



Neurogenesis of Cerebral Cortex Projection Neurons 12

Robert F. Hevner

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Abstract

The cerebral cortex develops from the dorsal telencephalon (or pallium) at the rostral end of the embryonic neural tube. Neurons in the cerebral cortex consist of two main types, projection neurons (PNs; 75–85%) and interneurons (15–25%), derived from separate germinal zones in the pallium and subpallium (basal telencephalon), respectively. This chapter focuses on neurogenesis of PNs, which differentiate with glutamatergic neurotransmitter phenotype and make long axonal projections including those to subcortical structures, as well as commissural and ipsilateral cortico-cortical connections. During development, PNs are generated in the ventricular zone (VZ) and subventricular zone (SVZ) of the pallium, and migrate mostly radially to populate cortical columns. The

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R. F. Hevner (✉)

Department of Pathology, University of California San Diego, La Jolla, CA, USA

e-mail: rhevner@ucsd.edu

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primary source of cortical PNs are radial glia progenitors (RGPs), which serve dual functions as both neural stem cells (NSCs) and guides for PN migration. Some PNs appear to be generated directly from RGPs by asymmetric mitosis, but the majority of PNs are derived indirectly via intermediate progenitors (IPs), a type of transit-amplifying cells that are produced from RGPs, then divide once or a few times to produce PNs. Differentiation from RGP → IP is regulated by Delta-Notch signaling and neurogenic transcription factor expression. Programmed cell death significantly regulates RGP numbers during early neurogenesis, and PN numbers at later stages. In humans and other large species, some RGPs detach from the ventricular (apical) surface and migrate away from the ventricle to form basal RGPs (bRGPs) that accumulate in a thick “outer” SVZ (OSVZ), where genesis of IPs and PNs is augmented. Regional differences in OSVZ proliferation contribute to gyrus formation. Finally, areal and laminar identity of PNs are largely programmed by intrinsic spatiotemporal patterning, beginning in cortical progenitors.

Keywords

Radial glia · Intermediate progenitors · Pax6 · Tbr2 · Tbr1

Brief History

The developing cerebral cortex (embryonic pallium) was first studied histologically in the nineteenth century by anatomists such as Wilhelm His and Santiago Ramón y Cajal (reviewed by Rakic 2003; Hevner 2006). Those early researchers described radial glia, which were thought to be glial progenitors (“spongioblasts”), and reported that mitotic divisions occurred predominantly at the ventricular surface of the ventricular zone (VZ), where neurons were thought to be produced by distinct “germinal cells.” By 1970, when cortical neurogenesis was reviewed by the Boulder Committee (convened by the American Association of Anatomists), concepts had evolved to include the possibility that neurons and glia might be produced from the same progenitor cells, and a consensus nomenclature was introduced for the transient zones and cell types of developing brain (Boulder Committee 1970). Moreover, mitotic activity was recognized away from the ventricular surface, in the newly designated SVZ, where gliogenesis was presumed to occur. Also, the “inside-out” sequence of cortical neurogenesis from deep to superficial layers had been defined during the 1960s.

In the twenty-first century, huge advances have occurred in understanding the cellular and molecular basis of cortical neurogenesis. RGPs were identified as NSCs and the ultimate source of cortical PNs and glia, while IPs were implicated as critical cells for differentiation and amplification of virtually all cortical PNs. Research on cortical development in humans and other species with gyrencephalic brains (such as ferrets) revealed the contributions of bRGPs and the OSVZ to cortical growth and gyrification. Genetic studies in the past 10 years have shown how disorders such as

autism, epilepsy, and intellectual disability arise from mutations that perturb cortical development. In many cases, such mutations have been found to arise *de novo* during gametogenesis. Most recently, single cell gene expression profiling has provided a catalog of progenitor cell and PN subtypes in mouse and human neocortex.

At this point, in the third decade of the twenty-first century, we have a firm grasp on the essentials of PN neurogenesis and differentiation, from molecular to morphological levels. This knowledge will lay the foundation for future research, especially in the arena of new treatments for human neurological disorders, as discussed in the “[Outlook](#)” section.

Developing Cortex Has Epithelial, Zonal, and Regional Organization

Like other regions of the vertebrate CNS, cerebral cortex develops from the neural tube as an epithelium with apical (ventricular) and basal (meningeal) surfaces. Initially, the pallium consists of neuroepithelial cells (NECs), which form a pseudostratified neuroepithelium. Then, near the onset of neurogenesis, NECs differentiate by acquiring some glial properties to become RGPs (Taverna et al. 2014). Like NECs, RGPs divide at the ventricular surface, where mitotic figures are numerous, but their nuclei move away from the ventricle during interphase, in a process known as interkinetic nuclear migration (Fig. 1).

