in generating particular phenotypes? 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, as the studies of Harlow and coworkers clearly indicate (Harlow and Zimmerman, 1959; Kerr et al., 1969; Mckinney et al., 1971; Suomi et al., 1971; Suomi and Harlow, 1972; Suomi et al., 1976), regardless of genetic makeup, early experience plays a major role in the social and personal phenotype that emerges in an individual. Further, modern 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, due to changes in activity patterns across sensory receptor arrays. Activity-dependent system level changes take the form of functional map reorganization (Recanzone et al., 1992, 1993; Recanzone, 2000), and in the developing nervous system include large sensory domain shifts, changes in functional map organization and changes in connectivity (e.g. Kahn and Krubitzer, 2002; see Krubitzer and Kahn, 2003, for review). Presumably, these types of structural and functional changes in the nervous system account for the behavioral differences observed in Harlow's monkeys. However, exactly how neural changes translate into global changes in behavior is not known.

This review will focus on some of the features associated with complex brains and discuss the evolutionary (inherent, genetic contributions) and activity-dependent mechanisms that give rise to these features including increases in cortical sheet size, changes in cortical domain and cortical field specification, and the potential activity-dependent intracellular mechanisms that regulate the structure and function of neurons in development. Our goal is not to identify specific genes that regulate the features associated with complex brains, but to determine which features are likely to be genetically regulated, which features are likely to be regulated by activity, and how these two mechanisms act in concert to produce the wide variety of phenotypes observed in mammalian neocortex.

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. It is important to note that an increase in cortical sheet size that is proportionate to the increased size of the rest of the brain is not necessarily associated with increased complexity (for example in brush-tailed possums). Indeed, a number of mammalian species that have large bodies have large brains and a large neocortex that is not considered complexly organized. On the other hand, the neocortex has expanded disproportionately compared to the rest of the brain in most primates and cetaceans (Fig. 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).

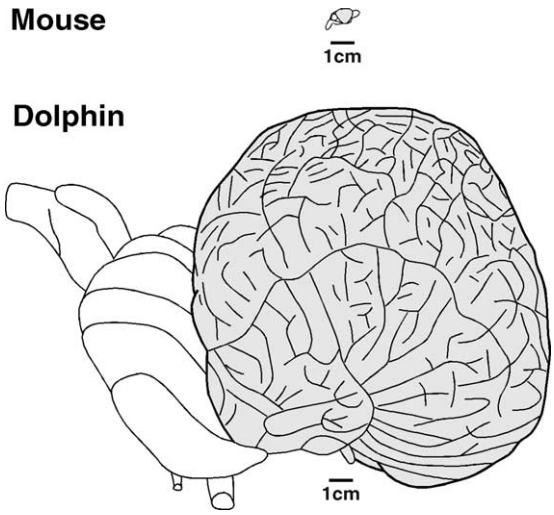

Fig. 2. A comparison of the mouse and dolphin brain drawn to scale illustrates the dramatic differences in the size of the neocortex. The difference in size is even larger in magnitude than is illustrated here, since the dolphin brain contains a number of fissures in which the neocortex is buried. An increase in the size of the cortical sheet relative to the rest of the brain is one of the most salient features associated with complex nervous system organization in mammals. Rostral is right, medial is up, scale bar = 1 cm.

While the selective pressures that lead to an enlarged cortical sheet are not clearly understood, it has been proposed that frugivory (fruit eating), longevity, and sociality are associated with the evolution of an enlarged brain in primates (Allman, 1999). For example, because fruits are distributed in space and time, particular strategies for remembering the location, color, and shape of the fruit would be necessary, as well as adapting plans for harvesting. It has also been proposed that longer life spans lead to an increased brain size, which in turn endows the animal with a greater ability to deal with extreme environmental fluctuations. Finally, sociality is also a factor that may contribute to large brain size. The ability to cope with complex social interactions, both overt and subtle, may promote the increase in the size of the neocortex. Indeed, there is a strong correlation between social group size and the size of the neocortex in particular (Dunbar, 1995; Hakeem et al., 1996; see Allman, 1999, for review). Further, sociality is one feature that all mammals with a relatively large neocortex (such as most anthropoid apes and cetaceans) appear to have in common.

Although all of these conjectures are viable, they are only correlative and not necessarily causal, and they may not explain variations in brain size between species with similar behaviors. Further, it is difficult to link direct changes to the cortex, like the expansion of one portion of cortex compared to the others, with any of these global features of social organization, economy or longevity. Therefore, it is

not surprising that determining the selective pressures that led to an expanded cerebral cortex are elusive. However, it is clear that larger brains that are more complexly organized generate more complex behavior. Regardless of the selection pressure that led to an enlarged cortical sheet, it seems that an increase in the size of the neocortex is a necessary step in the evolution of complex mammalian nervous systems. Therefore, to appreciate brain evolution and the factors that contribute to a complex phenotype, it is important to understand the types of developmental mechanisms that could give rise to an expanded cortical sheet, the genes which instruct these processes, 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, and two (not necessarily mutually exclusive) hypotheses have been generated about why the increase is disproportionate. One suggestion is that more cells are generated in development. Kornack and Rakic (1998) proposed 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 increase in the size of the cortical sheet (Fig. 3). Kornack's comparative analysis on the kinetics of cell division in monkeys and rodents (Kornack, 2000; Kornack and Rakic, 1998) revealed that in macaque monkeys, the period during which cell division occurs is 10 times longer in macaque monkeys than in mice and the cell cycle duration is two to five times longer 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.

Alternatively, it has been proposed that the cortical sheet can increase in size by decreasing 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 which regulate its production, may be involved in the determination of cortical sheet size in different lineages. β-catenin, an intracellular

