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Evolution and Ontogenetic Development of Cortical Structures

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Abstract

The cerebral cortex controls our unique higher cognitive abilities. Modifications to gene expression, progenitor behavior, cell lineage, and neural circuitry have accompanied evolution of the cerebral cortex. This chapter considers the progress made over the past thirty years in defining potential mechanisms that contribute to cortical development and evolution. It discusses the value of model systems for understanding elaboration of cortical organization in humans, with an emphasis on recent technical and conceptual advances. It then examines our current understanding of the molecular and cellular basis for cortical development and evolution; discusses how neuronal fates are specified and organized in lamina, columns, and areas; and revisits the radial unit and protomap hypotheses. Finally, it considers our current understanding of the development, stability, and plasticity of cortical circuitry. Throughout, it highlights the profound impact that new technological advances have made at the molecular and cellular level, and how this has changed our understanding of cortical development and evolution. The authors conclude by identifying critical and tractable research directions to address gaps in our understanding of cortical development and evolution.

Group photos (top left to bottom right) Debra Silver, Pasko Rakic, Christopher Walsh, Takao Hensch, Elizabeth Grove, Michael Stryker, Tarik Haydar, John Rubenstein, Zoltán Molnár, Mriganka Sur, Nenad Sestan, Maria Antonietta Tosches, Wieland Huttner, Debra Silver, Mriganka Sur, Maria Antonietta Tosches, John Rubenstein, Pasko Rakic, Zoltán Molnár, Nenad Sestan, Michael Stryker

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Introduction

The cerebral cortex is generally considered the biological substrate of our unique cognitive abilities, including memory, complex reasoning, and advanced language. Over the course of evolution, the neocortex has undergone a disproportionate number of changes relative to other brain regions, suggesting that anatomical, cellular, and molecular modifications of the cerebral cortex may have gone hand in hand with human cognition. This great advance in computing power, however, has come at a price, as complex cognitive and psychiatric disorders also appear to be largely unique to humans. The study of cortical evolution is thus crucial as it can inform fundamental principles governing how the brain works as well as elucidate mechanisms relevant to human health.

The cerebral cortex is derived from the dorsal telencephalon or pallium, which has been traditionally divided into medial, dorsal, and lateroventral areas. A distinct feature of mammals is their six-layered cortex, termed "neocortex," considered to be a substrate for our highest cognitive functions, including abstract thinking and language. The medial pallium, archicortex, or hippocampus consists of three layers and is involved in short-term memory and cognitive spatial mapping functions. Some lateroventral pallium areas also contain three layers that receive inputs directly from the olfactory system. The neocortex is organized in the radial dimension into neuronal layers that are further divided into sublayers. Historically, it is well established that the neocortex is tangentially composed of functional areas that control sensory, motor, and cognitive capacities (Brodmann 1909).

Neocortical anatomical features correlate with complex behavior, such as language and an ability to develop and use tools and technology, which distinguishes humans from other species (Geschwind and Rakic 2013; Molnár and Pollen 2013). Relative to nonhuman primates, humans also possess a higher brain to body ratio, more neurons, greater degree of brain lateralization (Lewitus et al. 2014; Sousa et al. 2017a), and a complex pattern of gyri and sulci (Borrell and Götz 2014). In addition, these cortical features are derived in humans during a longer gestational period and an extended adolescence up to the third decade of life (Petanjek et al. 2011).

As we reflected on the question of what is uniquely human, it became apparent that our understanding of human-specific cortical features still remains inadequate. Yet, when compared to the issues discussed at the Dahlem Workshop on neocortex (Rakic and Singer 1988), research over the past thirty years has given rise to enormous progress in our understanding of cortical development and evolution.

Technological Breakthroughs Advancing Our Understanding of Cortical Development and Evolution

Conceptual advances in cortical development and evolution have coincided with major technological breakthroughs. The first advance, in molecular

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neuroscience, now enables us to pinpoint genomic, epigenomic, transcriptomic, and proteomic features of development and evolution. Recent implementation of single-cell transcriptomics (scSEQ) has led to comprehensive classification of cortical cell types, progenitor states, and developmental trajectories across species (Camp et al. 2015; Macosko et al. 2015; Bakken et al. 2016; Tasic et al. 2016; Nowakowski et al. 2017; Mayer et al. 2018; Mi et al. 2018; Tosches et al. 2018). A second advance is our ability to manipulate genes, cells, and circuits using various approaches: genomic engineering, viral transduction, electroporation, optogenetics, RNAi, and cell transplantation. More recently, CRISPR/Cas9 technology allows for constitutive and conditional mutagenesis, as well as manipulation of promoters and enhancers to control gene expression precisely (Cong et al. 2013; Wang et al. 2013; Kalebic et al. 2016; Tsunekawa et al. 2016; Yang and West 2016). Major advances in microscopy have further enabled real-time visualization of genetic manipulations. Further, progress in cortical development and evolution has been propelled by new and accessible advances in model systems, including macaques, marmosets, and ferrets, which have a more complex cortical organization than commonly studied rodents (Homman-Ludiye and Bourne 2017; Johnson et al. 2018). Moreover, we now possess the ability to generate brain organoids *in vitro* for diverse species, including the great apes and notably humans (Lancaster et al. 2013; Camp et al. 2015; Mariani et al. 2015; Mora-Bermudez et al. 2016; Otani et al. 2016; Giandomenico and Lancaster 2017). These technological advances have laid the groundwork for thirty more years of deciphering even deeper mechanisms of cortex development, evolution, and human cortical disorders.

Theories Underlying Human Cortical Evolution

A number of theories have been put forth to explain human cortical evolution (Geschwind and Rakic 2013; Molnár and Pollen 2013). One theory posits that the duration of gestation and infancy can explain cortical differences, due to prolonged neurogenesis and a differential impact of experience. Humans have by far the longest neurogenic period among primates (Petanjek et al. 2011). Mathematical modeling of cortical progenitor lineages suggests that a longer neurogenic period in humans is sufficient to explain the increased cortical neuron number compared to other great apes (Lewitus et al. 2014; Picco et al. 2018). Consistent with this, human babies born prematurely, with a reduced gestation period, are at elevated risk for neurological deficits, including learning and communication disabilities. However, in comparison, synaptogenesis proceeds intrinsically according to the day of conception rather than the birth date (Bourgeois et al. 1989).

A second theory posits that human-specific traits, such as higher cognition and abstract thinking, are associated with a disproportionately large cerebral cortex. It is notable that some cetaceans, such as dolphins, also have extra large and complex neocortices and are considered highly intelligent (Sousa et al.

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2017a). Indeed, the importance of brain size for human cognition is not easily reconciled with disorders of microcephaly in which patients have disproportionately smaller brains, yet retain human-like social behavior and in some cases language (Sousa et al. 2017a). Thus, it may be more relevant to consider the extent to which the prefrontal cortex (PFC) is enlarged. Indeed, the PFC, together with some association areas of the parietotemporal lobes, is the most expanded brain structure in primate evolution (Goldman-Rakic 1987), but its relative size in humans is still a matter of debate (Wise 2008; Elston et al. 2011; Gabi et al. 2016). Thus, beyond cortical size, several additional factors, including the pattern of connectivity and large subcortical white matter, have been posited to shape human brain evolution (Rash et al. 2019):

1. Quantification of neuronal nuclei has shown that relative to chimpanzees and rodents, human brains have more cortical neurons (Herculano-Houzel et al. 2007; Gabi et al. 2016). The embryonic telencephalic vesicles of human and nonhuman primates, however, are disproportionately enlarged relative to rodents, even before the first neurons have been generated (Bystron et al. 2008). This indicates that cortical expansion may initiate in the neuroepithelium (Rakic 2009).
2. Beyond neuronal number, neuronal diversity and morphological differences distinguish humans and primates. For example, a subtype of enlarged pyramidal neurons, von Economo cells, are enriched in humans and other great apes, and are hypothesized to promote rapid communication (Nimchinsky et al. 1999). In humans, some pyramidal neurons have been described with extensive branching, which could augment neuronal activity (DeFelipe 2011). Rare subpallial-derived interneurons expressing dopamine biosynthesis genes and capable of producing dopamine *in vitro* are also enriched in the human striatum, yet absent in the nonhuman African ape neocortex (Sousa et al. 2017a). In addition to structural differences, homologous human neuronal cell types have undergone molecular changes that may have changed their physiological properties (Sousa et al. 2017a). Also, the number and size of astrocytes and oligodendrocytes are greater in primates compared to rodents (Oberheim et al. 2009).
3. Primates have thicker and more complex supragranular layers, thought to promote increased connections between cortical regions (Marin-Padilla 2014). Further, humans have robust white matter connecting language regions of the perisylvian cortex, which is smaller or absent in nonhuman primates (Rilling et al. 2008). Likewise, differences in the number and composition of functional areas and their asymmetry may also influence cortical capacity, particularly with regard to language skills (Chance 2014).
4. Finally, noncortical structures, such as the cerebellum, are greatly expanded in humans, with a disproportionate increase in granule cell

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number (Weaver 2005; Ito 2008; Barton 2012; Barton and Venditti 2014; Sokolov et al. 2017). Further, higher-order nuclei within the thalamus are massively enlarged in primates and may mediate cortico-cortical interactions (Sherman and Guillery 2011).

How Model Systems Have Contributed to Understanding Cortical Development and Species Differences

Mouse Models

Different animal model systems afford distinct advantages to study cortical development and evolution. Mice have been a historical model of choice, in large part due to their genetic tractability and the fact that they share key features with humans (Clowry et al. 2010). During development, both species undergo similar cellular processes with comparable temporal progression. They also have homologous cell types and, in many cases, utilize identical molecular programs.

The mouse cortex, however, is the product of its own unique evolutionary forces that resulted in a small body and lissencephalic brain, a nonlaminated lateral geniculate nucleus, and lateral-set eyes with minimal binocular vision. The PFC of mice is limited in size, containing medial, orbitofrontal, and cingulate areas but probably no equivalent of the primate dorsolateral PFC (Preuss 1995). Beyond cortical size and a limited number and diversity of higher-order cortical areas, mouse brains are lissencephalic. In addition, compared to primates which contain >40% white matter, mice only have about 5% (Herculano-Houzel et al. 2010), perhaps due to their smaller brain size and the relatively shorter distances that neuronal signals traverse.

