Review Article

How neural stem cells contribute to neocortex development

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The mammalian neocortex is the seat of higher cognitive functions, such as thinking and language in human. A hallmark of the neocortex are the cortical neurons, which are generated from divisions of neural progenitor cells (NPCs) during development, and which constitute a key feature of the well-organized layered structure of the neocortex. Proper formation of neocortex structure requires an orchestrated cellular behavior of different cortical NPCs during development, especially during the process of cortical neurogenesis. Here, we review the great diversity of NPCs and their contribution to the development of the neocortex. First, we review the categorization of NPCs into different classes and types based on their cell biological features, and discuss recent advances in characterizing marker expression and cell polarity features in the different types of NPCs. Second, we review the different modes of cell divisions that NPCs undergo and discuss the importance of the balance between proliferation and differentiation of NPCs in neocortical development. Third, we review the different proliferative capacities among different NPC types and among the same type of NPC in different mammalian species. Dissecting the differences between NPC types and differences among mammalian species is beneficial to further understand the development and the evolutionary expansion of the neocortex and may open up new therapeutic avenues for neurodevelopmental and psychiatric disorders.

Introduction

The neocortex is the largest part of the mammalian cerebral cortex and is the seat of higher cognitive functions, such as thinking and language in human. The neocortex is characterized by six layers of specialized neurons, which are generated during development from neural progenitor cells (NPCs) [1–6]. In the developing central nervous system, specifically the rostral end of the neural tube that generates the cerebral cortex, a single-layer of neuroepithelial cells (NECs) forms the neuroepithelium, which exhibits marked apical-basal polarity and a pseudostratified appearance due to the apical → basal → apical migration of the NEC nuclei in the course of the NEC cell cycle [7–9], as is further discussed below. Before cortical neurogenesis, NECs undergo symmetric proliferative divisions, which increase their abundance and result in an expanded germinal zone [10,11].

To briefly introduce the NPCs emerging subsequently, around the onset of mammalian cortical neurogenesis, NECs transform into apical (or ventricular) radial glia (aRG). While the cell bodies of aRG reside in the ventricular zone (VZ), aRG grow and extend a process from the VZ in the basal direction, through multiple cortical zones, to the basal-most side of the developing neocortical wall [1,12]. With the progression of neurogenesis, an increasing proportion of aRG switch from symmetric to asymmetric cell division [1,13,14]. These divisions result in the self-renewal of the aRG and the generation of a basal progenitor (BP) or, rarely a post-mitotic neuron [15–18]. BPs comprise basal (or outer) radial glia (bRG) [19–21] and basal intermediate progenitors (bIPs) [15–17], the cell bodies of which reside in the subventricular zone (SVZ). Both bRG and bIPs are the major sources of cortical neurons.

Cortical neurons fall into two principal classes, (i) excitatory projection neurons, which are generated by the NPCs, mostly the BPs, in the neocortex itself, and (ii) inhibitory interneurons, which are born in the ganglionic eminences and then migrate into the developing neocortical wall. The excitatory cortical neurons that eventually reside in the six layers of the neocortex are sequentially generated in an 'inside-out' manner, that is, deep-layer neurons are born first, and upper-layer neurons are born later [22,23]. Newborn neurons in the developing neocortex migrate along the radial processes of aRG and bRG to their final destination, the cortical plate (CP). Within the CP, cortical neurons mature and extend their axons and dendrites to form synaptic connections with subcortical or intracortical structures [24].

The size of the mature neocortex is primarily determined by the abundance and proliferative capacity of the diverse NPCs during cortical development. Due to the interspecies differences in NPC type, abundance, division mode and proliferative capacity, the size and morphology of the neocortex are remarkably diverse among mammalian species. In this review, we discuss recent findings on key cell biological and gene expression features of cortical NPCs and their contribution to mammalian neocortex development and evolution.

Principal classes of NPCs

The wall of the neural tube, and later the wall of the developing neocortex, possesses an intrinsic tissue polarity, with contacts to (i) the lumen of the neural tube (later of the lateral ventricles) on the apical side, and (ii) the basal lamina on the basal side (Figure 1). Based on the location of mitosis, NPCs can be categorized into two different principal classes: apical progenitors (APs) and BPs (Figure 1) [1].