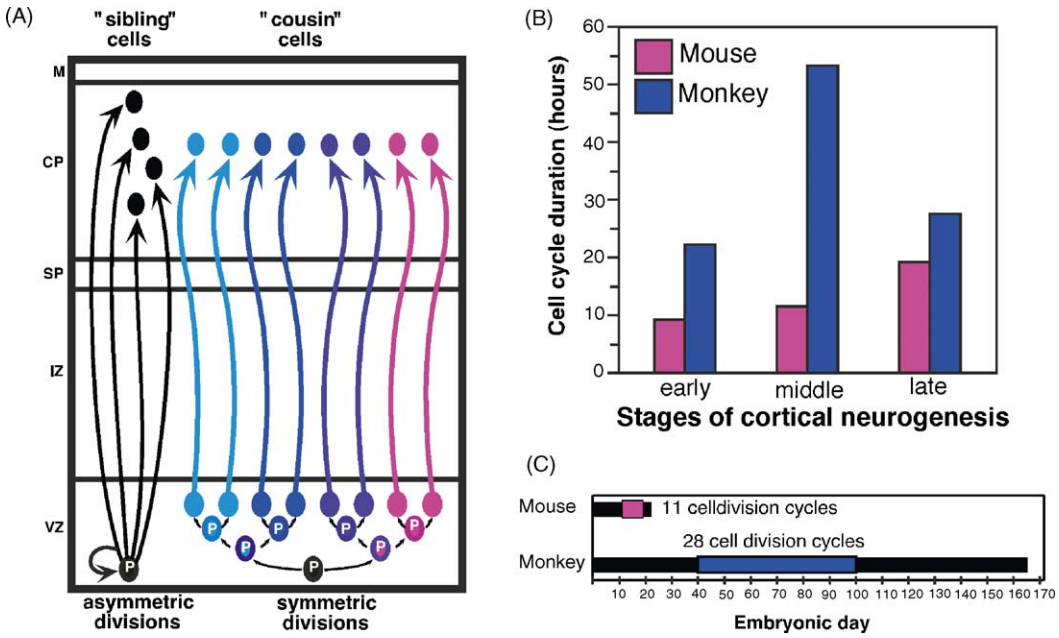

Fig. 3. Illustrations of how specific patterns of cell division in the ventricular zone (VZ) give rise to the patterns of clonally related neurons in the neocortex. In part A, asymmetric divisions from a single progenitor cell (P) (black arrows) generate "sibling" cells that migrate sequentially to different layers of the cortical plate (CP). This type of cell division determines cortical thickness. Symmetric divisions from a single progenitor cell (colored arrows) generate several progenitor cells that in turn simultaneously generate "cousin" cells that 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 differs dramatically in the mouse (pink) and the rhesus monkey (blue). In part 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 (E) day E11 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 (data used to construct this figure is taken from the work of Kornack and Rakic, 1998 and Kornack, 2000).

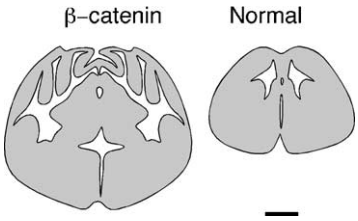

Fig. 4. An example of how changes in gene expression can dramatically alter the size of the cortical sheet. To the left is a coronal view of the cortex of a transgenic mouse in which the regulatory gene, *β-catenin*, was overexpressed, shown next to an age-matched normal mouse (right). In this study by Chenn and Walsh (2002), the animals that overexpressed *β-catenin* had massive increase in horizontal growth of the cortical sheet, which caused the normally lissencephalic cortex to became gyrencephalic. The results of this study indicated that the increased cortical size was due to a two-fold increase in the proportion of progenitor cells that re-entered the cell cycle and continued to divide (data used to construct this figure is taken from the work of Chenn and Walsh, 2002). Dorsal is to the top, scale bar = 1 mm.

protein that transduces *Wnt* signals (see Peifer and Polakis, 2000, for review), is expressed in neuroepithelial precursor cells (which will become the neocortex) in the ventricular zone during neurogenesis (Chenn and Walsh, 2002). These investigators demonstrated that transgenic mice that over-express 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 in these animals that the normally lissencephalic cortex became gyrencephalic (Fig. 4). This enlarged cortical sheet in the transgenic brains could be due to an increased mitotic rate, as proposed by Kornack and Rakic (1998), a decrease in cell death, or changes in the fraction of cells that continue to divide compared to those that leave the cell cycle and differentiate. A clever series of experiments (Chenn and Walsh, 2002) indicated that *β-catenin* did not change the rate of mitotic division nor decrease cell death. Rather, the investigators found that the proportion of progenitor cells that re-entered the cell cycle and continued mitotic division was increased two-fold.

While β-catenin appears to be a promising candidate for regulation of cortical sheet size, it is not the only one. Another gene, *Brain factor-1* (*BF-1* or *Foxg1*), is expressed in telencephalic progenitor cells (Tao and Lai, 1992) and has been demonstrated to regulate progenitor cell proliferation and differentiation in the neocortex of immature mice (Hanashima et al., 2002). In turn, *BF-1* is positively regulated by *Fgf8* (Shimamura and Rubenstein, 1997) while negatively regulated by BMP4 (Furuta et al., 1997). An increase in BMP4 expression is correlated with a decrease in cell proliferation in mouse telecephalic explants. Therefore, cortical sheet size can be altered via genes that regulated *BF-1* expression. The gene *Fgf2* is also involved with regulating cortical sheet size by positively regulating cell proliferation and neurogenesis in immature cortex. For instance, microinjections of FGF2 into the ventricle of embryonic rats substantially increased the number of cortical neurons as well as cortical volume (Vaccarino et al., 1999). In contrast, *Fgf2* knock-outs had smaller cortices and fewer cortical neurons (Raballo et al., 2000).

All of this data on developmental cascades involved in cortical sheet size determination indicate that alterations in any one or some combination of these genes and proteins could account for differences in the size of the cortical sheet in different mammals. Thus, the disproportionate increase in cortical sheet size in some mammals could be regulated, in part, by β-catenin, BF-1 (Foxg1), FGF2, FGF8, BMP4, or other proteins whose temporal and spatial patterns of expression vary slightly in different lineages. It should be noted that different genetic mechanisms may be responsible for similar aspects of cortical size regulation and organization in different mammals. Because many mammals with large neocortices have been evolving independently for tens of millions of years, and changes in one lineage obviously occur independent of the changes in another, a search for a ubiquitous mechanism that increases cortical sheet size, for instance, may prove fruitless. This, of course, implies that the mechanisms that regulate cortical sheet size in the mouse may not be the same as those employed by primates or other mammals with a disproportionately large neocortex.

Several hypotheses regarding why this increase is disproportionate have been proposed. A comparative analysis by Finlay and Darlington (1995) suggests that the order of neurogenesis is highly conserved in mammals, and neural structures that are produced later in development, such as the neocortex, are disproportionately larger. Kornack (2000) proposed that the disproportionate allocation of the neocortex compared to other telencephalic structures is due to a shift in regional boundaries of gene expression in the embryonic telencephalon.

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. No other extant mammal exhibits such a disproportionate increase, and the primate and cetacean lineages are very distantly related. Thus, 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 if this process occurs via homologous genetic mechanisms or if there is more than one way in which the neocortex can change in size.