Consistent with their epithelial properties, RGPs are extensively attached to each other along the apical surface by adherens junctions (AJs), which form a belt-like zonula adherens. The AJs serve not only as structural elements, but also as signaling centers for pathways such as Notch, and as molecular boundaries separating apical from basal plasma membrane. The apical plasma membrane, which accounts for <1% of the total plasma membrane surface on RGPs, is exposed to the ventricular surface, harbors the primary cilium (a specialized nonmotile cilium used for signaling), and incorporates apical polarity complexes PAR, CRB, and Mals/Pals (Taverna et al. 2014; Bedogni and Hevner 2021). The basolateral plasma membrane (>99%) expresses a basal polarity complex consisting of DLG, LGL, and SCRIB. At the basal (pial) surface, RGPs bind to the basement membrane using molecules such as dystroglycan to bind laminins and other extracellular matrix molecules produced by meningeal precursor cells. Interestingly, RGPs do not have classical tight junctions, as they do not express occludin, but RGPs do have gap junctions scattered on their surfaces. In the case of bRGP cells, it is unclear whether they retain apical properties or how they organize apicobasal polarity, since they are no longer attached to the ventricular surface.

For neurogenesis, RGPs divide asymmetrically to self-renew and produce new IPs and PNs (Fig. 1). Some IPs remain in the ventricular zone (VZ) with RGPs, retain attachments to the ventricular surface, and exhibit short radial morphology as apical IPs (aIPs); other IPs lose their ventricular attachment, migrate to the SVZ, and convert to short multipolar morphology as basal IPs (bIPs). New PNs migrate to the

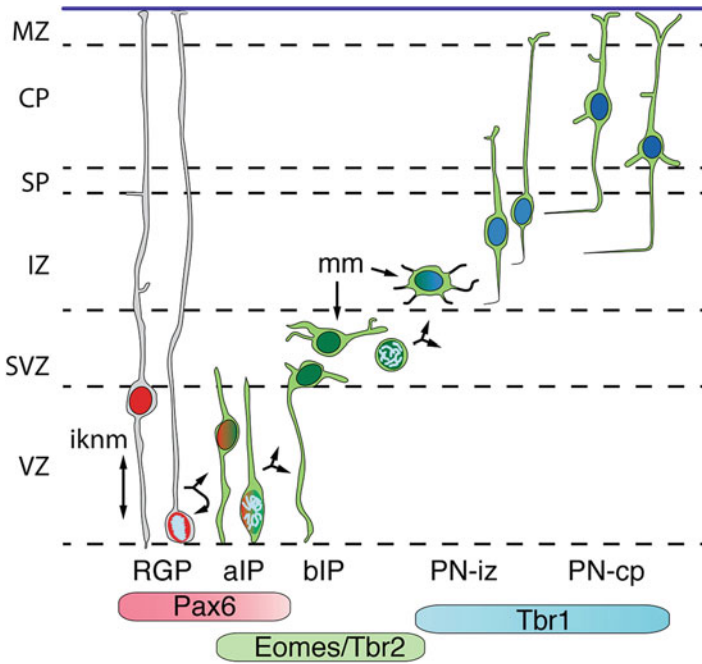


Fig. 1 Zonal organization, lineage differentiation, and transcription factors in cortical neurogenesis. The ultimate source of cortical projection neurons (PNs) are radial glial progenitors (RGPs), which undergo interkinetic nuclear migration (iknm) within the ventricular zone (VZ). Dividing at the ventricular surface, RGPs may produce projection neurons (PNs) directly, but more often produce intermediate progenitors (IPs) that initially remain attached at the surface as apical IPs (aIPs), which then divide or differentiate as basal IPs (bIPs) in the subventricular zone (SVZ). The bIPs extend radial processes into the VZ to contact RGPs, and short tangential processes for multipolar migration (mm). The bIPs divide to generate postmitotic PNs, which transiently continue multipolar migration in the intermediate zone (IZ) then switch to bipolar morphology and migrate radially past the subplate (SP) to the top of the cortical plate (CP), just below the marginal zone (MZ). PN-iz and PN-cp indicate PNs in the IZ and CP, respectively. At each stage of differentiation from RGPs to IPs and PNs, the cells express distinct sets of transcription factors such as Pax6, Tbr2, and Tbr1. This diagram is modeled on developing mouse neocortex during mid-neurogenesis at embryonic day (E) 14.5, but applies to early stages in human as well. Not shown are the basal RGPs (bRGPs), which are prominent later in development of larger-brained species