APs

APs form, and are integrated into, the apical junctional belt and undergo mitosis at (or close to) the lumenal surface of neural tube (or the lateral ventricles). The confinement of AP mitoses to the apical domain of the VZ reflects their epithelial nature, specifically the presence of an apical primary cilium (discussed further below) whose basal body is involved in tethering the centrosomes (i.e. the future mitotic spindle pole bodies)
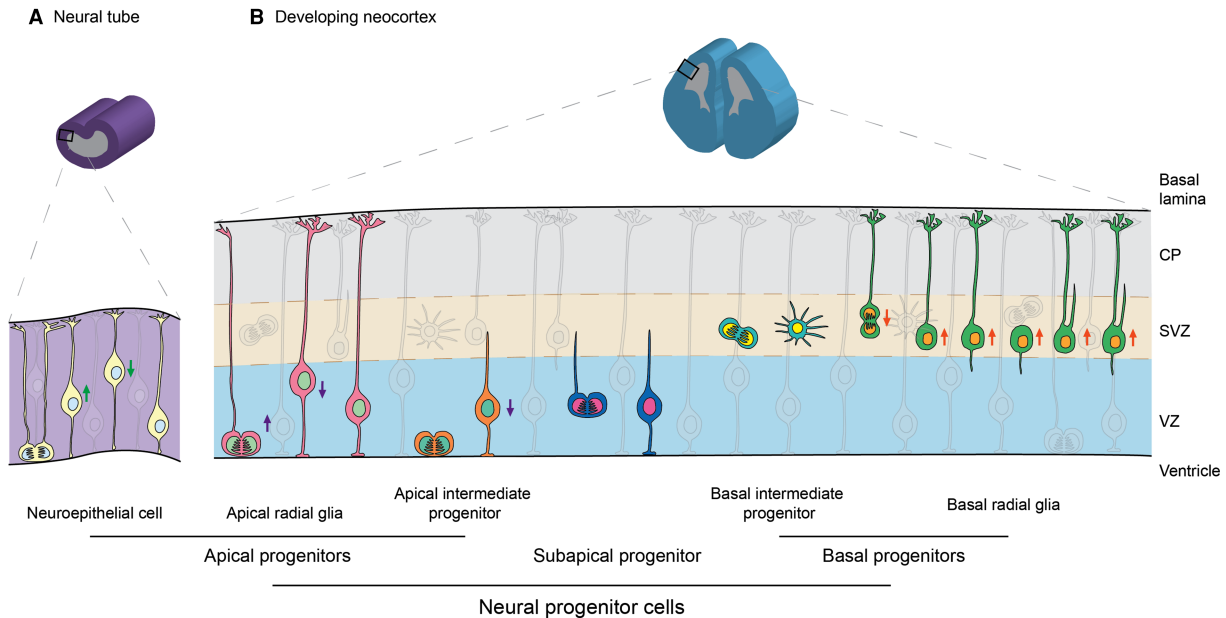
Figure 1. Principal classes and types of neural progenitor cells (NPCs) in the neural tube and the developing neocortex.
(**A**) Neuroepithelial cells (NECs) in the neural tube. Green arrows indicate the interkinetic nuclear migration during the cell cycle. (**B**) NPCs in the developing neocortex. Apical radial glia (aRG) (red), apical intermediate progenitors (aIPs) (orange) and subapical progenitors (SAPs) (blue) in the ventricular zone (VZ). Purple arrows indicate the interkinetic nuclear migration during the cell cycle. Basal intermediate progenitors (bIPs) (cyan) and basal radial glia (bRG) (green) in the subventricular zone (SVZ). Orange arrows indicate the mitotic somal translocation of bRG. Different morphotypes of bRG include bRG with a basal process, bRG with a basal process and an apical process, bRG with an apical process, bRG with bifurcated basal processes and bRG with apical and bifurcated basal processes.