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 that 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 cord 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., 1992a,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 review). At a finer level of detail, expression domains of genes within the diencephalon, such as *Otx1*, *Otx2* and *Wnt3*, coincide with anatomical divisions, such as the dorsal and ventral thalamus and pretectum, and are also involved in specifying these smaller subdivisions of the central nervous system (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 dorsoventral, 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 Marin and Rubenstein, 2002; Rubenstein et al., 1999; and Levitt et al., 1997, for reviews). Recent studies by Bishop et al. (2000) demonstrate that regulatory genes such as *Emx2* and *Pax6* are also involved

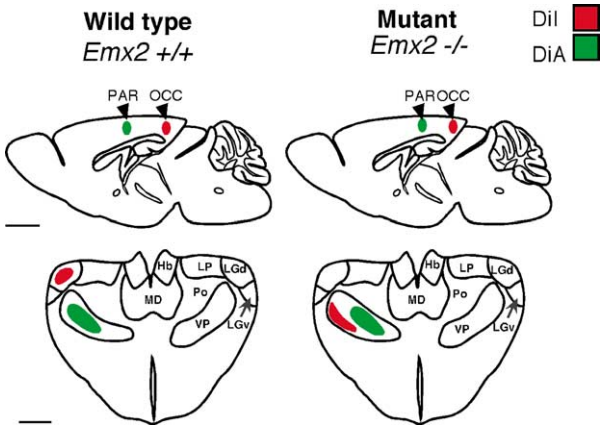

Fig. 5. Thalamocortical projections in *Emx2* wild-type (+/+) and mutant (−/−) mice as revealed by anatomical tracers placed into the cortex. *Emx2* is a regulatory gene that is expressed in a low rostral to high caudal gradient in mouse cortex during the late embryonic period. In both wild type (top left) and *Emx-2* deficient (mutant; top right) mice, the post-mortem tracer Di-A (green) implanted into the somatosensory cortex (PAR) retrogradely labeled cells in VP, the ventroposterior nucleus of the somatosensory thalamus (bottom left). However, 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 *Emx-2* deficient mice, there was a caudal shift in the thalamocortical projection patterns and presumably somatosensory cortical fields. Thus, *Emx-2* appears to be a gene involved in guiding thalamic afferents to the appropriate cortical location. The top row is an illustration of a lateral view of the brain, rostral is to the left, medial up. Green and red ovals in top row represent Di-A and Di-I injection sites into the parietal (PAR) and occipital (OCC) regions of the neocortex, respectively. Bottom row depicts areas in which retrogradely labeled cells were observed in coronally sectioned thalamic tissue (data used to construct this figure is taken from the work of Bishop et al., 2000). Bottom row, dorsal is to the top. Scale bar = 1 mm.

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. 5, Bishop et al., 2000). Fukuchi-Shimogori and Grove (2001) demonstrate that electroporation of the molecule FGF8, which is thought to serve as a signaling marker of rostral cortex and appears to function in part through repression of *Emx2* expression (Crossley et al., 2001), results in a posterior shift of anterior cortical fields and an anterior–posterior elongation of cortical fields.

Several recent experiments also demonstrate that *Fgf8* is essential for patterning of the rostral neocortex. A study by Garel et al. (2003) showed that a reduction of *Fgf8* levels in mutant mice shifted gradients of several cortical molecular markers (including *Emx2*, *Id-2*, *Cad-8* and *COUP-TF1*) rostrally, thus modifying the molecular identity of rostral cortex. However, the position of thalamic inputs in this cortex was not altered. Finally, when FGF8 is electroporated into a separate caudal location in the developing mouse cortex, in some instances, an ectopic barrel field forms caudally (Fig. 6b; Fukuchi-Shimogori and Grove, 2001). These data indicate that particular molecules (regulated by

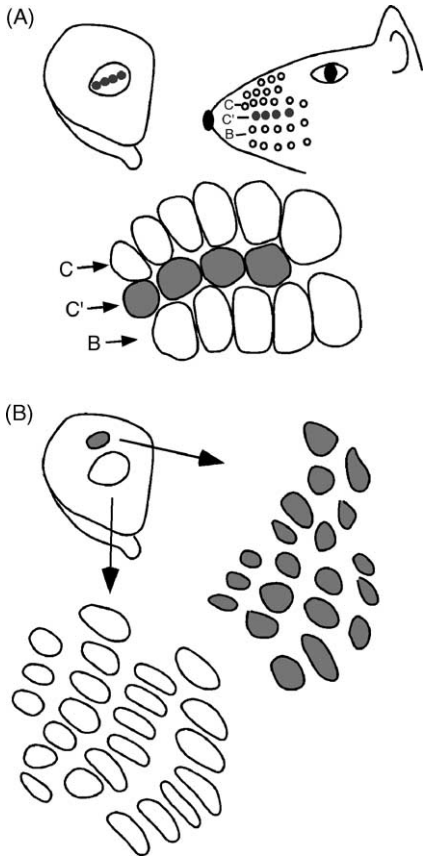

Fig. 6. Two potential ways in which extra representations of whiskers (barrels) may be generated in cortex. The first method (A) was reported over a decade ago by Welker and Van der Loos, who demonstrated the importance of peripheral morphology on the formation of cortical fields. Mice that were selectively bred to have an extra row of whiskers (top right) had an extra row of whisker representations in the cortex, indicated by C′ (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 FGF8 was inserted into a selected location caudal to normal location of expression, and an ectopic barrel field formed caudal to the barrel field in S1 (gray area; data used to construct this figure is taken from Welker and Van der Loos, 1986, and Fukuchi-Shimogori and Grove, 2001).

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. 7). These expression patterns are proposed to mark functional boundaries of cortical areas, but there is no direct evidence to support this contention.

All of 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 combinatorial actions of genes intrinsic to the neocortex that operate 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.

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 precursors (Butler and Molnár, 2002; Molnár and Butler, 2002). It has been proposed that medial neocortex (and the sensory cortices therein) arises from the corticostriatal junction and that these fields are homologous to the anterior portion of the dorsoventricular ridge of sauropsids (birds and reptiles), while the more lateral portions, which contain non-primary fields, have different origins. This suggests that the rules of arealization for primary fields and non-primary fields may

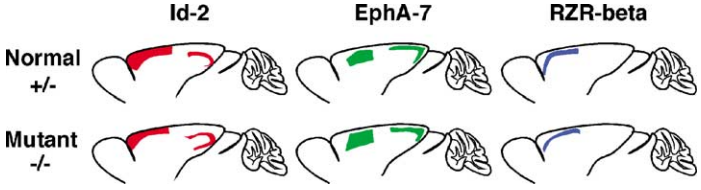

Fig. 7. Expression patterns of genetic markers in normal (+/−; top row) and *Gbx2* mutant (−/−; bottom row) mice at postnatal day 0. In *Gbx-2* deficient (−/−) mice, thalamic differentiation is disrupted, and thalamic axons do not form connections with the cortex. In the normal animal, *Id-2* (left column), *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. Thus, in normal and mutant animals, similar laminar and rostro-caudal boundaries of gene expression were observed for each gene marker. These results demonstrate that thalamic input (and the patterned activity it relays to cortex) is not necessary for some aspects of cortical arealization (data used to construct this figure is taken from Miyashita-Lin et al., 1999). Illustrations depict a lateral view of the neocortex, rostral is to the left and medial is up.

be different, and each may be more or less influenced by activity versus genes.

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 primary cortical fields is compelling, 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/or 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. 8 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. 9a). 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. 9b; 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 ventral 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).