cortical plate (CP) while concurrently extending axons in the intermediate zone (IZ), or developing white matter. Other zones of developing neocortex include the marginal zone (MZ) and the subplate (SP), which contain mostly transient populations of PNs, as described below. In species with larger or gyrencephalic cortex, delamination of bRGP cells from the ventricular surface culminates in expansion of the OSVZ, where genesis of IPs and PNs continues during middle and late stages of neurogenesis. In humans, the OSVZ is discerned from gestational weeks (GW) ~12–26 (Malik et al. 2013; Cadwell et al. 2019).

Projection Neurons Are Generated Sequentially in Regional and Laminar Gradients

A distinguishing feature of cerebral neocortex is its organization into six histological layers, defined in part by the different sizes and packing densities of PNs. This basic hexalaminar organization is modified across the cortical surface, such that different cortical areas exhibit characteristic variations of each layer, as depicted in the well-known cortical area map of Brodmann.

Simple histological stains revealed that in humans and other mammals, the cortex initially develops along a rostralateral-to-caudomedial gradient, as measured by thickness of the CP in embryos (rostralateral thicker). With the introduction of cell birthdating methods in the 1960s, it was further discovered that neurons in different cortical layers are produced in an “inside-out” or deep-to-superficial gradient. Accordingly, layer 6 PNs are produced first and layer 2 PNs last. (Layer 1, also known as the marginal zone, is a special case because it contains very few or no PNs, except Cajal-Retzius neurons during development.)

Cell birthdating methods are based on the incorporation of radiolabeled thymidine, or chemically labeled thymidine analogs such as bromodeoxyuridine, into DNA during S phase of mitosis. When the label is administered as a pulse, it is incorporated into cells that are actively synthesizing DNA. During the subsequent chase period, unincorporated label is excreted, so that only newly generated cells retain label in their nuclei. If the labeled cell continues to divide, the label is progressively diluted, so only cells that were “born” on the pulse day and did not further divide are labeled heavily. Since neurons are postmitotic, their birthdays are easily determined. In mice, cortical PN neurogenesis spans embryonic days (E) 10.5–17.5. In humans, PN neurogenesis spans GW 5–28 (Bystron et al. 2006; Malik et al. 2013).

Cajal-Retzius Cells and Subplate Neurons Are Early-Born, Mostly Transient PNs

The complex structure and synaptic circuitry of the mature cortex do not form *de novo*, but begin with a temporary scaffolding known as the preplate (PPL), or primordial plexiform layer. The PPL is comprised of the earliest-born cortical PNs, known as Cajal-Retzius cells and SP neurons. In mice, the PPL is most prominent on E12.5. These PPL neurons form synaptic networks that incorporate newly arriving PNs and afferent axons. These temporary circuits organize and sustain new PNs during early development, until all the components of mature circuits are present. In addition, Cajal-Retzius neurons regulate PN migration by synthesizing an extracellular matrix protein called Reelin. Absence of Reelin in mice causes overall inversion of the cortical layers, and in humans is one cause of lissencephaly, a cortical malformation with smooth brain lacking gyri. SP neurons are key temporary targets of newly arriving thalamocortical and cortico-cortical axons (Molnár et al. 2020).

As new PNs arrive, they form the densely cellular cortical plate (CP) and split the PPL into MZ and SP zones, containing Cajal-Retzius cells and SP neurons, respectively. The SP zone is especially thick in primates, probably reflecting more extensive cortico-cortical connections than in smaller species such as mice. Postnatally, most Cajal-Retzius neurons undergo programmed cell death, but some survive and persist in layer 1 (mature counterpart of the MZ). Likewise, most SP neurons die, but some persist in white matter as “interstitial cells” or “white matter neurons.”

Most Projection Neurons Differentiate from Radial Glia Via Intermediate Progenitors

The localization of mitotic activity at the VZ ventricular surface and in the SVZ of developing cortex gave rise to various theories about cortical progenitors. One theory, for example, held that neurogenic progenitors divide at the VZ ventricular surface, and gliogenic progenitors in the SVZ. Another held that deep layers are produced from VZ divisions, and upper layers from SVZ mitoses. Not until the twenty-first century, with the advent of new methods, was the true nature of cortical “germinal cells” discovered.