to the apical plasma membrane during the entire interphase of the cell cycle [25,26]. As APs progress through the cell cycle, they exhibit a characteristic movement of their nucleus called interkinetic nuclear migration (INM) [7,8]. After mitosis at the apical surface, APs move their nucleus basally during G1 to the basal side of the neuroepithelium or VZ, where they undergo S phase. This is followed by the migration of the nucleus apically during G2 for the next round of mitosis at the apical surface. Due to the limited size of the ventricular surface, the number of AP mitoses is constrained. However, due to INM, the nuclei of interphase APs are away from the ventricular surface, which increases the space for apical mitosis and hence allows for an increase in the number of AP mitoses per unit time [27]. This in turn allows for an increased generation of APs and/or other NPCs and neurons. Another class of NPC that does not show the INM characteristics of APs are the sub-apical progenitors (SAPs). Similar to BPs, SAPs undergo mitosis at an abventricular location, typically in the basal VZ and SVZ, which avoids crowding at the ventricular surface. In the developing mouse brain, SAPs are abundant in the developing ganglionic eminences but rare in the developing neocortex. However, SAPs are abundant in the developing neocortex of gyrencephalic mammals, such as sheep and marmoset [28].

In this context, it should be mentioned that the spatial extent of INM, that is, the confinement of INM to the apical-basal range of the VZ, had been thought to reflect an AP-intrinsic mechanism. However, recent evidence suggests a mechanical collaboration between APs and AP-derived cells, including BPs and newborn neurons. Specifically, when selectively ablating BPs and neurons in the SVZ by conditional expression of diphtheria toxin upon *in utero* electroporation, the nuclei of APs migrated beyond the basal boundary of the VZ, i.e. into the SVZ. Moreover, these 'overshooting' APs showed prolonged S phase and did not return apically [29].

Another unique characteristic of APs is the presence of an apical domain, notably an apical plasma membrane, which directly contacts the cerebrospinal fluid (CSF) in the ventricle. This allows APs to receive signals from the CSF, notably through their primary cilium. The primary cilium is an organelle that protrudes from the apical plasma membrane of APs into the lumen of the ventricle, and that plays a crucial role in progenitor fate specification and neurogenesis [30,31]. Embryonic CSF contains a variety of secreted (or released) factors that have established roles in cortical development [32]. These factors act through binding to specific receptors expressed on the primary cilium plasma membrane to regulate the cell biology and behavior of APs. A prominent example is lysophosphatidic acid (LPA) in the CSF. LPA promotes the proliferation of NPCs through activation of the LPA receptor 1, which is localized on the ciliary membrane of APs [33]. Given the down-regulation of functional tight junctions prior to the onset of neurogenesis [34], certain components of the CSF may diffuse into the intercellular space between NPCs. This raises the possibility that newborn BPs as daughter cells of APs, which form a basolateral primary cilium [35], may sense certain extracellular signals in the CSF via this primary cilium. The important role of primary cilia in NPC behavior and cortical development in general is also highlighted by patients suffering from ciliopathies, a developmental disorder due to the dysregulation of ciliogenesis. For example, functional loss of the ciliary membrane-localized enzyme inositol polyphosphate 5 phosphatase E (Inpp5e), which hydrolyzes the phosphatidylinositol polyphosphates PIP2 and PIP3, results in an increase in neuron generation from APs at the expense of BP production [36]. This leads to a premature neurogenesis, which eventually results in a reduction in the total abundance of neurons generated during cortical development.

BPs

BPs delaminate from the apical junctional belt and translocate their cell bodies to an abventricular location for mitosis, typically in the subventricular zone (SVZ) [1,25,37]. The delamination of BPs is controlled by a wide range of molecules, including (i) transcription factors such as insulinoma-associated 1 (Insm1), which promotes delamination by repressing the apical junctional belt-specific protein Plekha7 [38]; (ii) centrosome proteins such as AKNA, which destabilizes apical microtubule–actin–apical junction complexes and promotes constriction of the apical endfeet and cell delamination [39]; (iii) the microtubule-associated protein called leucine zipper putative tumor suppressor 1 (Lzts1), which inhibits microtubule polymerization and activates the actomyosin systems; this in turn stimulates a coordinated cytoskeletal rearrangement resulting in cell delamination [40].