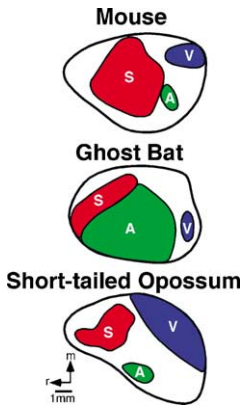

Fig. 8. Primary cortical areas in three species of mammals that have approximately the same size cortical sheet, but different amounts of cortex allotted to different sensory systems, related to specialized sensory receptor arrays and use of particular sensory receptor arrays. For example, in the mouse, 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 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 depending on use (activation from peripheral receptors), certain aspects of organization are conserved in these brains, even in the absence of apparent use. The similarity in relative location of cortical domains and fields therein suggests that the geographic organization and overall pattern of thalamocortical projections of the brain is constrained by developmental mechanisms. On the other hand, the differences in size, shape, and detailed organization of primary cortical fields indicate that input from the periphery is a crucial factor in guiding many of the details of organization of the neocortex. Medial is up and rostral is to the left, scale bar = 1 mm.

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. 6 and 9). 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. 6a). The authors noted that the relation-

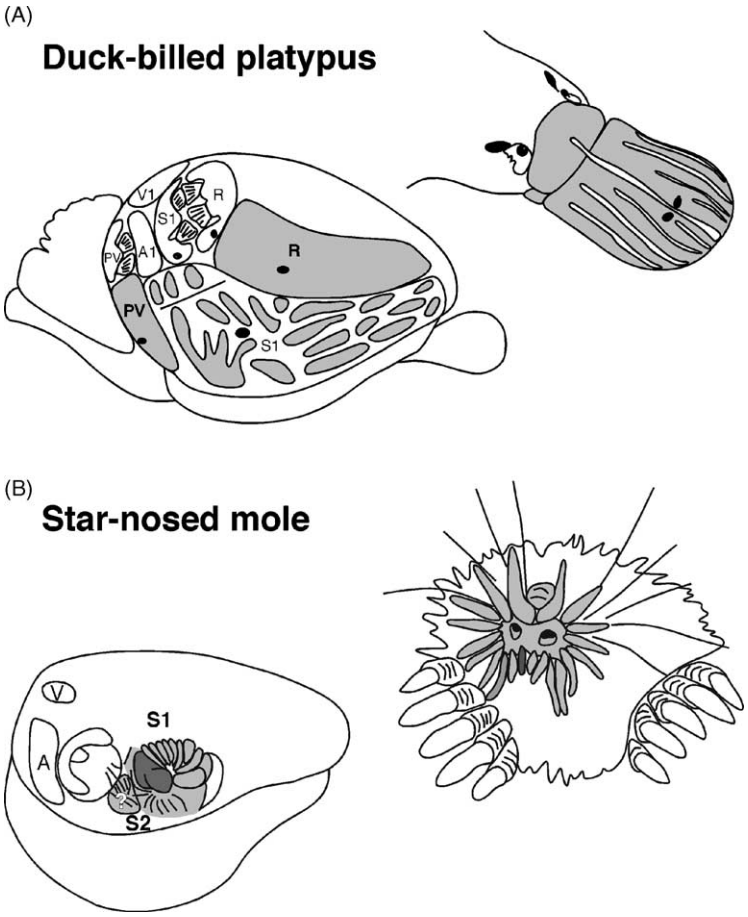

Fig. 9. Cortical representation of the bill of the duck-billed platypus (A) and the nose of the star-nosed mole (B). Both mammals have evolved specializations in peripheral morphology and use of specialized body parts, which is accompanied by changes in cortical organization. The large bill of the platypus has an enormous representation in the cortex that spans several cortical fields (gray). In fact, the majority of cortex in each field (S1, R, and PV) is devoted to processing inputs from the bill. 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 surround and have been likened to a fovea. In the cortex, these rays form isomorphic representations that appear band-like in both S1 and S2 (gray). One of the rays of the star-nosed mole (number 11: dark gray) is utilized preferentially compared to the other rays, and has an even larger representation in the cortex (dark gray) 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 (data used to construct this figure is taken from the work of Krubitzer et al., 1995 and Catania and Kaas, 1997b). Medial is up and rostral is to the right.

ship between peripheral innervation density and cortical isomorph was not linear and suggested that other factors, such as patterned activity, contribute to some aspects of organization, including size of the isomorphic representation.

More recent studies in the star-nosed mole by Catania and Kaas (1997a,b) support the findings of Welker and Van der Loos (1986). In star-nosed moles that naturally possess an additional nose appendage or ray, there is an additional isomorph of this appendage in the neocortex. Furthermore, the eleventh, ventromedial ray is preferentially used in tactile exploration. Although it is the smallest ray with the fewest number of sensory end organs, it has the largest sensory representation in S1 of the neocortex and the greatest area of cortical innervation relative to the size of any of the other rays (Fig. 9b). While this study does not directly demonstrate use-dependent magnification of a specific cortical representation, it does suggest that preferential use, as opposed to innervation density alone, contributes to the construction of some attributes of cortical isomorphs.

This relationship between peripheral activity 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 appear to be in direct conflict. For instance, studies in which *Fgf8* was electroporated into a caudal region of cortex clearly demonstrate the emergence of a new, ectopic barrel field (Fig. 6a; 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. 6b). 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. However, as with changes in cortical sheet size, there may be several ways in which cortical representations are modified in evolution—some due to intrinsic changes at a molecular/genetic level in the CNS and others to alterations in peripheral morphology and associated use.

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 LGd, MGd and VP, and primary sensory areas, such as V1, A1, and S1, respectively. Finally, some aspects of cortical architecture such as the laminar arrangement of the neocortex, the presence of a koniocellular layer, and the 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 indicates 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.

5. Theories of cortical field addition

Most theories of the development of the neocortex do not consider the dynamic nature of developing nervous systems over time in different lineages. While the molecular mechanisms involved in the generation of cortical areas within a particular species have begun to be elucidated, how these mechanisms are altered in different lineages to generate variable phenotypes is unclear. Such alterations must occur, but we do not know if these alterations are due to the addition or loss of an allele, or to more subtle changes in the spatial and temporal patterns of expression in genes thought to be involved in particular aspects of arealization.