Beyond these structural differences, common inbred lab mice are overfed and understimulated, factors which could affect the precision of cortical responses. Thus, generation of knockout strains may select for the fittest animals and mask biological insights, failing to model disease phenotypes. For example, *ASPM* mutations are associated with severe human microcephaly (Jamuar and Walsh 2015), yet *Aspm* knockout in mice results in mild microcephaly (Pulvers et al. 2010). By contrast, the same genetic perturbation in gyrencephalic ferrets causes profound microcephaly and preferential loss of frontal areas, as seen in humans (Johnson et al. 2018; see also Coulter and Walsh, this volume). Phenotypic discrepancies may be amplified when modeling complex human psychiatric disease. Yet, compared to other model species, such as great apes, which are subject to ethical and legal hurdles, mice are superior for studying behavior. Thus, we emphasize that for elucidating basic principles of development and circuitry, the mouse remains invaluable (Goffinet and Rakic 2000).

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New Primate Models: *In Vivo*, *Ex Vivo*, and *In Vitro* Toolsets

Much of our classical understanding of cortical function and development has employed nonhuman primates, particularly the Old World primate, the rhesus macaque (Geschwind and Rakic 2013). Relative to thirty years ago, our knowledge base today has been driven by a deeper examination of a range of primate models. Remarkable progress has also been made through direct studies of human fetal and adult samples obtained via surgery, postmortem, or tissue banks. While *ex vivo* human brain slices are invaluable for investigating cellular and molecular aspects of development, they are less amenable to longer-term studies and lack important extrinsic cues. Moreover, access to human tissue remains a major hurdle. Thus, recent efforts toward developing additional primate models, such as marmosets (Homman-Ludiye and Bourne 2017), offer complementary approaches for future studies.

The ability to generate induced pluripotent stem cells (iPSCs) has transformed traditional primate model approaches, enabling investigation of evolutionary differences within species-specific contexts. Several groups have established iPSC lines from human and nonhuman primate somatic cells, which can be readily directed toward a neural fate to model early developmental stages (Eiraku et al. 2008; Lancaster et al. 2013; Marchetto et al. 2013; Gallego Romero et al. 2015; Heide et al. 2018). Three-dimensional organoids have revealed new evolutionary differences between humans and nonhuman primates (Camp et al. 2015; Mora-Bermudez et al. 2016; Otani et al. 2016). For example, such comparisons led to the identification of the first differences concerning cortical progenitor cell behavior between human and other great apes—the specific lengthening of metaphase during human apical progenitors mitosis (Mora-Bermudez et al. 2016). However, there remain limitations with current protocols for generating organoids, including lack of vasculature for long-term culture, lack of basement membrane and cerebral spinal fluid, as well as lack of standardization across labs. Given the current pace of research, continued optimization of organoid protocols will likely overcome many of these technical hurdles.

Which Molecular and Cellular Processes Shape Development and Are Evolutionarily Divergent?

Genetic Basis for Cortical Development and Evolution

With the complete sequencing of the genomes of humans and most major mammalian species comes the promise of discovering specific molecular changes that make each species unique. Delivering on this promise, however, remains a complex, multifaceted challenge. Genome-wide approaches have collectively uncovered human-specific features including structural variations

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(e.g., chromosomal deletions and duplications) and point mutations in coding and noncoding regulatory regions (Lui et al. 2011; Borrell and Reillo 2012; Geschwind and Rakic 2013; Dennis et al. 2017; Florio et al. 2017; Sousa et al. 2017a). Many of these have been empirically demonstrated to affect protein structure, gene function, and/or expression as well as to influence diverse aspects of the neocortex (see Lorente-Galdos et al., this volume).

Changes in gene regulatory regions are strongly linked to brain evolution (King and Wilson 1975). For example, the vast majority of 510 annotated human-specific deletions reside within noncoding regions (McLean et al. 2011). Evolutionary changes to noncoding regulatory elements are frequently located near genes implicated in neural development, whereas coding changes do not show the same bias (Haygood et al. 2010). Over 3,000 human accelerated regions (HARs), sequences that have undergone rapid positive selection in humans, reside mostly in regulatory elements (Pollard et al. 2006a, b; Lindblad-Toh et al. 2011; Capra et al. 2013). To date, HARs have been implicated in diverse aspects of cortical function, ranging from progenitor proliferation to control of neuronal spine density (Capra et al. 2013; Boyd et al. 2015; Reilly et al. 2015; Doan et al. 2016). While HARs are poised to fine-tune human cortical development, annotated functions for the vast majority of HARs and other human-specific coding and noncoding elements are lacking.

Approximately one-third of mouse and human enhancers are predicted to diverge between species (Nord et al. 2013; Reilly et al. 2015; de la Torre-Ubieta et al. 2018). Indeed, epigenetic profiling for histone acetylation and methylation marks in humans, macaques, and mouse neocortices reveal promoters and enhancers that have gained human-specific activity (Silbereis et al. 2016; Mitchell and Silver 2018). Importantly, more than 4,600 human telencephalic enhancers have been identified, but only a subset have demonstrated activity (Visel et al. 2013).

In comparison, transcriptional circuits have been well defined in the developing mouse neocortex. These circuits highlight both individual and redundant transcriptional regulation. For example, the transcription factors FEZF2, SOX5, SATB2, and TBR1 control specification of different subtypes of excitatory projection neurons, whereas multiple Dlx factors are present during development of perhaps all forebrain GABAergic neurons, projection and local circuits (Leone et al. 2008; Kwan et al. 2012b; Greig et al. 2013; Hu et al. 2017). However, while the human genome is estimated to harbor 400,000 enhancers and 70,000 promoters (ENCODE Project Consortium 2012), it remains largely unclear how transcriptional networks control 20,000 protein-coding genes, including human-specific cortical development (Nord et al. 2015; Emera et al. 2016).

Transcriptome comparisons of developing and early postnatal human, chimpanzee, and macaque brains indicate prevalent, global differences in gene expression and splicing among primates (Johnson et al. 2009; Kang et al. 2011; Fietz et al. 2012; Konopka et al. 2012; Lui et al. 2014; Miller et al. 2014; Pletikos et al. 2014; Sousa et al. 2017a). Sequencing of isolated cells

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has further reinforced these differences (Pollen et al. 2014, 2015; Florio et al. 2015; Johnson et al. 2015). For example, a recent *in silico* screen of five transcriptome data sets led to the identification of 15 human-specific genes with preferential expression in progenitors (Florio et al. 2018). One of these human-specific genes, *ARHGAP11B*, has been shown to amplify basal progenitors in the mouse neocortex and is implicated in cortical folding and expansion (Florio et al. 2015, 2016). Thirty-five human genes with progenitor-enriched expression have primate-specific orthologs, constituting a resource of candidates which may exert key roles in neocortical development during human evolution (Florio et al. 2018). Several prominent signaling pathways (STAT, mTOR, Notch, WNT, FGF, SHH) have also been implicated in human-enriched progenitors (Lui et al. 2014; Pollen et al. 2014, 2015; Nowakowski et al. 2017). Taken together, these discoveries indicate molecular support for species-specific patterns of gene expression and give us the ability to interrogate functionally a finite number of genes. Future progress will rely on our ability to exploit this knowledge base to understand cell fate specification, heterogeneity, and circuitry across development and evolution.

Neural Progenitors: Building Blocks for the Cortex and Underlying Cortical Evolution

Initial stages of corticogenesis involve early patterning of the neural plate and early neurula. As of 25 days postcoital, species differences are already visible. At this stage, relative to the posterior neural plate, the anterior neural plate is larger in humans than in mice (Bystron et al. 2008). This suggests that the human neural plate, the anterior neural ridge, the rostral patterning center, or the prechordal plate may secrete factors to control specification of anterior structures.

The founder population for the neocortex is neuroepithelial progenitor cells (NPCs/NECs), which are arguably the most impactful progenitor for brain expansion (see Figures 5.1a and 5.2). They are critical for amplifying the precursor pool via symmetric proliferative divisions near the ventricular cavity, termed the ventricular zone (VZ) (Rakic 1972). The proliferative pool is further augmented by the emergence of the subventricular zone (SVZ), which is particularly enlarged in primates, including humans, where it was initially identified and named (Bystron et al. 2008). Empirical support for NPC function was shown by increasing proliferation or decreasing apoptosis of founder cells in mice, which causes enlargement of the cortical surface and convolutions (Kuida et al. 1998; Chenn and Walsh 2002). Genetic manipulation that increases founder cells in mice also can enlarge frontal brain regions (Assimacopoulos et al. 2012).

The radial unit model, put forth more than thirty years ago (Rakic 1988b), proposes that increasing the size and proliferative capacity of neural precursors close to the ventricular cavity helps explain the beginning of the

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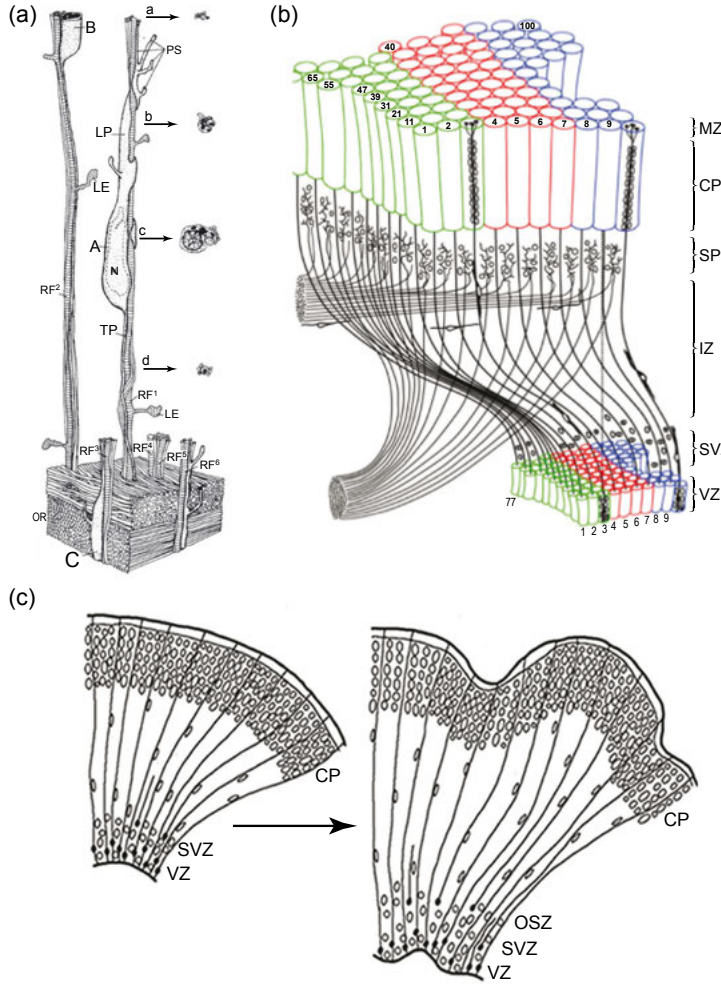