As a result of the basal migration of the delaminated BPs to the SVZ, the SVZ can grow in radial thickness, especially in species developing an expanded neocortex such as human [2,19,20,37,41]. In fact, a seminal study showed that in such species, an inner SVZ (ISVZ) and an outer SVZ (OSVZ) can be distinguished, with the OSVZ, in particular, growing in thickness [3,41]. By being able to undergo mitosis virtually anywhere along the radial dimension of a thickening SVZ, BPs can massively increase the number of their mitoses, in contrast with APs whose mitosis is limited to the apical domain of the VZ [15–17,19,20]. This makes BPs a pivotal
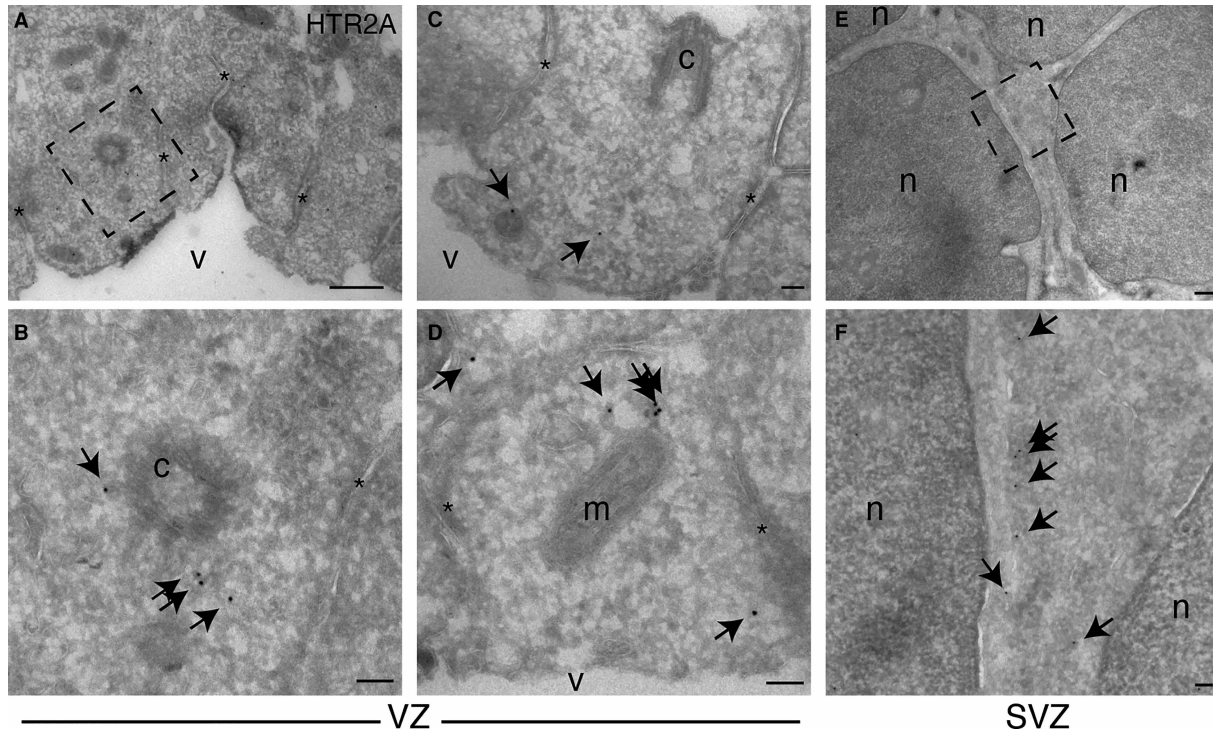
Figure 2. Transmission electron micrographs of fetal (gestational week 12) human neocortex showing the localization of serotonin receptor 2a (HTR2A) in NPCs by immunogold labeling.
Fetal human neocortical tissue was fixed and subjected to ultrathin cryosectioning (70 nm) following a previously described protocol [35]. Sections were immunogold-labeled using rabbit-anti-HTR2a antibody (1:200, Abcam, ab140524, RRID: AB_2858218) and Protein-A–10 nm gold. Immunogold labeling of HTR2A was observed in three independent fetal human neocortical tissues. (**A**) Overview of the ventricular area of the VZ of fetal human neocortex. (**B**) Higher magnification of the boxed region in A. Note the pericentriolar immunogold labeling. (**C,D**) Representative images of the ventricular area of the VZ showing an unlabeled basal body (**C**) and HTR2A-labeled cytoplasmic vesicles (**C,D**). (**E**) Representative image of densely packed nuclei in the SVZ. (**F**) Higher magnification of the boxed region in **E**. Note the immunogold labeling for HTR2A next to the plasma membrane. Scale bars 500 nm (**A,E**), 100 nm (**B–D,F**). Arrows, gold particles; v, ventricle; asterisks, adherens junctions; c, centriole; m, mitochondrion; n, nucleus.