Initially, our ideas regarding cortical field evolution were based almost predominantly on comparative observations. These observations (see Krubitzer, 1995; Krubitzer et al., 1995; Krubitzer and Huffman, 2000, for review), as well as studies done several years ago on the grey-headed flying fox (Krubitzer and Calford, 1992; Krubitzer et al., 1993), prompted us to speculate on the types of anatomical and functional changes that may occur in different lineages over time to generate the variability in neocortical number observed in extant species. Specifically, we proposed a way in which cortical fields might be added in evolution (Krubitzer and Calford, 1992; Krubitzer et al., 1995; Krubitzer, 1995). We observed that all mammals have a constellation of cortical fields that are present, even in the absence of apparent use (Figs. 1 and 12, see Section 1). We also observed that in the somatosensory cortex of the flying fox some fields were interdigitated within other fields, and some were completely embedded in other fields (Fig. 10). At first, we believed this to be an example of modularity within the field, much like CO blobs in V1 of monkeys. Not surprising, this led us to re-evaluate existing data on “modularity” in cortical fields. Our theory was that the cortex was a relatively homogenous structure early in development and that a cortical field represented some pattern of connectivity from the thalamus and ipsilateral and contralateral cortex. Over time in evolution, these patterns must shift and change, possibly

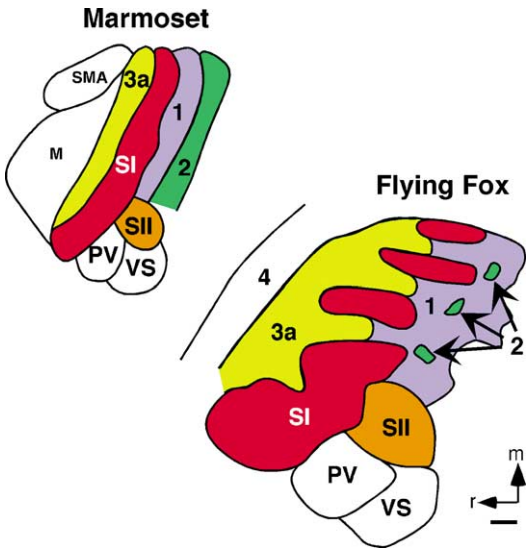

Fig. 10. The organization of somatosensory cortex in the marmoset and the flying fox. In the marmoset, as in other mammals, the somatosensory regions SI (red), 3a (yellow), area 1 (violet), and 2 (green) are contiguous regions, each containing a representation of the entire somatosensory surface. In contrast, in the flying fox, SI is interdigitated with areas 3a and 1. Further, area 2 consists of small islands that are completely embedded within area 1 (data used to construct this figure is taken from the work of Krubitzer and Kaas, 1990 and Krubitzer and Calford, 1992). Rostral is to the left, medial is up. Scale bar = 1 mm.

due to additions or alterations in peripheral sensory arrays. This results in a change in the geographic location of homologous cortical fields and the emergence of new patterns of connectivity (new cortical fields). If this were the case, then one might argue that there is nothing inherent about a cortical field (Fig. 11).

This hypothesis appeared to fit well with comparative observations for several reasons. First, the constellation of cortical fields that we identified (VI, AI, and SI) was observed in all mammals examined, but the overall size, shape, and some aspects of geographic location of homologous cortical fields were shifted and reconfigured across lineages (Figs. 1

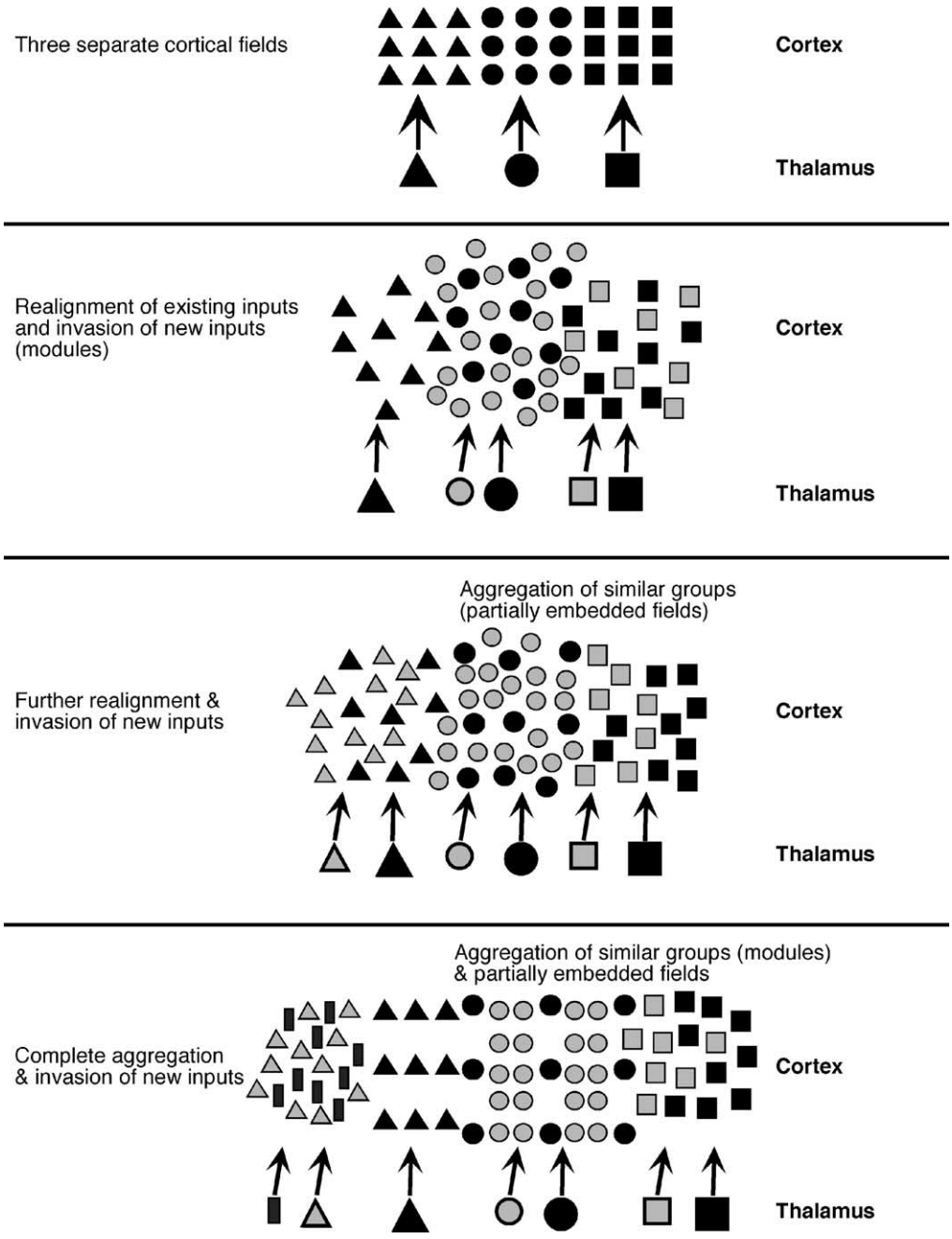

Fig. 11. Illustration of how cortical fields might be added or modified in evolution. (Top) A hypothetical stage of cortical field evolution where three separate fields (triangles, circles, and squares) have similar input from three distinct thalamic nuclei. In the row below, specific cortical regions are invaded by new thalamic afferents (smaller gray circles and squares), which causes a realignment of existing inputs. Next, new thalamic inputs continue to invade the cortex, similar groups of afferents aggregate, and existing inputs continue to realign. (Bottom) For some fields, there is complete segregation of related fields; while in other cortical fields there is partial segregation of afferent input, and the field remains embedded within another field (modified from Krubitzer, 1995). This process can occur in either direction. The invasion of new thalamic afferents or a new combination of thalamic afferents may be due to intrinsic changes in the cortex, discorrelation of activity in the thalamus, or modifications of peripheral apparatus. This illustration depicts the influence of thalamic input on cortical organization, but does not take into account the influence of cortico-cortical and callosal connections on this process.