Figure 5.1 Interpretation of the radial unit hypothesis. (a) Three-dimensional reconstruction of migrating neurons, based on electron micrographs of semi-serial sections of the occipital lobe of the monkey fetus; reprinted with permission from Rakic (2003). (b) Representation of the radial unit hypothesis; reprinted with permission from Rakic (1988b). (c) Graphic explanation for cortical expansion and elaboration during evolution. An expanded cellular sheet due to increased proliferation or decreased cell death of radial units is associated with transformation from a lissencephalic (left) to gyrencephalic brain; reprinted with permission from Geschwind and Rakic (2013).

distinct cytoarchitecture and enlarged neocortical size of higher mammals (Figure 5.1b). A larger progenitor pool would ultimately generate more cortical neurons and a bigger brain (Bystron et al. 2008). The radial unit model is supported experimentally (Kuida et al. 1998; Chenn and Walsh 2002; Pattabiraman et al. 2014) and provides a basic cellular explanation for how

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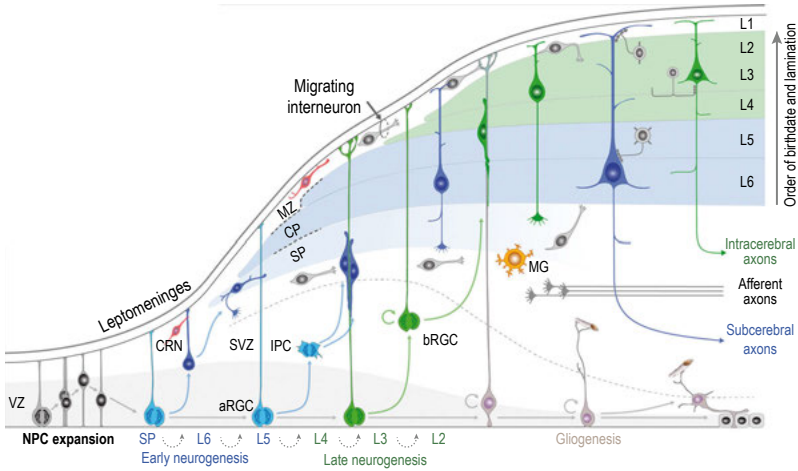

Figure 5.2 Illustration of the development of cerebral neocortex with diverse progenitors. Prior to the onset of neurogenesis, neuroepithelial progenitor cells (NPCs) in the ventricular zone (VZ) of the developing neocortex divide symmetrically to expand the progenitor pool. Later, NPCs transform into apical radial glia cells (aRGCs), which line the VZ; from there they extend a long radial process stretching to the basal surface. aRGCs asymmetrically divide to generate another aRGC and either a nascent projection neuron, basal intermediate progenitor cell (IPC), or basal radial glial cell (bRGC). Basal progenitors in the subventricular zone (SVZ) also generate neurons. The nascent projection neurons migrate radially from the VZ along the RGC basal process into the cortical plate (CP). The earliest born neurons migrate to form the preplate. Later-migrating neurons split the preplate into the marginal zone (MZ) and subplate (SP). The MZ also consists of Cajal-Retzius neurons (CRNs), which originate from multiple sites in the forebrain. As neurogenesis proceeds, diverse subtypes of neurons are generated through successive asymmetric divisions of RGCs. Early-born projection neurons settle in the deep layers (layers 5 and 6) and later-born projection neurons migrate past older neurons to form more superficial layers. Thus, radial neuronal migration in mammals occurs in an inside-first, outside-last manner. Mature subcerebral projection neurons extend axons to the striatum, thalamus, brainstem, and spinal cord, and mature upper layer projection neurons project axons within the cerebrum. In contrast, cortical interneurons originate in the subcortical forebrain and tangentially migrate in the MZ, intermediate zone, and SVZ. At the end of neurogenesis, the radial scaffold is dismantled and most of the RGCs become gliogenic, generating cortical and subependymal zone astrocytes and giving rise to a layer of ependymal cells.

the cerebral cortex expands in surface area as a sheet during development and evolution (Figure 5.1c). In light of this model, we consider the current knowledge base for cortical specification; for further details, see Tyler and Haydar (this volume).

After the neural plate closes, cortical neurons begin their genesis from populations of neural progenitors (Figure 5.2) (Lui et al. 2011; Taverna et al. 2014; Matsuzaki and Shitamukai 2015). NECs give rise to apical (ventricular) radial glial cells (aRGCs), which produce cortical neurons, largely via indirect divisions (Malatesta et al. 2000; Noctor et al. 2001; Tamamaki et al. 2001). After

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the last division, newborn neurons migrate through the expanding intermediate zone (IZ) to enter the cortical plate and settle in an inside-to-outside pattern (Angevine and Sidman 1961; Rakic 1974 reviewed in Bystron et al. 2008).

A few molecular markers, such as GFAP, faithfully distinguish the highly related aRGCs from NECs (Choi and Lapham 1978; Levitt and Rakic 1980). Both NECs and aRGCs exhibit epithelial features, apical-basal cell polarity, and contact the ventricular surface and the basal lamina. However, interkinetic nuclear migration is distinct between NECs and aRGCs (Taverna and Huttner 2010). At the neuroepithelial stage, there is essentially one zone of cells, and NEC nuclei migrate between the ventricular surface and the basal lamina, in concert with cell-cycle progression (Sidman and Rakic 1973). In contrast, at the aRGC stage, aRGCs span several zones with a basal process that emerges from the cell body in the VZ. Interkinetic nuclear migration of aRGCs remains confined to the VZ (Lui et al. 2011; Geschwind and Rakic 2013). Thus, the absence or presence of a basal process distinguishes NECs and aRGCs, respectively (Delaunay et al. 2016). Notably, the plasma membrane composition of the aRGC basal process is distinct from the aRGC apical process (Taverna et al. 2016).

An important concept in the cortical development and evolution field is the granular classification and diversification of progenitor cell types and, in particular, the enlarged pool of basal progenitors (Fietz et al. 2010; Hansen et al. 2010; Shitamukai et al. 2011; Betizeau et al. 2013; Pfeiffer et al. 2016). aRGCs generate basal progenitor populations composed of basal/outer radial glia cells (bRGCs/oRGs), basal intermediate progenitors (bIPCs), and aIPCs (Stancik et al. 2010). As suggested by the name, aRGCs divide in the VZ whereas basal progenitors divide in the SVZ (Bystron et al. 2008). aRGCs can also become detached from basal and apical surfaces as human cortical development progresses (Sidman and Rakic 1973; Smart et al. 2002; Lukaszewicz et al. 2005; Nowakowski et al. 2016). It has been suggested that human and rodent aRGCs may be structurally and genetically different (Rakic 2003).

Evolutionary differences suggest that bRGCs have a significant role in neuronal production in primates. Initially observed by Golgi staining of human and monkey embryos (Schmechel and Rakic 1979), bRGCs are characterized morphologically by a basal process and, in some cases, by a short apically directed process which does not reach the ventricular surface (Betizeau et al. 2013; Florio and Huttner 2014; Rash et al. 2019). Primates possess an expanded and elaborate SVZ composed of outer and inner SVZs (oSVZ and iSVZ, respectively) and containing about 50% bRGCs and 50% bIPCs (Smart et al. 2002). Gyrencephalic nonprimate mammals, such as sheep, ferrets, and cats, also tend to have an expanded SVZ with significantly more bRGCs than lissencephalic mammals. Nevertheless, both the lissencephalic primate marmoset and the gyrencephalic rodent agouti also possess abundant bRGCs (Garcia-Moreno et al. 2012). In contrast, mice contain few bRGCs, which show markedly reduced proliferative capacity compared to human bRGCs (Wang et al. 2011a;

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Wilsch-Bräuninger et al. 2016). Instead, in mice, neurons are produced primarily from aRGCs and bIPCs (Vasistha et al. 2015). Indeed, analysis of basal progenitors is an area where there was consensus among us that mouse is a poor model (Liu et al. 2014).

Importantly, although basal progenitors are considered the predominant neurogenic cell, the proportion of neurons born from bRGCs or bIPCs in humans is unknown. *In vitro* studies demonstrate that human bRGCs undergo expansive symmetric proliferative divisions before producing neurons destined for layers II/III as well as the majority of astrocytes and oligodendrocytes (Pollen et al. 2015). In the nonhuman primate macaque, bRGCs initially produce neurons which increase the thickness and complexity of superficial layers. However, after cortical neurogenesis stops, around E100, bRGCs produce astrocytes and oligodendrocytes (Rash et al. 2019).

Above the radial glia, at the pia mater, a milieu of basement membrane and meninges reside. ECM-mediated activation of integrins promotes basal progenitor proliferation (Fietz et al. 2010; Stenzel et al. 2014). In fact, some differences in proliferative potential between mouse and human cortical progenitors may derive from differential expression of genes encoding components of the extracellular matrix (ECM) (Fietz et al. 2012). Both mouse and human aRGCs, which are endowed with proliferative potential, show substantial endogenous expression of ECM genes. In contrast, highly proliferative human basal progenitors, but not mouse bIPCs, sustain expression of ECM genes.

Progenitors and Evolution: Where Do We Go Next?

Single-cell profiling of cortical glutamatergic neurons has uncovered a remarkable diversity both within cortical layers and across cortical areas, including at least 13 transcriptomically defined glutamatergic types in layer 5 of the mouse visual cortex (Tasic et al. 2016). It remains unclear, however, whether these glutamatergic types are produced by distinct sets of progenitors which change over the course of neurogenesis, and/or if they are predisposed to form gyri. Another question is whether cell cycle plays an instructive role in progenitor fates in primates. Indeed, progenitor cell cycle diverges across cortical areas in monkeys (Lukaszewicz et al. 2005) and varies between humans, nonhuman primates, and rodents (Kornack and Rakic 1998; Dehay and Kennedy 2007; Geschwind and Rakic 2013). This is relevant since in mice, cell cycle can modulate progenitor symmetric versus asymmetric divisions (Lange et al. 2009; Pilaz et al. 2009; Arai et al. 2011; Okamoto et al. 2016; Pilaz et al. 2016).

To what extent do differences in progenitor heterogeneity influence neuronal diversity and cortical traits? The historical view of multipotent progenitors has also been challenged with recent evidence of fate-specified progenitors driving expression of specific neuronal subtypes (Franco et al.