NPC class for neocortical expansion during evolution [25,37,42–44]. Of note, as a result of their delamination, BPs are further remote from, though not necessarily inaccessible for, pro-proliferative signals in the CSF. However, BPs in the SVZ may increasingly receive signals from the embryonic blood stream, the extracellular matrix and factors secreted from neighboring BPs or neurons. Indeed, serotonin derived from the maternal blood and the placenta may be delivered via the embryonic blood stream to the developing neocortex, where it promotes BP proliferation through the serotonin receptor 2A (HTR2A) [45]. Indeed, consistent with this finding, HTR2A is expressed on NPCs in the fetal human neocortex (Figure 2) [45].

Types of NPCs
Based on cell polarity and cell morphology as well as expression of characteristic markers, APs can be categorized into two main types — NECs and aRG, and also BPs can be categorized into two main types — bRG and bIPs.

NECs and aRG
Many molecular and morphological features, including the epithelial feature of apical-basal polarity, are shared between NECs and aRG. Both NECs and aRG are bipolar, with an apical process contacting the ventricular surface and a basal process reaching the basal lamina. However, a specific mitotic behavior can distinguish NECs from aRG in fetal human neocortex. During the early developmental stage, human NECs completely

retract their basal fiber and round up at the apical surface before division. Daughter cells generated from these divisions can extend new basal processes and re-establish their contact with the basal lamina. In contrast, aRG retain their basal fiber throughout mitosis, and one of the daughter cells generated from aRG can inherit the basal processes directly [46]. In contrast with the latter report on fetal human neocortex, in embryonic mouse neocortex, NECs do not retract their basal process for mitosis, but rather mitotic NECs can split their basal process in the basal-to-apical direction by the cytokinesis machinery before anaphase, and both daughter cells can inherit one basal process after its splitting [47]. Of note, asymmetric inheritance of the basal process has also been reported for symmetric divisions of retinal NECs [48], and the split basal process can be asymmetrically inherited by one of the daughter cells in developing zebrafish embryos [47]. The bipolar property of APs, maintained by polarity-related proteins including Llgl1(or Lgl1, lethal giant larvae homolog 1 [Drosophila]), is important for the presence and correct location of apical and basal membrane components and the overall organization of the neuroepithelium and the VZ of the early developing neocortex. Two recent studies independently reported that the deletion of Llg1 in mouse led to overproduction and ectopic deposition of neurons at the ventricular surface, accompanied by the disruption of the junctional adhesion belt [49,50]. Another main difference between NECs and aRG is that aRG, but not NECs, express astroglial markers such as the brain lipid-binding protein (BLBP), the glutamate aspartate transporter (GLAST) and vimentin, which serve as important markers to distinguish aRG from NECs [51,52]. Another type of APs are the apical intermediate progenitors (aIPs, also known as short neural precursors), which share some of the cell biological characteristics of APs, including contact with the ventricular surface and undergoing one round of INM before mitosis at the ventricular surface. However, the basal processes of aIPs are typically confined within the VZ and do not reach the basal lamina.

bRG and bIPs

The two main BP types, bRG and bIPs, differ greatly in cell polarity and marker expression. As to *Cell Polarity*: bRG share some features, such as basal polarity, with NECs and aRG, and exhibit bipolar or monopolar morphology. Bipolar bRG extend an apically directed process and a single basal process or bifurcated basal processes, whereas monopolar bRG extend either only a single basal process (or bifurcated basal processes) or only an apically directed process (Figure 1) [19,20,28,53,54]. As with aRG, the basal process of bRG is retained through mitosis in most cases [19,20,55,56]. In contrast, bIPs are multipolar and may exhibit several short processes extending into any direction (Figure 1) [15–17,54]. As to *Marker Expression*: bRG are highly related to aRG in terms of marker expression and express proteins such as the transcription factors Sox2 and Pax6, and astroglial markers [19–21]. The most commonly used marker for bIPs is the transcription factor Tbr2, however Tbr2 is also expressed in a portion of bRG in mouse [56–58]. Two new markers, phosphacan (PTPRZ1) and tenascin C (TNC), which are expressed in bRG but not bIPs, have been identified by single-cell RNA sequencing [52]. In addition, phosphorylated vimentin is found in the radial processes of bRG at mitosis. Therefore, another useful tool to distinguish mitotic bRG from mitotic bIPs is immunostaining for phosphorylated vimentin [19,21]. Finally, in addition to these aspects of cell polarity and marker expression, bRG have been reported to exhibit a particular form of nuclear movement just before mitosis called mitotic somal translocation (MST) [20,59,60]. The biological significance of MST is not yet fully understood; however, an intriguing possibility for MST is to move the bRG nucleus and cell body further away from the signaling sources in the VZ and closer to the signaling sources from the basal lamina.