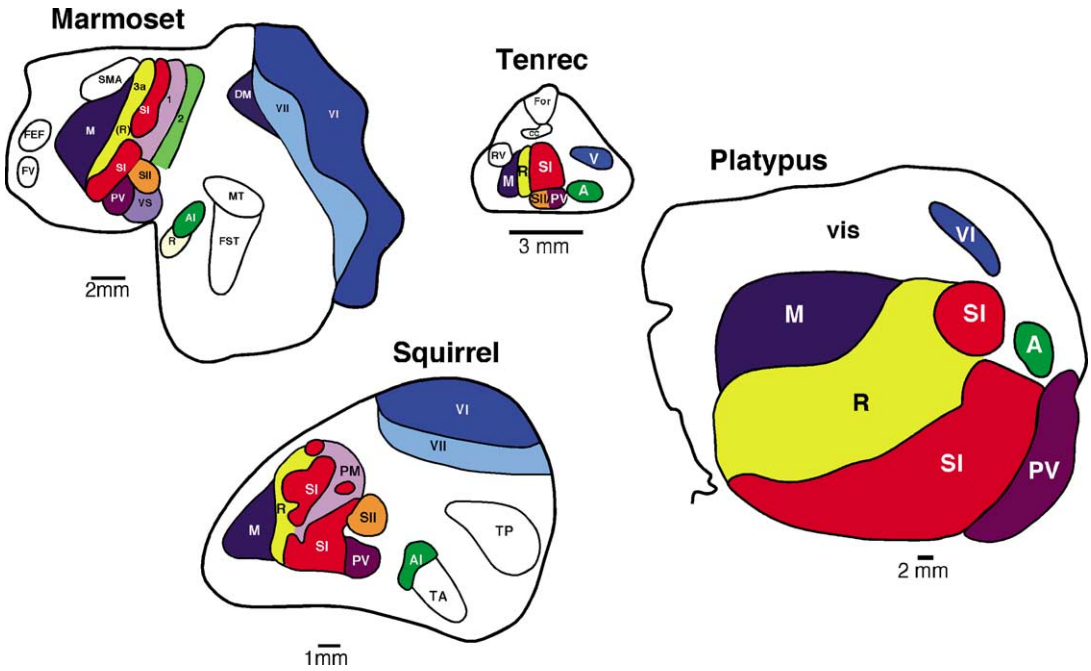

Fig. 12. Variations in cortical field organization of different mammals with vastly different lifestyles. In all mammals observed, there are cortical fields that are common (e.g. SI, VI, AI, SII, PV, and M), and patterns of callosal and subcortical connections are fairly constant across different lineages, despite differences in size, shape and geographic location of different fields. However, there are large shifts in the geographic location of homologous fields as well as changes in their size and shape. Rostral is to the left, medial is up.

and 12). Second, modularity, which we define as small architectonic (or histochemical), neuroanatomical and physiological territories either fully embedded or partially embedded within cortical fields, was observed across species in all sensory systems (see Krubitzer, 1995).

We reasoned that if our hypothesis that cortical fields are specified predominantly by thalamic input was correct, then manipulating the size of the cortical sheet (e.g. making it significantly smaller) prior to the arrival of thalamic input would result in a shift of thalamocortical afferents as well as other cortical connections on the reduced sheet. To test this theory we ablated up to two-thirds of the caudal portion of the cortical sheet very early in development (Huffman et al., 1999), well before the arrival of thalamocortical afferents (Molnár et al., 1998). Our prediction was that with a reduced cortical sheet, cortical fields would shift rostrally, and individual fields would be compressed on the remaining cortical sheet. This turned out to be the case (Fig. 13a). An unexpected finding was that a reduction in the size of the cortical sheet very early in development induced a corresponding reduction in the thalamus and midbrain. These structures were normally organized, but nuclei and layers were compressed (Fig. 13b and c). These changes were consistent with the types of changes observed in other mammals with a relatively smaller cortical sheet.

Our initial proposition of cortical field evolution, which we believed these studies would help validate, skirted the issue of the mechanisms by which cortical field additions or contractions might occur. Further, in its original formulation, our theory was based on several assumptions, one of which was incorrect. Most notably, this theory assumed that thalamocortical afferents play a dominant role in cortical field addition and modification, that the periphery and associated activity are critical to the formation and evolution of cortical fields, and that cortex is initially homogeneous.

This latter assumption was incorrect and has since been reconfigured in light of molecular data demonstrating that the developing neocortex is not homogeneous. As discussed in Section 3, a number of signaling centers have been identified, and genes are expressed preferentially in different regions of the cortex prior to the arrival of thalamocortical afferents and even in the absence of such inputs (Miyashita-Lin et al., 1999; Nakagawa et al., 1999). Thus, thalamic input is not necessary for some aspects of cortical development. Even if our theory regarding cortical field addition is accurate, the specific mechanisms involved in cortical field addition and modification are unknown. Based on the data outlined in this review, several scenarios are plausible. First, there may be some change to the cortex itself, such as a genetically mediated expansion of the cortical sheet (see Section 2). An expansion of the developing neocortex may be sufficient to induce new patterns of thalamocortical and corticocortical connections to form. Another possibility is that there is a change in the dorsal thalamus, possibly induced by alterations in the expression patterns of some ephrins and their Eph receptors that are involved axon guidance and tissue border formation during early stages of thalamic development (Feldheim et al., 1998; Frisen et al., 1998; Kullander