Early histological descriptions of RG cells, noting their bipolar morphology with processes attached to pial and ventricular surfaces, had inferred their likely involvement as guide wires for PN migration. Indeed, subsequent studies confirmed that migrating PNs stay closely apposed to RG fibers (Rakic 2003). In the current century, RGPs were implicated in neurogenesis by genetic lineage tracing in mice, which revealed that RGPs also function as NSCs to generate cortical PNs, as well as astrocytes and oligodendrocytes. Thus, RGPs play dual roles in cortical development, as both NSCs and guidance cues. During neurogenesis, RGPs divide either symmetrically to self-duplicate, or asymmetrically to self-renew and produce a more differentiated neuronal precursor. At the end of neurogenesis, RGPs switch to production of astrocytes and oligodendrocytes, and undergo terminal differentiation.

While RGPs may produce neurons directly, the subsequent discovery of IPs indicated that at least some PNs or subsets are produced by an “indirect” RGP → IP → PN pathway (Fig. 1). Indeed, further research showed that the vast majority of PNs in mouse neocortex, including Cajal-Retzius and SP neurons, are produced from RGPs indirectly via IPs (Kowalczyk et al. 2009). In contrast to RGPs, which can produce multiple cell types (neuronal and glial) and have high proliferative capacity, IPs are committed to produce only glutamatergic PNs and have low proliferative capacity. Accordingly, RGPs are considered to be a type of NSC, and IPs a type of transit-amplifying cell. On the basis of morphological and molecular criteria, IPs can be further divided into apical (aIP) and basal (bIP) subtypes, which occupy the VZ and SVZ, respectively (Fig. 1). The aIPs have short radial bipolar morphology and transiently remain attached to the ventricular surface, while bIPs have short multipolar morphology and occupy the SVZ.

As might be expected, RGPs, IPs, and PNs express very different transcriptomes, including specific transcription factors (TFs) in each cell type. Well-known TFs in

the PN lineage include Pax6, Tbr2, and Tbr1, expressed by RGPs, IPs, and PNs, respectively (Fig. 1). These TFs actually form a feedforward activating cascade (Pax6 \rightarrow Tbr2 \rightarrow Tbr1), such that each TF drives expression of the TF for the next stage. Also, it should be noted that Pax6 is not specific to RGPs, but is also expressed by aIPs. More specific markers of RGPs include other TFs, such as Sox9 (Bedogni and Hevner 2021).

The gene expression network for PN neurogenesis is extraordinarily complex, but consists of core pathways modified by regional and temporal heterogeneity. Key regulators of the fundamental RGP decision to divide symmetrically (producing two RGPs) or asymmetrically (one RGP and one IP) include Delta-Notch signaling, and expression of neurogenic (or IP-genic) TFs. In this system, Notch receptors (Notch1, Notch2, Notch3) are expressed on RGPs (including bRGPs), and Delta ligands on IPs. Also, IPs express activating factors like Mib1 that are required for Delta presentation. Upon binding Delta1 (the major active Delta isoform in IPs), Notch signaling from the cell membrane to the nucleus is activated in RGPs and their differentiation to IPs is inhibited. When Delta1 levels are low (e.g., due to limiting numbers of IPs), Notch signaling is reduced in RGPs, and IP-genic TFs are derepressed, so differentiation of IPs and PNs is increased. Some of the key TFs that drive IP genesis in cerebral cortex include Pax6, Neurogenin2, and Insm1. Subsequent IP \rightarrow PN differentiation is driven by the expression of a different set of TFs, including Tbr2, NeuroD1, and (again) Insm1.

Even though IPs are dividing progenitor cells, their commitment to neuronal differentiation is evident from their gene expression (Bedogni and Hevner 2021). For example, IPs express genes such as HuC, NeuN, and vGluT2, which are all typically expressed in neurons. In addition, genetic lineage tracing in mice has shown that Tbr2⁺ IPs produce glutamatergic PNs exclusively (Mihalas and Hevner 2018).

Spatiotemporal Gradients of Gene Expression Drive Areal and Laminal Identity

Superimposed on the basic program for IP and PN differentiation are multiple gradients of gene expression, linked to arealization and specification of laminar identity. Regional gradients of gene expression are seen across rostrocaudal and mediolateral axes of the VZ, SVZ, and CP. These observations support the *protomap hypothesis* of arealization, which proposes that a rough map of cortical areas is established in RGPs, and propagated into IPs and PNs using distinct modules of genes. An alternative model, the *protocortex hypothesis*, proposes that immature cortical regions are equipotent, and arealization is driven by innervation from specific thalamic and cortical connections. Current evidence supports a model where the initial areal map is determined genetically, then modified by axonal innervation (Cadwell et al. 2019).