Proliferative capacity of NPCs

The proliferative capacity of NPCs is determined by the number of division rounds an NPC undergoes before consuming itself, as well as by the identity of the daughter cells generated during division. As to the mode of cell division, cortical NPCs can divide either symmetrically or asymmetrically, depending on the identity of the daughter cells when compared with each other, and can undergo either self-renewing or self-consuming division, depending on the identity of the daughter cells compared with that of the mother cell. Thus, there are four principal modes of NPC division, (i) symmetric proliferative ($1A \rightarrow 2A$), (ii) symmetric consumptive ($1A \rightarrow 2B$), (iii) asymmetric self-renewing ($1A \rightarrow 1A + 1B$), and (iv) asymmetric consumptive($1A \rightarrow 1B + 1C$) (with A, B, C indicating different cell types) (Figure 3A).

In principle, all four main NPC types are able to undergo symmetric proliferative division to generate two daughter cells sharing the same identity, which is also the same as that of the mother cell. Symmetric proliferative divisions of NECs, which lead to two NEC daughter cells each, prior to the onset of neurogenesis are
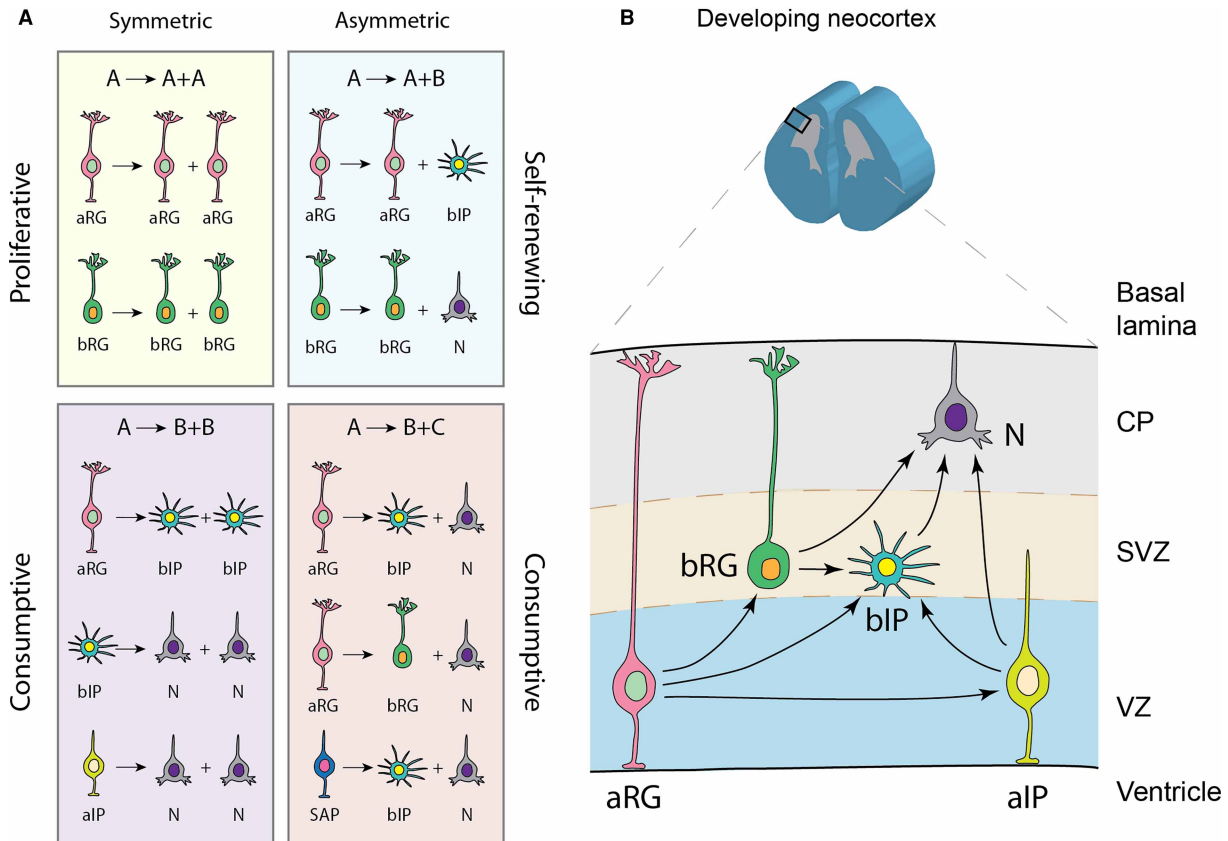
Figure 3. Different modes of NPC division and the lineage relationships of NPCs.
(**A**) The various modes of division of NPCs. Symmetric divisions can be either proliferative or consumptive. Asymmetric divisions can be either self-renewing or consumptive. Examples are given in each of the four division mode boxes. (**B**) Lineage relationships of NPCs in the developing neocortex. Arrows indicate the lineage progression. Abbreviations: aRG, apical radial glia; bRG, basal radial glia; aIP, apical intermediate progenitor; bIP, basal intermediate progenitor; SAP, subapical progenitor; N, neuron; VZ, ventricular zone; SVZ, subventricular zone; CP, cortical plate.