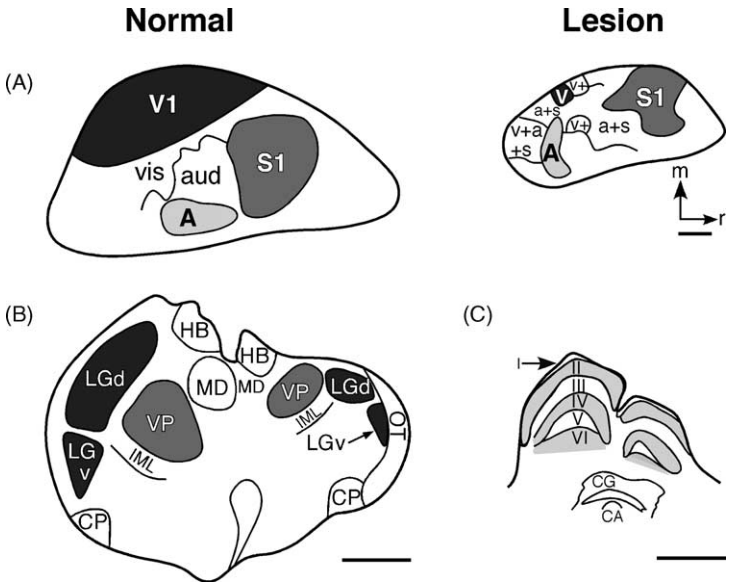

Fig. 13. The effects of cortical reduction early in development in the short-tailed opossum. In part A, the cortical fields of a normal animal were identified electrophysiologically and myeloarchitectonically. In animals that underwent unilateral lesions to the caudal 2/3 of the neocortex prior to the formation of thalamic afferents (B), cortical field organization was extensively altered onto the reduced cortical sheet. There were also changes observed subcortically; the dorsal thalamus (B) and superior colliculus (SC) (C) were significantly smaller on the side of the brain ipsilateral to the lesion. In the dorsal thalamus, the size of some of the individual nuclei (including the LGd and VP) were substantially reduced and shifted dorsally. In the SC, the layers appeared to be compressed (data used to construct this figure is from Huffman et al., 1999). CA, cerebral aqueduct, CG, central gray, CP, cerebral peduncle, HB, habenula, LGd, dorsal division of the lateral geniculate nucleus, LGv, ventral lateral geniculate nucleus, MD, mediodorsal nucleus, OT, optic tract, VP, ventroposterior nucleus. Dorsal is up, and in A, rostral is to the right. Scale bar = 1 mm.

and Klein, 2002; Lyckman et al., 2001; Sestan et al., 2001; Wilkinson, 2001). This may induce a discorrelation between different groups of thalamocortical afferents, which in turn results in fractured patterns of input or modules in the cortex. Finally, there may be a change in peripheral morphology, such as alterations in the size and shape of an appendage, or the number, type and density of sensory receptors. It should be noted that any or all of these are viable candidates for inducing the types of shifts in connectivity that we proposed earlier in this section. Indeed, it seems likely that there is more than one way to developmentally alter the cortical phenotype in evolution. The types of central and peripheral changes described above may occur either singly or in some combination but are unlikely to occur simultaneously.

6. Testing theories of cortical domain specification: studies of bilaterally enucleated opossums

We can test the extent to which peripheral input and associated activity play a role in specifying cortical domains by making changes to the peripheral receptors, similar to the types of changes that occur naturally in evolution. As noted above, 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 activity 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 results 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 (Kahn and Krubitzer, 2002; Dunn et al., 2001). Electrophysiological recordings in these enucleated opossums after they reached adulthood revealed that “visual cortex” was substantially reduced (Fig. 14). Further, cortical regions normally involved with visual processing, including area 17 (V1 in normal animals), had been captured by auditory and somatosensory inputs, and contained neurons that responded to a different sensory modality compared to normal animals. Thus, there were dramatic shifts in cortical domain territories, as large as, or larger than, those produced by genetically modifying intrinsic signaling centers (see Section 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 brains of bilaterally enucleated animals using neuroanatomical tracing techniques indicated that cortico-cortical and thalamocortical connections of area 17 were largely preserved. In addition, gross positional organization in terms of mediolateral and rostrocaudal organization of the cortex was maintained. Finally, although area 17 appeared to be substantially reduced in size, its cortical architecture was similar

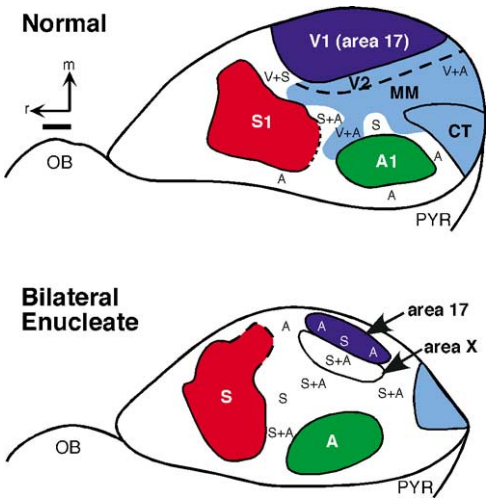

Fig. 14. Illustration of a dorsal view of the neocortex of a normal (top) and bilaterally enucleated (bottom) adult short-tailed opossum. Bilateral enucleations were performed early in development, prior to the formation of the retino-geniculo-cortical pathway. Despite the absence visual of input from 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, suggesting some genetic component to cortical organization. However, 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 domain specificity are mediated by activity from peripheral receptors. These data are consistent with the nervous system organization of animals in which input from specific sensory systems are naturally reduced or eliminated, such as the blind mole rat or anophthalmic (eyeless) mice. Dark blue in top (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 (data used to construct this figure is taken from the work of Kahn and Krubitzer, 2002).

to normal animals. These results indicate that peripheral input plays a significant role in assigning cortical domains and that cortical areas are susceptible to dramatic changes in organization and size, while position, shape, architecture and some aspects of connectivity of at least primary fields are likely to be mediated by intrinsic genetic signals (see Kahn and Krubitzer, 2002, for review of related literature).

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 micro-ophthalmic and covered with skin. In these animals, as in the bilateral enucleated animals, a geniculo-cortical pathway is still present (Cooper et al., 1993; Bronchti et al., 1991), and neurons in the LGN and "visual" cortex respond to auditory stimulation (Bronchti et al., 1989). In anophthalmic (eyeless) mice, large changes in subcortical connections are apparent in that the LGN receives auditory and somatosensory inputs (Asanuma and Stanfield, 1990; Bronchti et al., 2000).

While some of the results from comparative studies of the neocortex and data in bilateral enucleated and naturally a-visual animals seem at variance with studies of gene expression described earlier, there are several important observations that are consistent between data sets. First, the global geographic relationships of primary sensory fields are maintained. That is, the primary visual area (V1) is located caudally, while the primary somatosensory (S1) area is located more rostrally, and the primary auditory area is located more laterally, between V1 and S1. This geographic relationship is maintained even in brains where the size of the primary sensory field is reduced either naturally or experimentally as a result of loss of peripheral inputs or a reduction in activity from peripheral receptors. Second, some aspects of connectivity are maintained even in the absence of use or loss of a sensory system. For example, blind mole rats still maintain a retino-geniculate-cortical pathway. Further, in these animals, as well as in anophthalmic mice and bilateral enucleates, connections between the dorsal division of the lateral geniculate nucleus (LGNd) and V1 are still maintained, although reduced. 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, fits well with 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 for the relative location of cortical fields with respect to other cortical fields. These types of signaling centers arose early in evolution, and certainly constrain the evolution of the mammalian neocortex since their action directs developmental cascades in which any event is contingent upon a prior event.