Interestingly, the genes that drive areal and laminar identity include several components of the basic neurogenic program. Notably, Pax6, Tbr2, and Tbr1 are expressed in high rostral–low caudal gradients, and along with dozens of other

genes, regulate rostrocaudal identity and arealization. The overlap of rostralizing and neurogenic TFs may in part account for the overall rostrocaudal gradient of cortical neurogenesis, described above.

Since neurons for different cortical layers are produced sequentially (the inside-out gradient), laminar identity of PNs is determined by temporal changes in gene expression within cohorts of RGPs, IPs, and PNs. Again, the genes that determine laminar identity overlap with some that control arealization and neurogenesis. For example, Polycomb repressive complex 2 (PRC2), an epigenetic system for gene regulation by histone modification, regulates neurogenesis, as well as regional and laminar identity beginning in RGPs. High levels of PRC2 activity restrict genesis of IPs and PNs; spatial gradients of PRC2 activity (high caudal) promote occipital cortex specification (Miró et al. 2009); and temporal regulation of PRC2 (high early) controls the progression of laminar fates (Telley et al. 2019).

Together, the available data on PN neurogenesis support an integrated view of the radial unit and protomap hypotheses, in which RGPs, via IPs, produce columns of cortical PNs that are born with defined transcriptomic and epigenetic programs for areal and laminar differentiation (Rakic 1988; Cadwell et al. 2019). Extrinsic influences, including secreted factors, afferent innervation, and membrane electrical activity, then act on the intrinsic programs to ultimately define cortical areas and layers.

Intermediate Progenitors Regulate RGP Differentiation and Interneuron Migration

The discovery of IPs prompted novel hypotheses and mechanistic insights into cortical development. Since the SVZ is most prominent during middle-to-late neurogenesis, one hypothesis was that IPs are specialized for genesis of upper layer PNs. However, multiple studies showed that IPs produce the majority of PNs for all cortical layers, including Cajal-Retzius and SP neurons (Kowalczyk et al. 2009; Mihalas and Hevner 2018). Another hypothesis suggested that local modulation of IP genesis plays an important role in the development of gyri and sulci. This hypothesis has merged with the discovery of bRGPs in the basal progenitor hypothesis of gyrogenesis, discussed below. Certainly, one basic role of IPs is to amplify neurogenesis by allowing mitotic activity outside the VZ, probably in a regulable manner.

One way that IPs regulate neurogenesis is by expressing Delta proteins that activate Notch signaling in RGPs, as mentioned above. Although it was initially thought that IPs usually divide once to produce two PNs, clonal analysis in mice has actually shown that the number of daughter PNs from an IP varies considerably, from one to at least eight (Mihalas and Hevner 2018). (In contrast, RGP clones from early neurogenesis may contain dozens of PNs.) Remarkably, approximately half of IP-derived clones contain only one PN, indicating a substantial fraction of asymmetric daughter PN death (Mihalas and Hevner 2018). Accordingly, relatively high

levels of apoptosis have been reported in the SVZ during neurogenesis; and microglial cells surveil the SVZ, ingesting some IPs (Cunningham et al. 2013). Thus, the potential for IPs to amplify neurogenesis appears to be balanced by a significant quantity of regression by apoptosis and microglial engulfment. RGP numbers are likewise regulated by apoptosis, albeit mostly prior to neurogenesis.

Along with regulating PN neurogenesis, IPs also help to balance the numbers of PNs and interneurons in developing cortex. In the SVZ, bIPs produce SDF-1 (*Cxcl12*), a factor that promotes interneuron migration tangentially through the SVZ. Also, bIPs have the molecular machinery to secrete glutamate, a neurotransmitter that likely activates migrating interneurons (Bedogni and Hevner 2021).

Intermediate Progenitors Regulate PN Axogenesis

One purpose of daughter PN apoptosis may be as a mechanism for competitive integration of PNs into cortical circuits. Axon selection and fasciculation occur in new PNs shortly after they are born, preceding migration out of the inner IZ (Fig. 1). Upon axon selection, new PNs migrate toward the CP as the axon grows tangentially, in a process described as the “touch-and-go” (T&G) model (Funahashi et al. 2014). IPs appear to play a major role in initiating this process.