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2012; Garcia-Moreno and Molnár 2015; Gil-Sanz et al. 2015). Further, fate-mapping technologies and abilities to manipulate gene expression in mice have demonstrated that different precursor types can generate neurons of similar cortical layers (Tyler et al. 2015). Recent scSEQ studies have also uncovered molecules unique to aRGCs, bRGCs, and IPCs in humans (Pollen et al. 2014; Florio et al. 2015; Johnson et al. 2015; Pollen et al. 2015; Johnson and Walsh 2017; Nowakowski et al. 2017). Using new technologies to interrogate cellular morphology, profile gene expression, and perform lineage analyses in nonhuman primates as well as humans, we are optimistic that key questions can be addressed.

Given their clonal capacity, bRGCs are predicted to play a significant role in increasing the size and complexity of superficial layers II/III and may contribute to the expansion of cortical surface and formation of convolutions in humans (Wilsch-Bräuninger et al. 2016). However, most studies of bRGC clonal output have been done *in vitro*; thus in future studies, it will be critical to assess neurogenic potential of bRGCs *in vivo* (Mariani et al. 2012; Pollen et al. 2015). Likewise, it will be valuable to understand the nature of the few bRGCs found in mice. In this light, recent findings suggest the mouse dorsomedial telencephalon contains an oSVZ with abundant bRGCs (Huttner and colleagues, unpublished).

Thus far there is no correlation between the presence of convolutions and an oSVZ, as convolutions develop in species lacking an oSVZ and, likewise, some species with gyri and sulci lack an oSVZ (Garcia-Moreno et al. 2012; Hevner and Haydar 2012). Further, the thousandfold increase in the surface area of the human cerebral cortex occurs across all layers without a comparable expansion of thickness (Bystron et al. 2008; Geschwind and Rakic 2013). Notably, there is evidence that neurogenesis is complete in the Macaque even prior to gyrification (Rash 2019). Additionally, secondary and tertiary gyri form postnatally, long after neuronal generation and migration is complete. Therefore, the subsequent enlargement of cortical surface is likely due to neuronal growth in volume and their dendrites, formation of neuropil, and addition of protoplasmic astrocyte and oligodendrocytes (Rakic 2009; Rash et al. 2019). Thus, the extent to which bRGCs contribute separately to expanded cortical surface has been debated by the examination of its evolutionary history (Hevner and Haydar 2012).

How can we explain cortical surface expansion? It has been suggested that in organisms with larger and gyrencephalic brains, the radial unit is more conical or wedge shaped (Fietz and Huttner 2011). However, this model may not fully consider the fact that for each conical summit with larger superficial layers and larger surface area, there is a valley where the situation is reversed. Thus, enlargement of the oSVZ, which generates mostly layers II and III, is important but not sufficient to explain expansion of cortical surface during human evolution.

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Radial Neuronal Migration and Clonal Dispersion

The basal processes of aRGCs and bRGCs provide scaffolds for newborn glutamatergic neurons to migrate into the cortical plate. The increasing distance and emergence of primary convolutions add to the importance of RGC scaffolding to guide neurons to their proper columnar and areal positions (Rakic 1988b) (Figure 5.1). In humans, neurons migrate centimeters, whereas in mice this distance is much shorter. During their radial migration, neurons undergo polarity changes, transitioning from a multipolar to a bipolar morphology (Gal et al. 2006). Neurons migrate in an inside-out fashion such that the earliest born neurons ultimately reside within the deepest cortical layers while later born neurons form superficial layers (Angevine and Sidman 1961; McConnell and Kaznowski 1991). Histological studies and thymidine labeling suggest that inside-out corticogenesis is conserved among mammals and that the relationship between time of origin and cell position is sharply defined (Rakic 1974). Nonetheless, we still do not fundamentally understand why neurons migrate in an inside-out fashion and segregate into different laminar structures in the cortical plate.

The past thirty years have, however, yielded important new insights into neuronal dispersion. Lineage-tracing studies performed in mice, including using a technique called mosaic analysis with double markers (MADM), show that labeled clones of excitatory neurons are relatively constrained (Gao et al. 2014; Hansen et al. 2017) (Figure 5.3a). Columnar or cylindrical territories contain clones of up to 100 cells measuring 500 μm or less. In the future it will be valuable to measure more cells by inducing recombination at earlier developmental stages (i.e., prior to E10). Importantly, MADM studies are consistent with investigation of Tis21+ biPCs and their progeny, which distribute across all cortical layers (Kowalczyk et al. 2009). Likewise, they corroborate experimental manipulation of ephrin molecules in mice, which show that clonal intermixing occurs within functional columns but that large dispersion may be relatively rare in monkeys (Torii et al. 2009) (Figure 5.3b).

In contrast, recent preliminary studies in the human cortex reveal evidence of a small, but significant amount of dispersion of newborn cells (Lodato et al. 2015; Woodworth et al. 2017) (Figure 5.3c). These studies used long interspersed nuclear elements (LINEs) which spontaneously transposed in about 5% of cells, followed by whole genome sequencing of areas 17 and 18. The researchers detected LINE-induced mutations in adjacent cells and found that a very small number of cells (<1%) were labeled but primarily localized in columns, with some clonal intermingling. Once labeling was achieved in 1–3% of cells, however, there was more clonal dispersion. Going forward, somatic clonal studies may allow a quantitative grasp on neuronal distribution in the brain and on patterns of cell division. In some mammals, such as ferrets, clonal dispersion may be more profound (Reid et al. 1997; Ware et al. 1999; Reillo et al. 2011). Since these species diverged from the human phylogenetic tree

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millions of years before rodents, their evolution may have proceeded differently. Regardless, direct comparisons of clonal dispersion, using assays such as piggyback or CRISPR to label cells, could be performed in parallel in mice and nonhuman primates.

Interneuron Generation and Migration

An additional concept in our understanding of cortical development is the recognition that inhibitory GABAergic interneurons have a different pattern of neurogenesis and migration than excitatory neurons. They are generated primarily in the medial (MGE) and caudal (CGE) ganglionic eminence, as well as in the preoptic area and lateral (LGE) regions, which produce predominately olfactory bulb interneurons (Anderson et al. 1997; Wonders and Anderson 2006; Batista-Brito and Fishell 2009; Gelman and Marin 2010; Welagen and Anderson 2011). As in the dorsal telencephalon, GABAergic interneurons are generated via radial glia and transit amplifying progenitors (Turrero Garcia and Harwell 2017). Initially, newborn interneurons undergo tangential migration, moving along distinct routes from the LGE/CGE to the dorsal telencephalon via the SVZ, IZ, and marginal zone (MZ), before migrating radially to their final positions in the cortical plate (Glickstein et al. 2007; Brown et al. 2011; Sultan et al. 2014, 2016; Harwell et al. 2015; Mayer et al. 2015; Tischfield et al. 2017). Interneuron migration is governed by molecular cues including Semaphorin3a/f, CXCL12, Neuregulin1, Robo, and Ephrin (Marin et al. 2001; Flames et al. 2004; Andrews et al. 2007; Sanchez-Alcaniz et al. 2011; Wang et al. 2011b; Steinecke et al. 2014).

As MGE-derived cortical interneurons mature, they gain morphological and molecular diversity to become two main subclasses that express either parvalbumin (PV) or somatostatin (SST) (Wamsley and Fishell 2017). PV interneurons primarily target the cell body and axonal initial segment, whereas SST interneurons selectively target dendrites. CGE-derived interneurons, expressing VIP and Reelin, typically innervate other interneurons. The fate of the target cell may determine how inhibitory circuits are ultimately wired (Lodato et al. 2011; Ye et al. 2015).

Over the past thirty years, scSEQ and genetic studies in mice have significantly informed our understanding of interneuron fate specification. For example, progenitors and newborn neurons express markers characteristic of distinct interneuron subtypes (Mayer et al. 2018; Mi et al. 2018). Indeed, interneuron fates are specified by combinations of transcription factors (Hu et al. 2017). Additionally, and in contrast to excitatory neurons, sequencing experiments suggest the molecular identity of cortical GABAergic neurons does not depend on the cortical area in which they reside (Tasic et al. 2016).

To what extent are there species-specific interneurons or progenitors? Recent reports indicate that interneuron migration occurs in human brains for several months after birth, unmasking a novel population of late-born

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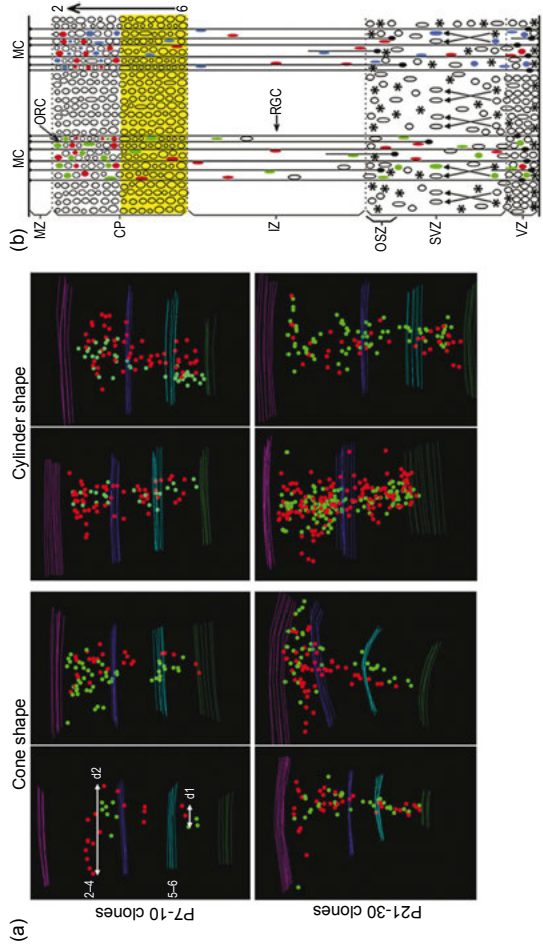