crucial to expand the founder NPC pool [10,11]. Similarly, aRG and bRG are able to generate two daughter aRG cells and two daughter bRG cells, respectively, expanding the NPC pool for neurogenesis. In fetal human neocortex, a major proportion of the bIPs is also able to generate two daughter bIPs, which is thought to be important for both neurogenesis and neocortex expansion in the context of evolution [61]. In contrast, in symmetric consumptive divisions, the two daughter cells share the same identity which, however, is different from that of the mother cell. Examples include one aRG generating two daughter BPs (two bRG or two bIPs), or one BP (bRG or bIP) generating two neurons. Of note, the latter type of symmetric consumptive division terminates the rounds of divisions of that particular NPC type.

In asymmetric self-renewing division, the two daughter cells generated from an NPC have different identities, but one of them shares the same identity as that of the mother cell. However, when both daughter cells have different identity, and this identity is also different from that of the mother cell, the NPC division is asymmetric consumptive. Typical examples of either type of asymmetric division are the generation of BPs from aRG, when either one aRG and one bIP or one bRG and one bIP are generated through an asymmetric self-renewing division or an asymmetric consumptive division of an aRG, respectively. The lineage relationships of NPCs in the developing neocortex are summarized in Figure 3B.

A proper balance of NPC proliferation vs. differentiation, resulting from an appropriate mixture of the various symmetric and asymmetric, self-renewing and consumptive, NPC division modes, is crucial for correct brain development. A concert of NPC-intrinsic and NPC-extrinsic factors is involved in maintaining the balance of division modes among NPCs to govern the proper formation of the neocortex during development. As to NPC-intrinsic factors, for example, a precise level of the orphan nuclear receptor Nr2f1 is necessary to

maintain the balance between proliferative and differentiative NPCs. In mice, high levels of Nr2f1 in NPCs of the posterior neocortex regionally promote their asymmetric neurogenic divisions. Upon loss of Nr2f1 function, Nr2f1 knockout mice showed prolonged NPC proliferation and delayed neural differentiation, which led to the expansion of the occipital neocortex [62]. As to NPC-extrinsic factors, cell surface molecules on NPCs such as ephrins have been found to promote, through cell–cell communication, the differentiation of other NPCs by binding to ephrin receptors on the latter. Indeed, signaling mediated by ephrin receptors triggers long-term alterations of epigenetic marks, which correlate with differentiation and suppression of NPC self-renewal [63].