Molecular profiling has shown that IPs express many molecules involved in neuron polarization, axogenesis, axon guidance, and intrinsic excitability (Bedogni and Hevner 2021). These include core planar cell polarity (PCP) and Wnt-Frizzled/PCP pathways, which appear to pattern IPs and new PNs along the planar axis, tangential to radial units. Consistent with this hypothesis, IPs and new PNs display multipolar morphology, and continuously probe the environment by dynamic extension and retraction of processes. This activity is known as multipolar migration (although actual distances migrated are minimal; see below). In mice, mutations that interfere with PCP signaling cause severe abnormalities in the formation of cortical axon pathways. Interestingly, mechanisms where progenitor cells are polarized and indirectly select axon pathways prior to the end of mitosis have been described in nonmammalian development, along with mechanisms that propagate polarization from progenitor to neuron (reviewed in Bedogni and Hevner 2021). So, the idea that cortical axon selection begins in dividing progenitor cells is consistent with previous studies.

Among the axon regulatory genes expressed by IPs are receptors for axon guidance molecules, such as *Unc5d*, *Dcc*, and *Epha4* (Bedogni and Hevner 2021). These, and some other molecules expressed by IPs, belong to the family of “dependence receptors,” so called because cell survival depends on ligand binding. In the absence of ligand, cell death is triggered. This suggests that IPs and new PNs may compete for limiting quantities of ligands, to avoid cell death. Besides axon growth, electrical activity is also important for PN survival, and this intrinsic activity actually begins in IPs, which express key molecules such as SCN3A (Smith et al. 2018).

Multipolar Migration Facilitates Clonal Dispersion, Layer Formation, and Axogenesis

Multipolar migration occurs predominantly in the SVZ and inner IZ, and appears to be the principal mode of cell migration for bIPs and newly generated PNs prior to axon selection and fasciculation (Tabata and Nakajima 2003). This “slow” mode of migration encompasses short radial and tangential movements of the cell body, while thin processes extend and retract in all directions, apparently probing the environment. Some cells do not even change position (migrate), but nevertheless exhibit the characteristic probing behavior. These observations suggest that multipolar migration serves additional purposes beyond migration.

One important purpose of multipolar migration appears to be the tangential (lateral) dispersion of clonally derived neurons, to facilitate cortical layer formation (Torii et al. 2009). Radial columns of neurons derived from proliferative units (as per the radial unit hypothesis) require some dispersion to distribute cells evenly among neighboring columns, and to form cortical layers of uniform thickness. The tangential dispersion is dependent on ephrinA-EphA signaling, as disruptions to this signaling pathway interfere with dispersion, and ultimately cause abnormal aggregation of neurons in clusters that distort the cortical layers (Torii et al. 2009).

Another important purpose of multipolar migration is to mediate contacts between new PNs and existing axons, as described in the T&G model (Funahashi et al. 2014). The multipolar migrating cells extend transient processes that contact nearby axons, which trigger polarization to define the axon and dendrite, and drive conversion from multipolar to bipolar radial migration toward the CP (Fig. 1). Interestingly, the polarization process is molecularly initiated in IPs, before final mitosis and PN differentiation (Bedogni and Hevner 2021). Significantly, cortical axon guidance is extremely dependent on PCP signaling, as shown, for example, in *Fzd3* null mice, in which defects of core PCP are associated with severe deficits in cortical axon pathways (Wang et al. 2002). A full suite of core canonical PCP and Wnt-Frizzled/PCP molecules are robustly expressed by IPs and new PNs (Bedogni and Hevner 2021). Intracortical Wnt signaling gradients originate from specific expression of Wnt factors in the cortical hem, a medial patterning center adjacent to the dentate gyrus neuroepithelium (Bedogni and Hevner 2021), and presumably provide the signals that pattern PCP.

For IPs, a third purpose of multipolar migration is to regulate neurogenesis and differentiation by Delta-Notch signaling. Some IP processes extend into the VZ, often to the ventricular surface where they contact RGPs (Nelson et al. 2013). The processes exhibit Delta1 protein on their surface, and lead to activation of Notch receptors on RGPs. In contrast to IPs, new PNs do not express Delta proteins and are not thought to have a significant role in Delta-Notch signaling.

Basal Progenitors Contribute to Cortex Gyrification

Gyrification of the neocortex and dentate gyrus is a mammalian brain feature found in all groups from monotremes and marsupials to placentals, indicating that the capacity for gyrification arose in stem mammals more than 200 million years ago

(Sun and Hevner 2014; Hevner 2016). With few exceptions, the degree of gyrification is highly correlated with brain mass, and indeed the largest mammals (whales) exhibit the greatest degree of cortex gyrification.