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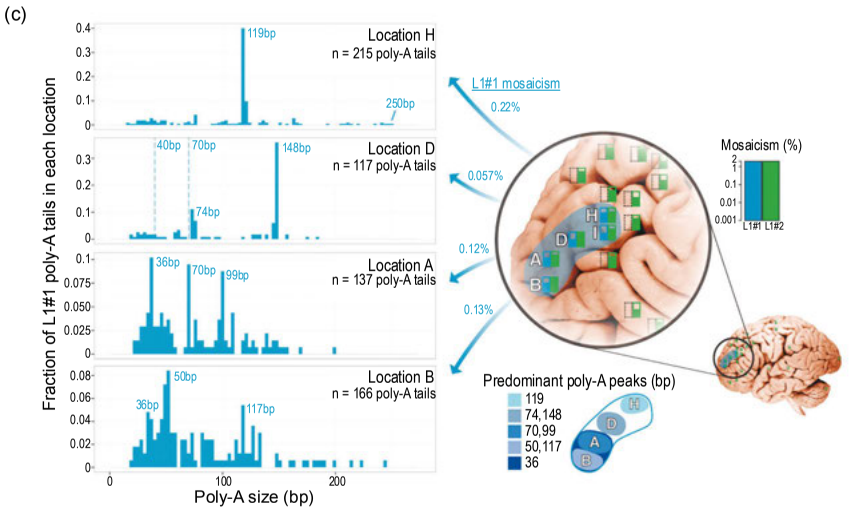

Figure 5.3 Clonal dispersion in mouse and human cortical development. (a) Clones of excitatory neurons in the mouse cortex, labeled using the MADM technique; clones (labeled in red and green) adopt conical or cylindrical shapes, ranging from 100–400 μm across at their widest point; reprinted with permission from Gao et al. (2014). (b) Clonal heterogeneity (red and green cells) within minicolumns (MC) due to intermixing within the subventricular zone (SVZ, arrows) before neuronal migration into the cortical plate (CP); reprinted with permission from Geschwind and Rakic (2013). (c) Two clones in the human cerebral cortex, labeled by spontaneous mobilization and insertion of two LINE elements. One LINE insertion, illustrated in blue, labels a clone limited to a gyrus, and about 1 cm across, labels from .05%–0.4 % of the neurons within this region. A second LINE insertion, labeled in green, is dispersed across the entire cortex among about 1–3% of neurons. The blue clone shows multiple somatic mutations of the polyA sequence of the LINE element, suggesting further subdivisions of the major clone with even more limited distribution in cortex, though each "subclone" again would include a small fraction (<0.4%) of neurons in the regions in which it is present. What remains to be understood is whether these lineage patterns represent fundamentally conserved patterns of clonal arrangement between species or fundamentally different patterns of dispersion; reprinted with permission from Evrony et al. (2015).

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inhibitory neurons (Paredes et al. 2016). Distinct GABAergic neuronal populations have also been described in humans (Raju et al. 2018). There are also reports of isolated GABAergic neurons in human and nonhuman primates that originate in proliferative zones of the dorsal telencephalon (Howard et al. 2006; Fertuzinhos et al. 2009; Radonjic et al. 2014). Consistent with this notion, in postnatal mice, a subpopulation of olfactory bulb interneurons is produced from aRGCs (Kohwi et al. 2007). This raises the interesting question of whether neocortical GABAergic interneurons could be produced by pallial progenitors. To date, however, human clonal studies have been biased toward investigating excitatory neurons. Thus we know very little about interneuron origin and dispersion and what defines their stop signals. The advent of scSEQ, human organoids, and human fetal tissue (e.g., Marinai et al., 2014) affords new methodologies to investigate human-specific features of inhibitory neuron development (Laclef and Metin 2018).

Transient Cells: Subplate Neurons and Their Contribution to Brain Development and Evolution

In mammals, the first postmitotic cell layer is the preplate (primordial plexiform, PP, zone), described first from Golgi analysis in cats and hypothesized to relate to the amphibian and reptile cortex (Marin-Padilla 1971). It also contains the first (pioneer) cortical neurons in humans (Kostovic and Rakic 1990; Meyer et al. 2000; Bystron et al. 2006) (Figure 5.4). Based on comparative anatomical studies, Marin-Padilla (1971) suggested that the PP layer later split, by the growing cortical plate, into layer 1 and subplate. The subplate, first discovered by Kostovic in humans (Kostovic and Molliver 1974), is a transient zone situated below the cortical plate, above the IZ (Rakic 1977; Bystron et al. 2008). While almost undetectable in marsupials, the subplate is a thin, distinct layer in mouse and rat (Rickmann et al. 1977), and a larger layer in carnivores (Luskin and Shatz 1985). The subplate zone is most expansive in human (Molliver et al. 1973) and nonhuman primates (Rakic 1977), particularly subjacent to the prospective association areas (Duque et al. 2016) (Figure 5.4). It has been proposed that later-born neurons split the MZ, subplate, and cortical plate (Marin-Padilla 1971). However, recent studies in primates, using H3-thymidine and BrdU to label cells at their birth and monitor their eventual positions, show a more complex picture in which subplate neurons are displaced by the arrival of new neurons (Duque et al. 2016). This enables the subplate to provide a constant platform upon which cortical afferents line up while the cortex is constructed.

During development of mammalian brains, subplate neurons form transient connections with the thalamus to establish cortical circuits. Over the last several decades, knowledge about the subplate has extended to include functional and molecular properties pointing to a structure with heterogeneous cell populations and a highly dynamic ontogeny (Antonini and Shatz 1990;

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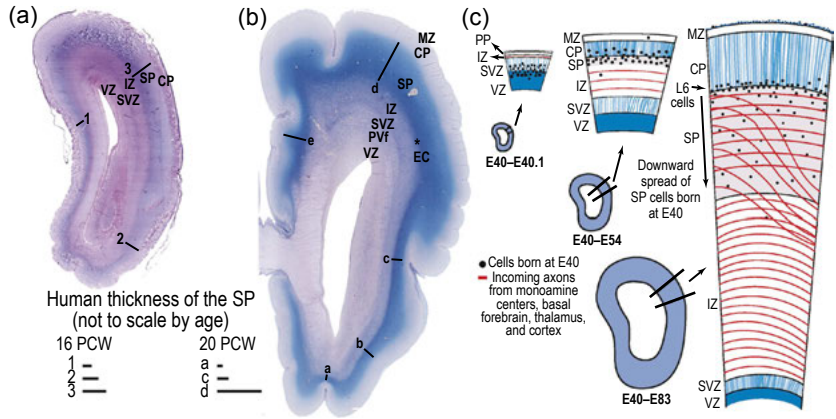

Figure 5.4 Comparative subplate (SP) development in mice and nonhuman primates: (a) and (b) show images of the human SP at 16 and 20 postconceptional week (PCW), depicting nonhomogeneous thickness of this structure. (c) Model of secondary expansion in the transient SP zone over the course of development in the Rhesus macaque. Reprinted with permission from Duque et al. (2016).

Hoerder-Suabedissen et al. 2009; Oeschger et al. 2012). H3-thymidine labeling in primates shows subplate neurons originate in the VZ (Duque et al. 2016); some glutamatergic subplate neurons, however, can also derive from the rostral medial telencephalic wall in mice (Pedraza et al. 2014). Further, gene expression patterns show homologies of cellular morphology, birthdating, and homology in the dorsal cortex/dorsal pallium of several amniote species. Thus, the subplate is hypothesized to contain both ancestral and newly derived cell populations (Montiel et al. 2011).

Postnatally, a large proportion of subplate neurons die; a small fraction, however, survive and become scattered below the cortex in the fiber layer in humans, or form a thin band of cells (layer 6b) in mouse (Hoerder-Suabedissen and Molnár 2015). In humans these cells are implicated in cognitive developmental disorders, such as autism and schizophrenia. Distinct subgroups of 6b neurons connect to select thalamic targets with known functions, providing a means to investigate subplate function (Hoerder-Suabedissen et al. 2009). Indeed, deep recording through all cortical layers suggests that in addition to neurons in cortical layers 1–6, subplate neurons also exhibit stimulus-driven responses (Pho et al. 2018). These neurons receive inputs from diverse brain regions and may project to nonprimary thalamic targets, thus suggesting that they represent a system for modulation of visual processing and brain states which persists into adulthood.

Beyond subplate neurons, additional transient glutamatergic neuronal populations include the Cajal-Retzius cells, pioneer neurons (Bystron et al.

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2008), and cortical-plate transient cells (Barber and Pierani 2016) (Figure 5.2). Lineage-tracing experiments have shown that Cajal-Retzius cells derive from the cortical hem, pallial septum, and pallial-subpallial boundary. They tangentially migrate into the cortex within the preplate and MZ, where they are implicated in radial neuronal migration, cortical lamination, and radial glia morphology via secretion of the extracellular matrix protein Reelin (Tissir and Goffinet 2003). In humans and nonhuman primates, Cajal-Retzius cells show great complexity and increased Reelin expression.

Organizational Features of the Neocortex: Areas and Layers

Debate of Protomap versus Protocortex Models of Arealization Enhanced Understanding of Cortical Development and Evolution

Three decades ago neuroscientists were debating the protocortex and protomap hypotheses. The protocortex (also called "tabula rasa") hypothesis proposes that the cortical plate initially has the same potential and that regionalization is controlled by external influences, such as axonal inputs from the thalamus (Creutzfeldt 1977). Today, the tabula rasa hypothesis has been largely disproven (e.g., O'Leary et al. 2013). Indeed, modern evidence indicates that cells generated within the VZ contain intrinsic information about their prospective laminar and areal fates. There are many levels of evidence, but one simple experiment is that X-ray irradiation, which ablates cells of the VZ/SVZ on a given day, produces a cortex in which neurons are missing from specific layers, with a sharp border between areas 17 and 18 (Algan and Rakic 1997). This shows that neurons are dedicated to specific layers and areas at the time of their genesis. When the protomap hypothesis, now accepted as the protomap model, was proposed, we did not know how initial positional information was imposed at the molecular level. This has changed significantly over the past thirty years.

To reconcile older descriptive and new experimental data, the protomap hypothesis suggests that the basic pattern of species-specific cytoarchitectonic areas emerges through synergistic, interdependent interactions between developmental programs intrinsic to cortical neurons that can be modified by extrinsic signals. Such signals can arise at later stages, supplied by specific inputs from subcortical structures (Rakic 1988b). Thus, neurons in the embryonic cortical plate—indeed in the proliferative VZ where they originate—set up a primordial map that preferentially attracts appropriate afferents and has the capacity to respond specifically to these inputs. Importantly, the protomap hypothesis is named "proto" (meaning modifiable) because it is not a fate map, since specific thalamic inputs, which arrive at given areas, are essential for proper cortical differentiation (Rakic 1988b). It is thought that species-specific functionally specialized areas are arranged in protomaps (Clowry et al. 2018).