Given this, it is not surprising that observations across mammalian species indicate that the position and general thalamocortical innervation patterns are invariant. Further, particular proteins (e.g. FGF8) may impart very specific features of cortical area identity, such as architecture, and also constrain evolution of the neocortex. This is consistent with observations that some aspects of architecture, connectivity, and geographic position of particular areas are invariant across mammals, regardless of the animal's sensory specializations or use of a sensory system.

There are still several outstanding questions generated by comparative, molecular and developmental manipulation studies that need to be addressed. For one, 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 post-synaptic 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? As discussed below, accumulating evidence indicates that several features of synaptic architecture and function may indeed be context dependent, and thus highly variable across species.

7. Potential mechanism for activity-dependent changes to nervous systems

The data presented throughout this review indicate that the neocortical organization of any particular species is highly adapted to the environment in which it resides. While the exact phenotype may not be selected for, the ability of neural tissue to be plastic or undergo functional and morphological changes may be selected for. Thus, plasticity appears to be built into evolving mammalian nervous systems and must be manifested at intracellular and molecular levels of organization. While the molecular basis for adult plasticity in terms of long-term potentiation has been well documented (Woody et al., 1978; Walters and Byrne, 1983; Manilow et al., 1988; Bonhoeffer et al., 1989; Bekkers and Stevens, 1990; Zalutsky and Nicholl, 1990; Castillo et al., 1994), there is less known about the molecular mechanisms that allow for large scale systems changes in the developing nervous system. However, 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 post-synaptic element (see McAllister et al., 1995, 1999; McAllister, 2001, for reviews), and they trigger local protein synthesis at the dendrite (Aakalu et al., 2001; see Zhang and Poo, 2001; for reviews).

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, which phosphorylates a transcription factor, CRE-binding protein or CREB (see Finkbeiner and Greenberg, 1998; West et al., 2001, for reviews). Phosphoralization of CREB can bind to the regulatory region of a gene and induce transcription. The transcript, mRNA, serves 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, NGF, NT3, and NT4/5, have been demonstrated to 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 (see Levi-Montalcini, 1987; Miller and Kaplan, 2001, for reviews) and stimulate cell migration of neurons out of proliferative zones (Borghesani et al., 2002). Neurotrophins also influence the growth of axons and dendrites, exerting very specific effects on neuronal differentiation. For example, BDNF increases the extent of axon outgrowth in cultured cerebellar granule cells (Segal et al., 1995) and enhances dendritic outgrowth of immature cerebellar Purkinje cells (Carter et al., 2002). Additionally, neurotrophin 3 (NT3) alters the pattern of neurite outgrowth of developing cerebellar granule cells and thus may be involved in fasciculation or branching of cell fibers (Segal et al., 1995). Some neurotrophins appear to act at very specific sites in the neuron. Recent studies in hippocampal slices of adult animals indicate that BDNF stimulates protein synthesis in dendrites (Aakalu et al., 2001). The local production of particular proteins has been proposed to be involved in determining dendritic and spine morphology as well as synaptic function.

Another way in which experience can alter neuronal structure and function during development is through the NMDA subtype of glutamate receptor (NMDAR). The NMDAR plays a critical role in experience-dependent reorganization and refinement of connections in the immature brain (see Constantine-Paton et al., 1990; Cramer and Sur, 1995; Katz and Shatz, 1996; Constantine-Paton and Cline, 1998, for reviews). In the developing CNS, synaptic strength can be regulated by activation of the post-synaptic NMDAR, either by non-NMDA AMPA currents or by structural alterations to NMDA receptor subunits. NMDA receptor channels are composed of two subtypes, the NR1 and NR2, and the NR1 subtype combines with NR2A-D receptor subunits. Each of these subtypes has distinct functional properties (see Fox et al., 1999, for review). In early development, NR2A-D sub-

units are expressed throughout the neonatal cortex, with the NR2B subunit most highly expressed in the neonatal forebrain. As the cortex matures, these are replaced by NR2A receptors, which have the fastest decay kinetics (Monyer et al., 1994; Sheng et al., 1994; Flint et al., 1997). It has been proposed that the conformational changes in the NMDAR subunit composition, and the associated functional changes can be triggered by sensory input (Fox et al., 1999). Direct evidence for this proposition comes from experiments in the rat visual system. Dark-reared rats showed a decrease in NR2A expression in the visual cortex compared to normal animals (Quinlan et al., 1999). Upon exposure to light, this effect in dark-reared animals was reversed. The authors proposed that the changes in NR2A subunit expression and cell kinetics could mediate plasticity by strengthening weak synapses by upregulating NR2A subunits. Conversely, these may be downregulated during development as connections become strengthened, or potentiated. These results support the view that sensory input can regulate NMDA receptor subunit expression, and they provide a model by which experience-dependent plasticity may occur in the developing cortex. As in the previous example of neurotrophins, the intracellular mechanisms that allow activity to regulate receptor subunit changes may be genetically mediated, but the ultimate phenotype is regulated by sensory input.

This entire processes or exact series of events by which activity promotes structural and functional changes in a neuron is far more intricate than we have described, and much of the basis 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 NMDA-mediated alterations to synapses or by 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 generate structural and functional modifications throughout the cell. Thus, there can be 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.

8. 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 dorsoventral coordinate system of the neocortex. Intrinsic signaling genes and molecules such as *Wnt*, *Shh*, *Fgf8* and BMP may set-up a combinatorial coordinate system that serves as a scaffold for incoming thalamocortical axons. Changes in peripheral morphology that ultimately control the types of patterned activity that the CNS can access are likely to be under genetic control. For example, 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 architecture of the pre- and post-synaptic elements and the intracellular machinery that allows for activity-dependent changes in the developing nervous system may 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 than 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 a specific sensory apparatus, which in turn influences the temporal and spatial patterns of neural firing at all levels of the central nervous system. Activity can indirectly alter the temporal and spatial patterns of gene expression via neurotrophins, for example, 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. Therefore, sensory domain enlargements, cortical field size, cortical magnification within cortical fields, and the connectivity of both cortical and subcortical structures are, to a large extent, dependent on neural activity from peripheral receptor arrays and activity from circuits within the CNS. 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 such as language and skill acquisition, and social and cultural learning that are not strictly tied to a particular sensory receptor array or associated behavior. These types of active influences can fundamentally alter the phenotype by changing patterns of synaptic efficacy and connectivity along the entire neuroaxis, and ultimately the organization and function of the neocortex. We hypothesize that much of the human neocortex that does not include the primary and second sensory and motor areas is occupied by cortex that is largely shaped by such active influences and 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 different for different species. While most of this review has focused on studies of non-human mammals, clearly the human brain is enslaved by the same genetic constraints and shaped by the same activity-dependent mechanisms as the brains of other mammals. Consequently, its future evolution will follow predictable paths. Although the precise specializations that may emerge cannot be known, the types of change possible and the mechanisms by which changes will be achieved are guided by the same mechanisms that sculpt the brains of other mammals.

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