The question of how gyri develop has intrigued investigators for decades. One pertinent negative concern is the old hypothesis that the cortical surface passively bends or “crumples” as it expands within the confines of the skull: gyri still form even if the skull constraints are released during development. Rather, gyrification is an intrinsic process in developing cortex, which involves multiple mechanisms. One simple mechanism, seen in medial temporal and occipital lobes, is folding of the entire cortical wall to appose adjacent ventricular surfaces, thus obliterating the enclosed lateral ventricular space (Sun and Hevner 2014). Other proposed mechanisms include local regulation of proliferation, neuron morphology (dendritogenesis), afferent innervation, synaptogenesis, and gliogenesis.

Studies of bIPs and bRGPs in developing cortex of ferrets, monkeys, and humans have demonstrated local differences in basal progenitor abundance, and in the thickness of the OSVZ, that appear to presage gyrus formation (Fig. 2a, b). For example, bIPs are locally abundant beneath developing gyri in ferrets (Toda et al. 2016). Such observations have added weight to the hypothesis that local growth and greater PN abundance drive gyrogenesis. However, one confounding fact is that most gyri form after the end of neurogenesis. In humans, most gyri and sulci form in the second half of gestation, while most PNs are generated in the first half of gestation. Thus, differential neurogenesis alone does not account for gyrus formation, but appears to be one important step in a multistep process.

In addition to increasing neurogenesis, bRGPs also support gyrus development through other mechanisms. Local amplification of bRGPs adds radial units to the CP, and adds new radial fibers for PN migration into the expanding gyral surface. At the end of neurogenesis, bRGPs convert to gliogenesis, producing astrocytes and oligodendrocytes that also contribute to gyrus growth.

Another likely contributing factor to gyrus development is local regulation of SP neurons. The SP is thicker beneath prospective gyri (Fig. 2c), and presumably attracts greater numbers of afferent axons from thalamus and distant cortical areas. The profusion of axon pathways within growing gyri (Fig. 2d) sustains cortical expansion and indicates a significant role for white matter in gyrogenesis, consistent with the increased proportion of white matter relative to gray matter in gyrencephalic brains.

Since humans and other species have stereotyped, consistent patterns of primary gyrus formation, it has been hypothesized that gyri and sulci may be patterned by genes. Indeed, numerous genes have been implicated directly or indirectly in gyrus formation. Some, such as *DCX*, are important for cell division and migration, and human mutations cause lissencephaly, or smooth brain lacking most sulci (Pilz et al. 1998). More recently, regulation of metabolic pathways has been implicated in the proliferation of bRG cells and gyrus formation. For example, the human-specific gene *ARHGAP11B* encodes a mitochondrial protein that promotes glutaminolysis (a metabolic pathway associated with proliferation) and drives bRG amplification to increase PN numbers (Heide et al. 2020). Nevertheless, since the capacity for gyrus formation arose in the earliest stem mammals (Sun and Hevner 2014; Hevner 2016),