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According to the protomap model, newborn radially migrating neurons carry positional information inherited from progenitors into the cortical plate, where subsequently thalamocortical axons and other afferents arrive in the cortex. Consistent with this, a mouse mutant with little or no thalamocortical innervation shows regional gene expression that marks prospective area borders (Miyashita-Lin et al. 1999). As further evidence of positional information, when *Emx2* expression is deficient in cortical progenitors, rostral areas expand and caudal areas contract, reflecting the low to high rostral-caudal gradient of *Emx2* expression. Loss of *Pax6*, expressed in the opposing gradient, has the opposite effect on area size (Bishop et al. 2000). Formation of specialized areas may also depend upon progenitor cell-cycle differences present between areas 17 and 18 (Lukaszewicz et al. 2005).

Another key finding was the recognition that the cerebral cortex is initially patterned by signaling centers which release diffusible proteins, including members of the fibroblast growth factor (FGF), Wnt, bone morphogenetic protein (BMP), and SHH families. FGFs promote telencephalic and neural identity in the neural plate in part by driving expression of the Foxg1 (BF1) transcription factor (Shimamura and Rubenstein 1997). Subsequently, a rostral telencephalic source of FGF8 patterns the area map along its rostral-caudal axis (Fukuchi-Shimogori and Grove 2001; Garel et al. 2003). FGF17 further promotes prefrontal and frontal area identity (Cholfin and Rubenstein 2007, 2008). As further evidence, an exogenous caudal FGF8 source induces duplicate sensory areas, which are oriented in mirror image to the endogenous areas (Assimacopoulos et al. 2012). Finally, a dorsal telencephalic signaling source, termed the cortical hem, rich in Wnts and BMPs, influences the dorsal to ventral axis (Caronia-Brown et al. 2014).

FGFs, Wnts, and BMPs operate in part by altering expression gradients of transcription factors such as CoupTF1, Emx2, Lhx2, Pax6, Pbx1, and Sp8 (Grove and Monuki 2013; O'Leary et al. 2013). Modifying the dosage of these transcription factors modulates the relative sizes of cortical areas (Garel et al. 2003; Hamasaki et al. 2004). Studies using advanced genomic methods have demonstrated the embryonic cerebral wall can be labeled by the activity of small enhancer elements in specific cortical progenitor domains. Relevant to this expression gradient, the activity of enhancer-like regulatory elements can be localized in small domains with sharp borders (Pattabiraman et al. 2014). Fate-mapping from these small domains provides evidence for a protomap of the cortex that is encoded by the integration of transcriptional information processed by gene regulatory elements (Pattabiraman et al. 2014). Cells are arranged largely in radial patterns from the ventricular to the pial surface (Figure 5.5).

There is broad agreement that thalamus inputs are essential for arealization and secondary area formation of cortical functional columns (Geschwind and Rakic 2013). Thus, the arrival of thalamic axons induces functionality, including, for example, anatomical barrels in somatosensory cortex, the morphology of layer 4 neurons, and some differential gene expression between areas.

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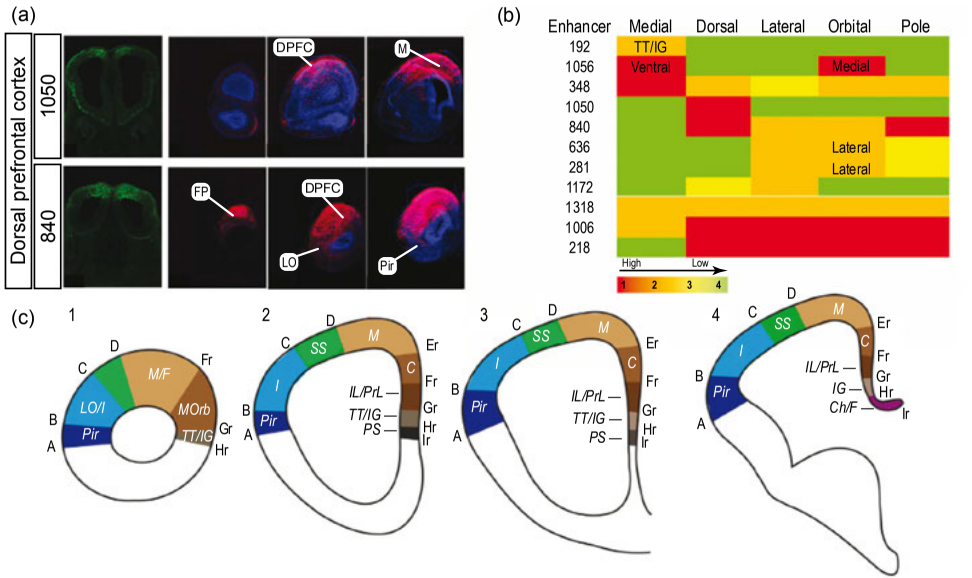

Figure 5.5 Fate-mapping analysis of enhancers with regional activity in the primordium of the mouse embryonic frontal cortex. (a) Coronal sections depicting two enhancers with activity in pallial progenitors that fate map to prefrontal cortex subdivisions. Shown are green fluorescent protein expression at E11.5 (left) and fate-mapping with tdTomato expression in an E17.5 rostrocaudal series (right). (b) Annotation of fate-mapping results from selected enhancers (y axis) in five regions of the frontal cortex (x axis). Different levels of density of tdTomato expression are estimated and described as high density (red), medium density (orange), low density (yellow), and negligible density (green). In some cases, subdomain expression is noted. (c) Deduced progenitor domain organization of the rostral E11.5 pallium. Abbreviations: C, cingulate gyrus; CR, Cajal-Retzius cells; DPFC, dorsal prefrontal cells; Er, entorhinal; FP, frontal pole; F, frontal; IG, indusium griseum; IL, infralimbic; LO, lateral orbital; M, motor; MOrb, medial orbital; Pir, piriform; PS, pallial septum; SP, subpallium; SS, somatosensory; TT, tenia tecta. Reprinted with permission from Pattabiraman et al. (2014).

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FGF8 can also induce thalamocortical projections, and when the cortex contains extra Emx2, which regulates FGF8, this alters the areal map (Fukuchi-Shimogori and Grove 2003). Integration of thalamic afferent inputs influences primary and secondary visual areas (Chou et al. 2013). Further, thalamocortical projections are attracted to area 17 and control barrel field formation but do not induce area 17 (Rakic et al. 1991). Likewise, an experimental decrease of geniculocortical afferents does not diminish area 17 but instead induces formation of a novel area with abnormal architecture and a sharp border to area 18 (Rakic 1988b; Rakic et al. 1991). This supports the notion that progenitors within areas 17 and 18 may be genetically predisposed to induce secondary areas. Indeed, progenitors under the influence of thalamic inputs show altered proliferation, which can influence secondary zone formation (Dehay et al. 2001).

A critical next step is to determine whether the area-patterning model worked out thus far in the mouse generalizes to other mammalian species. At similar developmental stages just after neural tube closure, the cortical primordium in ferret is equivalent to that of the mouse, and gene expression indicates similarly positioned sources of FGFs and Wnts (Grove and Jones, unpublished). Likewise, humans with mutations in FGF receptor 3 show abnormal cortical patterning, suggesting that areal patterning mechanisms by FGF may be conserved (Hevner 2005). It is clear that existing areas can be postnatally refined to accommodate new functional needs or opportunities. Qualitative differences in maps could be related to differential experience. For example, studies of the prehensile grip of monkeys have shown that manipulating biomechanics can modulate behavior and induce area 5 in new locations (Krubitzer and Stolzenberg 2014). Interestingly, functional area size can vary across humans, as exemplified by the visual cortex which shows threefold variation (Andrews et al. 1997). Novel maps may also be added during evolution by introducing new cells as the cortex expands. In more complex organisms, cortical expansion would thus coincide with new modules, which can serve as receptive units to process additional or slightly refined tasks.

Lamination

Cortical neurons are organized into six distinct layers which include layers I–III (supragranular layers), layer IV (internal granular layer), and layers V/VI (infragranular layers). Over the past three decades we have learned a great deal about molecular features of mammalian neuronal layers (Greig et al. 2013), including key transcription factors and gene expression networks which control both specification and maturation of glutamatergic type neurons. Remarkably, there is also a window of postmitotic development during which neuronal fates can be reprogrammed *in vivo*, as shown by manipulations of transcription factors in the mouse (Rouaux and Arlotta 2013; Lodato et al. 2015). Studies in mice have shown that sister neurons can be connected by gap junctions, but

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whether this is borne out in other species is unclear (Yu et al. 2012). In addition, it is enigmatic what drives distinct laminar organization across areas where neurons exhibit inherently different axonal projections. For example, in primates, layer IV is significantly thicker in the visual cortex relative to adjacent areas (Rakic 2009). This question is also relevant for understanding evolution, as relative to other primates, layers II/III are thicker and cytologically more diverse in humans, which could enable increased cortical-cortical connections (Hill and Walsh 2005; Rakic 2009).

Gyrification

Higher-order mammalian brains are gyrencephalic, having acquired cortical folds called gyri and sulci (Welker 1990), which allow expanded brain surface area. Gyrification is thought to require further expansion of initially larger cortical plate formed by proper areal distribution of neurons via radial migration (Rakic 2009). It also involves development of subcortical white matter that consists of various axonal bundles and numerous glial cells, which are particularly large in primates, including humans (Rash et al. 2019). The mechanisms of gyrification are evidenced by studies of the adhesion molecule FLRT, which is differentially expressed in humans (Del Toro et al. 2017). In developing ferrets, FLRT1/3 show differential expression in prospective gyri and sulci, and *FLRT1/3* double KO mouse have aberrant neuronal migration, associated with increased formation of gyri and sulci. Thus, reduced expression of specific genes which modulate neuronal migration may promote gyrification. Likewise, ECM components influence initial folding of the fetal human neocortex. Specifically, the ECM components HAPLN1, lumican, and collagen I cause a hyaluronic acid-dependent folding of fetal human neocortex tissue in an *in vitro* system (Long et al. 2018). Beyond gene expression, some modeling studies suggest that physical forces could also promote gyrencephaly (Tallinen et al. 2014).

Secondary and tertiary gyri in humans develop after neurons have been generated and have attained their final areal and laminar positions (Welker 1990; Kroenke and Bayly 2018). This suggests that human secondary and tertiary convolutions could be independent of increasing neuron number but rather an effect of neuronal enlargement and expanded neuropil and glial cells. In ferrets, which separated from the human phylogenetic tree before rodents, gyri develop postnatally (Kroenke and Bayly 2018). Thus, convolutions in carnivores and primates may be an example of analogy rather than homology. The most recent ancestor to all mammals is assumed to have already been gyrencephalic (gyrencephalic index of 1.3–1.4) (Lewitus et al. 2014). Hence, lissencephaly happens secondarily and is often associated with evolutionary dwarfism (e.g., the mouse is lissencephalic but originated from a larger and gyrencephalic ancestor).