Interspecies variations in NPC proliferative capacities

By adopting different modes of cell division, NPCs can exhibit different levels of proliferative capacity. Thus, the proliferative capacity varies among different NPC types and among the same NPC type in different species. For example, aRG across different mammalian species show similar levels of proliferative capacity. However, BPs, especially bRG, in different mammalian species display distinguishable levels of proliferative capacity [19–21,53,64]. In species with a relatively small and lissencephalic neocortex, such as mouse and rat, BPs, specifically bIPs, display a low level of proliferative capacity and typically undergo one round of consumptive division to generate two neurons [15–17]. In contrast, BPs, both bIPs and bRG, in fetal human neocortex are more proliferative and can undergo several cycles of proliferative and self-renewing divisions to generate more BPs before dividing to give birth to neurons [19–21,53]. Thus, the difference in proliferative capacity of BPs directly contributes to their higher abundance in human as compared with their lower abundance in rodents. By comparing the transcriptome of progenitors in prospective folds (gyri) versus fissures (sulci), a pioneer transcriptomic analysis in the developing ferret neocortex has identified genes whose expression correlates with the stereotypic patterning of progenitor proliferation and cortical folds. Differentially expressed genes showed a modular pattern along the OSVZ and VZ of the developing gyrencephalic ferret and human neocortex, but not in the developing lissencephalic mouse neocortex [64]. These expression modules along the OSVZ map faithfully to the prospective locations where the fissures and folds will form, which strongly suggests that some of the differentially expressed genes play a role in cortical patterning, especially cortical folding [64].

The different levels of abundance and proliferative capacity of BPs are thought to be a major reason for the diversity of neocortex size and morphology among different mammalian species. Recently, BP proliferative capacity has been shown to be linked to their morphology, especially the number of the processes that BPs have [54]. Overexpression of the morphoregulatory protein PALMD in the developing mouse neocortex led to the induction of processes in BPs, which in turn promoted BP proliferation. Conversely, the disruption of PALMD in fetal human neocortex reduced the number of processes and the level of proliferative capacity of BPs [54]. Besides their number, the nature of processes and their behavior in the course of the cell cycle appear to be relevant for BP proliferative capacity. Thus, mouse bIPs extend multiple short processes during interphase which however are mostly retracted for mitosis; yet, mouse bIPs exhibit very low proliferative capacity, typically dividing only once to generate two neurons [15–17]. In contrast with the multipolar bIPs with their multiple short processes, the long basal process of monopolar or bipolar bRG extends through multiple cortical layers, which allows these bRG to receive signals derived from various sources. Importantly, the bRG basal process may contact the pro-proliferation signal-rich basal lamina, which is thought to be crucial for maintaining the high proliferative capacity that bRG possess [19,20]. In line with this concept, the high proliferative capacity of APs across different species may be due to their bipolar morphology with radial processes. With their apical and basal processes extending across the entire cortical wall, APs can receive pro-proliferative signals not only from the CSF, but also from each neocortical zone and the basal lamina.

Concluding remarks

In summary, neocortex development is a well-orchestrated process, during which NPCs play a crucial role. The great diversity of NPC types impacts the difference in cortical size and morphology among mammals. Substantial progress has been made in the past decade to further dissect the cell biological features of NPCs and to uncover key molecules governing the balance between NPC proliferation and differentiation. In addition, recently identified NPC-intrinsic and -extrinsic factors have been implicated the interspecies differences in NPC proliferative capacity, which in turn has contributed to a better understanding of the development of the mammalian neocortex. All these studies have broadened our knowledge about the contribution of NPCs to neocortex development and laid down the cell biological and molecular foundation for further studying the evolutionary expansion of the mammalian neocortex. To this end, comparative studies using, besides the

mouse model system, fetal human neocortical tissue and the recently established human cerebral organoid technology might prove to be a rewarding strategy to understand neocortex evolution and may open up new therapeutic avenues for neurodevelopmental and psychiatric disorders.

Perspectives

- NPCs display a great diversity during neocortical development.

- A proper balance between NPC proliferation and differentiation, modulated by various cell-intrinsic and -extrinsic factors, is pivotal for the correct formation of the neocortex.

- Comparative studies using fetal human neocortical tissue and human cerebral organoids besides rodent model systems will be crucial to understand neocortex development and evolution.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

L.X. and W.H. wrote and edited the manuscript; M.W.B. performed HTR2A immunogold electron microscopy.

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Abbreviations

APs, apical progenitors; BP, basal progenitor; CP, cortical plate; CSF, cerebrospinal fluid; INM, interkinetic nuclear migration; LPA, lysophosphatidic acid; MST, mitotic somal translocation; NECs, neuroepithelial cells; SAPs, subapical progenitors; SVZ, subventricular zone; VZ, ventricular zone.

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