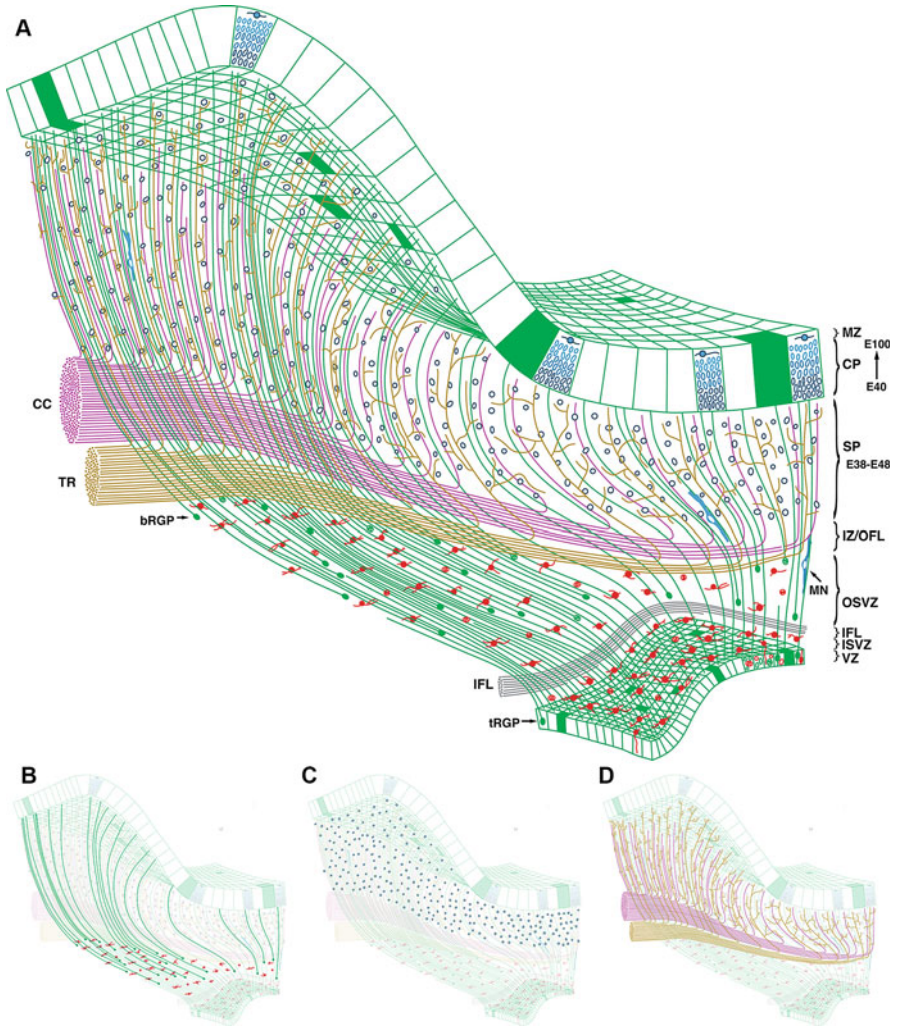


Fig. 2 Basal progenitors, the subplate, and afferent innervation contribute to development of gyri and sulci. (a) This schematic diagram, modeled after Rakic (2003), depicts the occipital cortex of fetal monkey at 80 postconceptional days (of 166 days total gestation), during development of the calcarine fissure (thin cortex at right). Compared to mouse, the primate brain has a more complex zonal organization with thicker subplate (SP), and an expanded subventricular zone (SVZ) divided into inner (ISVZ) and outer (OSVZ) portions, split by an inner fibrous layers (IFL). A subset of radial glial progenitors (RGPs; green cells with radial processes) detach from the ventricular surface and exit the ventricular zone (VZ) to become basal RGPs (bRGPs), located mainly in the OSVZ. Another subset of RGPs lose their basal attachment and become truncated RGPs (tRGPs). Intermediate progenitors (IPs; red cells) are present in the VZ as apical IPs (aIPs), in the ISVZ as basal IPs (bIPs), and in the OSVZ as bIPs. Projection neurons (PNs, blue) are indicated in the marginal zone (MZ), which contains Cajal-Retzius neurons; in the cortical plate (CP), with layers denoted by shades of blue; in the subplate (SP); and as migrating neurons (MNs) on radial glial fibers. The intermediate zone (IZ), or outer fibrous layer (OFL), contains axons including afferent cortico-

and many species (including elephants and whales) have larger brains than humans, the key regulators of gyrogenesis and brain growth are likely to be widely shared among mammals.

Outlook

One of the important outcomes of research on cortical development has been the production of cortical neurons and organoids from stem cells, including human-induced pluripotent stem cells (iPSCs). Cortical projection neurons can be produced from human iPSCs and upon transplantation into experimental animals, can integrate into cortical circuits (Tornerio et al. 2013). Future studies using iPSCs could potentially lead to cell therapies for neurological disorders, for example, replacing lost neurons in Alzheimer's disease. Also, cerebral organoids derived from iPSCs have been used to study neurogenesis, model human neurodevelopmental disorders, and even to study brain cancer and neurodegeneration. The increasing use of organoids provides a novel human experimental system that could have advantages over animal research. Given the medical potential, research using stem cells appears likely to expand.

Several important challenges remain in fundamental research on neurogenesis. The signaling pathways that control axon growth and guidance to specific targets (thalamus, brainstem, spinal cord, ipsilateral cortex, contralateral cortex) are still poorly understood, and evidently begin in IPs. The neurogenesis of allocortical areas, such as entorhinal cortex in the hippocampal formation, has received less attention than neocortex and will require further study to understand disorders affecting these regions, such as Alzheimer's disease. To understand neurogenesis of diverse PN types, single-cell profiling of progenitor cells and neurons is likely to continue playing an important role in neurogenesis research. The impact of microglia on PN neurogenesis and the potential impact of death receptors on PN apoptosis likewise remain important areas for further study.

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Fig. 2 (continued) cortical (CC) projections and the thalamic radiation (TR), which transiently innervate the SP before entering the CP. Embryonic (e) ages at right indicate neurogenic intervals for PNs in the SP and CP. (b–d) Modified images emphasize that developing gyri have more abundant basal progenitors (b), a thicker SP (c), and greater afferent innervation (d)

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