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Cortical Evolution and Lessons from Nonmammalian Vertebrates

An invaluable path to understand unique structural and functional features of the cerebral cortex is through comparative investigation with birds and reptiles. The comparison of amphibian, reptilian, bird, and mammalian embryos supports the hypothesis that the embryonic pallium is subdivided in homologous (medial, dorsal, lateral, and ventral) sectors that are demarcated by the co-expression of developmental transcription factors (Puelles et al. 2000; Brox et al. 2004). Although this early developmental body plan is conserved, differences of the reptilian, bird, and mammalian adult telencephalon suggest that developmental programs diverge at later stages (Figure 5.6).

Unlike fish and amphibians, a large portion of the reptilian pallium has a three-layered organization which emerged about 320 million years ago in

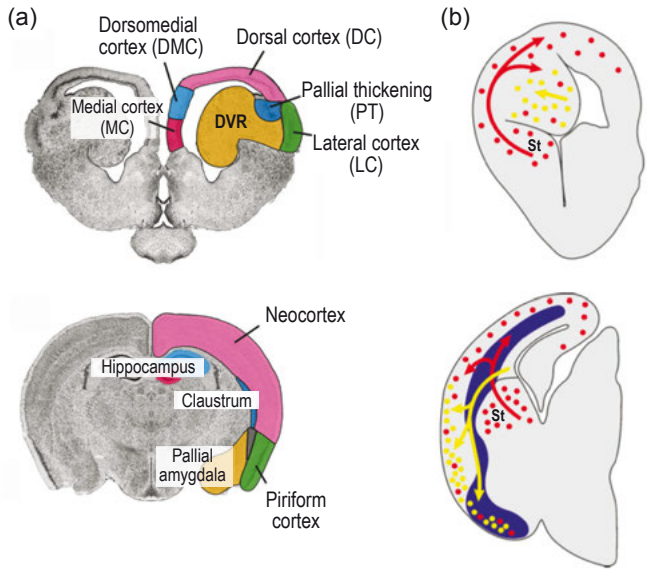

Figure 5.6 Reptile/mammalian homologies and differences in cortical organization and neuronal migration. Pallial regions in turtle (top) and mouse (bottom). (a) These regions are defined by neuroanatomy and transcriptomics. Colors represent proposed homologies, on the basis of current anatomical, developmental and transcriptomic data. (b) In mammals, the Pax6 territory is indicated in dark blue. Inhibitory, GABAergic neuronal precursors (red dots) originate from subpallial sources and migrate tangentially into the pallium in both mammals and sauropsids. Excitatory, pyramidal-type neuronal precursors (yellow dots) of the lateral migratory stream traverse the Pax6 territory to reach lateral pallial regions in mammals but remain *in situ* within the dorsal ventricular ridge (DVR) in sauropsids. Despite its extensive target area, the lateral migratory stream is considered to be a subset of the radially migrating pallial neurons. Interestingly, the tangentially migrating GABAergic cells have similar origin from Dlx gene expression territories from the medial ganglionic eminence (origin of red arrows) and they migrate dorsal to the cortex in mammal and DVR and dorsal cortex in reptiles.

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the amniote ancestor of mammals and reptiles. Genetic fate-mapping of the mammalian neocortex (Pattabiraman et al. 2014) supports the notion that this structure and the reptilian dorsal cortex develop from homologous embryonic regions (the dorsal pallium). The reptilian pallium harbors a nonlaminated region, called the dorsal ventricular ridge (DVR), which is derived from the ventral pallium (Karten 1969; Puelles et al. 2000; Butler and Molnár 2002; Tosches et al. 2018). This suggests that the DVR is unrelated to the neocortex. The DVR, however, harbors neocortical-like circuits, including the existence of thalamo-recipient neurons (Calabrese and Woolley 2015). This led to the "equivalent circuits" hypothesis, stating the homology of anterior DVR and neocortical layer 4 neurons (Karten 1969). Molecular analysis supports this idea (Dugas-Ford et al. 2012), indicating that different pallial regions expanded independently in the reptilian and mammalian lineages—ventral pallium (anterior DVR) versus dorsal pallium (neocortex)—resulting in the convergent evolution of gene expression and circuit architecture.

Notably, the reptilian cortex, which contains only a VZ, develops in an outside-in fashion (Blanton and Kriegstein 1991), in sharp contrast to the inside-out development of the mammalian neocortex (Angevine and Sidman 1961; Rakic 1974; McConnell and Kaznowski 1991). Further, there is an inversion of the corticogenesis gradient. In addition, recent evidence suggests that the tangentially migrating glutamatergic neuronal populations, such as Cajal-Retzius cells, subplate cells, and Dbx1 positive cortical neurons found in mammalian brains, do not exist in developing avian brains. This suggests that the emergence of these early neuronal populations in mammalian ancestors might have played a role in shaping the early development of dorsal pallium and could have triggered the evolution of the mammalian neocortex (Garcia-Moreno et al. 2018). Interestingly, inhibitory GABAergic neurons show similar tangential migratory behaviors in the reptiles and mammals (Cobos et al. 2001; Metin et al. 2007). However, excitatory pyramidal neuronal precursors of the lateral migratory stream traverse the Pax6 territory to reach lateral pallial regions in mammals but remain *in situ* within the DVR in sauropsids (Figure 5.6b). A few transcription factors are differentially expressed in the mammalian and bird ventral pallium, which might be responsible for the different migratory behaviors of ventral pallial derivatives (Garcia-Moreno et al. 2018; Yamashita et al. 2018).

Changes at the pallial-subpallial boundary might have participated in rerouting thalamocortical projections in our mammalian ancestors. In sauropsids, thalamic fibers reach the dorsal pallium through an "external" path, which traverses the ventral pallium. Conversely, mammalian thalamocortical projections arrive at the neocortex via the internal capsule (Bielle et al. 2011). Neuronal migration from the ventral pallium to the mammalian lateral amygdala, endopyriform nucleus, and claustrum present a rather difficult territory for thalamocortical projections. Early corticofugal projections which cross the pallial-subpallial boundary are thought to be important, as postulated in the

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handshake hypothesis (Molnár and Blakemore 1995). Further, the subplate/layer 6 handshake and basal ganglia play roles in directing corticothalamic topography and connectivity (Garel and Rubenstein 2004).

Genomic studies have enabled a comparison of glutamatergic cell types developing from the dorsal pallium (neocortex in mammals, dorsal cortex in reptiles) to clarify the evolution of neocortical layers (Tosches et al. 2018). ScSEQ data do not indicate simple one-to-one homologies between turtle cell types and individual cell types (e.g., layers) of the mammalian neocortex (Nomura et al. 2018). However, the turtle dorsal cortex contains cell types broadly similar, at the molecular level, to mammalian upper and deep layers. Likewise, turtle upper and deep layer neurons stratify according to birth order, with the former being deeper and the latter superficial (Ulinski 1986; Blanton and Kriegstein 1991).

These data suggest that neocortical glutamatergic neurons are new cell types that arose through the diversification of preexisting ones. Callosal projection neurons are an example of these new mammalian glutamatergic types (Garcia-Moreno and Molnár 2015). In mammals, these neurons originate from Emx2+ progenitors. Although less well defined, there is evidence that Emx2+ progenitors may exist in chick (Crossley et al. 2001). It thus remains possible that dorsal pallium progenitors vary across species, at least in part, at the level of gene regulatory networks, progenitor behavior, and heterogeneity (Garcia-Moreno and Molnár 2015). Notably, comparison of turtle and mouse data shows that the same classes of GABAergic interneurons exist in both species: MGE- and CGE-derived interneurons, including SST, PV-like, and VIP-like types (Tosches et al. 2018). This stands in stark contrast with the diversification of glutamatergic types and might reflect the existence of developmental constraints in the subpallium, where interneurons are born.

Synaptic Connectivity and Plasticity in Cortical Development and Evolution

Achieving the adult pattern of connections in each individual and species is activity dependent, wherein inhibition plays a key role. In the retina, for instance, cell connections are influenced by gradients of ephrins (Triplett and Feldheim 2012). However, ephrins play a crucial role in the formation of functional columns even before birth (Torii et al. 2009). Thus, before birth, innate patterns of neural activity, independent of sensory stimulation, set the stage for circuitry organization, similar to the influence of patterning centers.

The number of synapses in the developing human cerebral cortex is much higher than in adults (Huttenlocher and de Courten 1987), and similarly large overproduction occurs in developing nonhuman primates (Rakic et al. 1986). In the developing macaque, for example, the PFC contains as many as 60% more synapses than in adults (Bourgeois et al. 1994). This stage of exuberant synaptogenesis is followed by pruning which is prominent during puberty; in humans, however, this proceeds until the third decade of life (Petanjek et al. 2011). This

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might seem like an inefficient way to build a cortex, but recent theoretical analysis of network construction has shown that, paradoxically, this strategy leads to a more efficient network design compared to algorithms that do not depend on pruning (Navlakha et al. 2015). Once synapses form and circuits become functional, experience in the form of neural activity reshapes connectivity dramatically. This process of synaptic pruning followed by maturation is particularly potent during critical periods of brain development and is sensitive to environmental context. Great progress in cellular and molecular understanding has been made mainly in the mouse sensory cortex, where genetic manipulation has become a powerful dissection tool (Figure 5.7).

Proliferation and pruning of synapses is a hallmark of these late developmental stages from mouse to human (Rakic et al. 1986). In response to sensory deprivation, a gradual loss of dendritic spines is followed by their regrowth. Interestingly, spine motility is elevated by deprivation in a lamina-specific manner initially outside layer IV. Such events are likely enabled by extracellular proteases, such as tPA, and activated microglia. The subsequent regrowth and homeostatic strengthening of synaptic input may instead involve brain-derived neurotrophic factor (BDNF) and tumor necrosis factor secreted from neighboring neurons and astrocytes, respectively. Ultimately, synapses are largely converted from silent (NMDA only) to functional through PSD95-mediated AMPA receptor insertion and stabilization (Takesian and Hensch 2013).

Timing of the critical period is instead determined by the maturational state of fast-spiking, PV-positive inhibitory basket cells. Strengthening GABAergic synapses (by GAD65 expression, benzodiazepine exposure, BDNF overexpression, loss of PSA-NCAM, or Mecp2) can trigger premature plasticity. Slowing PV cell maturation by genetic deletion (Clock or a variety of

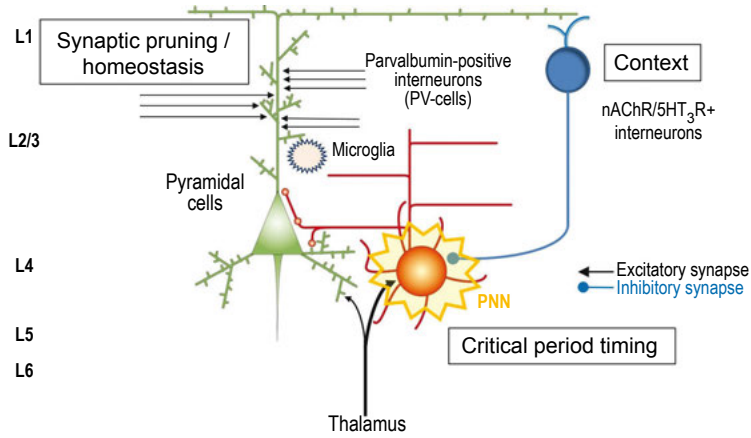

Figure 5.7 Overview of understanding of cortical circuitry. Findings over the last thirty years have given rise to details regarding synaptic pruning and maturation, critical period timing, and context in shaping cortical circuitry.

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autism-related proteins) delays plasticity. These findings reveal that critical period timing is itself plastic and not strictly determined by the age of the animal (Espinosa and Stryker 2012; Takesian and Hensch 2013; Sahin and Sur 2015). Transplantation of MGE-derived inhibitory precursors can reintroduce a second critical period later in life. As PV cells mature through the natural critical period, they enwrap themselves in a specialized extracellular matrix, the perineuronal net (PNN). This traps impinging synaptic boutons, such as thalamic and reciprocal inhibitory inputs, as well as a variety of noncell autonomous maturation and maintenance factors. Removal of the PNN can reopen critical period plasticity in adulthood (Hensch and Quinlan 2018). There are important homeostasis controls of excitation, as the firing of excitatory neurons can occur at the expense of inhibitory neurons.

Enriched environments can extend the critical period duration, while early life stress may accelerate closure. If mice are raised in an enriched environment, for example, the critical period of plasticity can be lengthened (Kaneko and Stryker 2017; Hensch and Quinlan 2018). Such ambient conditions may delay or accelerate the emergence of brake-like factors, such as PNNs, or increase neuromodulatory tone. Upper layer interneurons are enriched in ionotropic receptors for serotonin or acetylcholine (nicotinic) and send narrowly columnar input preferentially onto PV cells in layer 4 below. They also gradually express Lynx1 which dampens the action of nicotinic receptors after the critical period (Morishita et al. 2010; Takesian et al. 2018). This molecular brake can be overridden by gene deletion, acetylcholinesterase treatment, or exercise (running) to boost acetylcholine levels that enables plasticity in adulthood (Takesian and Hensch 2013).

Emergence of PNNs may serve as predictive biomarkers for novel critical period closure across brain regions (Takesian and Hensch 2013; Hensch and Quinlan 2018). Interestingly, localization of PNN components to astrocytes or non-PV cells in higher-order human brain regions in psychosis may not be captured in the mouse. Their molecular absence from more plastic, higher-order associational areas that are most vulnerable to Alzheimer's degeneration suggests a neuroprotective role for critical period closure. Similarly, by one year of age, Lynx1-deficient mice suffer neurodegeneration, a condition not typically seen in mice (Miwa et al. 2006). In the short term, however, pharmacological approaches to reopen critical periods may serve as a therapeutic strategy when increased plasticity may be desirable (e.g., stroke, recovery from brain injury).

Multiple lines of evidence suggest that neurons in the superficial layers of visual cortex in mice have prolonged plasticity after monocular deprivation, extending into adulthood. While neurons in the middle and deep layers of the cortex decrease responses to the closed eye and increase responses to the open eye, mainly during a well-defined "critical period" for ocular dominance plasticity, neurons in the superficial layers continue to exhibit plasticity until later (Frenkel et al. 2006; Espinosa and Stryker 2012). This increased propensity

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for plasticity may reflect layer-specific mechanisms of plasticity in cortex (McCurry et al. 2010).

How do molecular differences in species influence circuitry? Recent studies indicate that genetic modifications may modify circuitry over the course of evolution. For example, human-specific *SRGAP2* is implicated in control of the development of excitatory and inhibitory synapses. Notably, expression of the human ortholog in mice results in denser dendritic spines and delayed spine maturation (Charrier et al. 2012; Fossati et al. 2016). This may result from functional inhibition of ancestral *SRGAP2*. These modifications are thus hypothesized to impact circuit formation, cognition, and memory relevant for some developmental disorders.

Conclusion

In summary, over the last thirty years the following concepts emerged:

- Novel genomics, cell biological, and imaging approaches have led to recognition of additional distinctions between apical and basal progenitors during cortical evolution.
- During cortical development, genes are expressed at low resolution whereas enhancers define sharp expression boundaries.
- New technologies have enabled an unprecedented investigation of clonal dispersion in mice and humans.
- The protomap model has been realized with the discovery of thalamic and gradients of patterning factors, giving a mechanistic understanding of how patterning is established.
- Modules in evolution have enabled growth and duplication of cortical areas via transcription factor codes.
- Innervation and cortical circuitry are established via inhibitory–excitatory balance.
- Transcriptomic analyses of cerebral cortex have revealed convergent evolution of gene expression and circuit architecture.

To chart the way forward, we suggest the following approach:

Connect Genotype to Phenotype in an Evolutionary Context

Molecular neuroscience has enabled an understanding of genomic, epigenomic, transcriptomic, and proteomic features of cortical development and evolution. Yet linking these molecular changes to cortical phenotypes remains a significant challenge. These human-specific and evolutionary divergent cortical features include cortical areas, neuronal circuits, cortical neurons, neuronal processes (axon, dendrites), features of synapses, subcellular features within cortical neurons, cellular processes, reactions within cortical neurons,

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human-specific genes, RNAs, proteins, lipids, and carbohydrate structures. We propose that future research should focus on the following:

- Exploit and interrogate evolutionary differences between related species, such as the mouse and rat. Clear gene expression and cellular differences between mouse and rat make this a tractable approach in which to define the genetic underpinnings of evolution.
- Investigate different strains of the same species to correlate quantitative trait loci with transcriptomes, enhancer activity, and cellular and behavioral states. For example, the Peromyscus strains of deer and beach mice evolved differently, with the latter strain possessing a bigger PFC (Hu and Hoekstra 2017).
- Study genetics of human disorders, neurological features, and behaviors. By identifying and prioritizing variants associated with specific neurological features, this may inform evolution and be valuable for human health.
- Exploit organoid models to interrogate evolution "in a dish." This would enable species comparison of cortical development between human and other great apes, and experimental investigation of disorders of human neocortical development.
- Interpret findings in the context of sample origin. When studying human tissue, it is critical to ensure that human-specific differences are not due to technical reasons, such as using brains sourced from diseased or abnormal embryos or adults.

Interpret Gene Expression Differences to Understand Progenitor Cell Types, Lamination, and Arealization

Human-specific gene expression is driven by chromosomal deletions and duplications, alterations to coding and noncoding regions, and modifications to enhancer activity, but functional relevance of these human-specific changes remain to be elucidated. Great headway has been made in understanding progenitors, yet many questions remain unanswered, as noted in this chapter. Thus, we propose the following goals for future research:

- Identify additional features underlying the evolutionary increase in basal progenitor proliferative capacity, and clarify if bRGCs introduce an additional layer of clonal dispersion.
- Define progenitor heterogeneity both temporally and spatially. Use of single-cell omics technologies (transcriptomics, splicing, epigenetics, and proteomics) will be valuable toward understanding progenitor and cell identity and understanding human-specific aspects.
- Define how excitatory and inhibitory progenitors specify cell fate via both proliferative and neurogenic divisions. Investigate how neuronal

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migration is coordinated with progenitor proliferation. Clarify if clonal dispersion influences disease etiology and is expanded across primates.
- Link positional information at the level of progenitors to areal demarcation and cortical function: Are conserved or divergent signaling and patterning factors involved in control of primary and secondary maps?
- To fill in molecular details in support of the protomap, one needs to define factors downstream of FGF8, determine what axon guidance cues influence thalamic afferents, and whether these factors are at play in other species, including those with gyrencephalic brains. Do signals in the cortical primordium generate boundaries between primary and secondary areas?

Evolution of Ontogenetic Columns, Layers, Areas, and Gyrification

What are quantitative or qualitative differences in cortical area specification and function in humans versus other primates? To move beyond using cytoarchitecture to define areas, goals for future research include the following:

- Investigate the role of noncortical inputs in cortical development and evolution (e.g., vasculature, ECM, meninges, cerebellum, gut–brain axis).
- Despite access to new sequencing and imaging technologies, we lack a clear understanding of species anatomical differences. To compare cortical areas between humans and macaques, we need to explore using comparative MRI and electrophysiology, and take advantage of brain banks.
- To understand species differences in synapse complexity, one could use high throughput electron microscopy or determine synapse density per neuron using biochemical methodologies not reliant solely on immunohistochemistry or EM. This could employ quantification of NeuN+ neuronal nuclei and synaptophysin+ synaptosomes in cerebral cortex homogenates of humans and other species.
- Another important issue concerns the decreasing rate of adult neurogenesis during evolution (e.g., Arellano et al. 2018). We need to understand these differences and clarify why neurogenesis diminishes or does not occur in humans in order to understand the human capacity for retention of memory over many decades of life (Rakic et al. 1986).
- Gyrification allows the enormous increase of cortical surface and hence is a fundamental feature of cortical development and evolution, yet we know little about the mechanisms and specific-specific differences and similarities.

Understanding How Cortical Circuits Develop

For both cell- and position-specific circuitry, how do these develop, and what is the role of genes and activity? In addition, not only the quantity but quality

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of connections is important, as is the relationship between neuron number and types of connections. We propose that future research needs to pursue the following lines of enquiry:

- Determine if there is experience-dependent wiring among different species and if genes related to plasticity are upregulated or brake-like factors are absent in humans.
- Perturb circuits in mice to investigate function and likewise interrogate human neural function by longitudinal recordings. Overlapping genetics may enable clarification of common circuits.
- Define a comprehensive whole brain connectivity map in 200 μm patches. Use high throughput means (such as mapseq) to map the human brain by transfections of bar-coded factors (Kebschull et al. 2016).

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