Overview

Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex

Takashi Namba* and Wieland B. Huttner*

The evolutionary expansion of the mammalian brain, notably the neocortex, provides a platform for the higher cognitive abilities that characterize humans. Cortical expansion is accompanied by increased folding of the pial surface, which gives rise to a gyrencephalic (folded) rather than lissencephalic (unfolded) neocortex. This expansion reflects the prolonged and increased proliferation of neural stem and progenitor cells (NPCs). Distinct classes of NPCs can be distinguished based on either cell biological criteria (apical progenitors [APs], basal progenitors [BPs]) or lineage (primary progenitors and secondary progenitors). Cortical expansion in development and evolution is linked to an increased abundance and proliferative capacity of BPs, notably basal radial glial cells, a recently characterized type of secondary progenitor derived from apical radial glial cells, the primary progenitors. To gain insight into the molecular basis underlying the prolonged and increased proliferation of NPCs and in particular BPs, comparative genomic and transcriptomic approaches, mostly for human versus mouse, have been employed and applied to specific NPC types and subpopulations. These have revealed two principal sets of molecular changes. One concerns differences in the expression of common genes between species with different degrees of cortical expansion. The other comprises human-specific genes or genomic regulatory sequences. Various systems that allow functional testing of these genomic and gene expression differences between species have emerged, including transient and stable transgenesis, genome editing, cerebral organoids, and organotypic slice cultures. These provide future avenues for uncovering the molecular basis of cortical expansion.

How to cite this article:
WIREs Dev Biol 2017, 6:e256. doi: 10.1002/wdev.256

INTRODUCTION

The brain is the most complex organ in our body. With regard to the diversity of cell types, the cytoarchitecture and neural circuitry, this complexity is greatest in the neocortex, the seat of higher cognitive functions. Although certain fundamental aspects of neocortex structure are conserved among the various mammalian species, there are tremendous differences with regard to neocortex size and morphology, notably the absence or presence of folding (Figure 1). For example, the most popular experimental animals for studying the brain, that is, mouse and rat, have a smooth (lissencephalic) and, compared to us, relatively small neocortex. In contrast, many primates, including human, have a folded (gyrencephalic) neocortex exhibiting gyri and sulci, which is expanded in size compared to the rodent brain. Because of these features, many researchers are fascinated by a

*Correspondence to: namba@mpi-cbg.de; huttner@mpi-cbg.de

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Conflict of interest: The authors have declared no conflicts of interest for this article.

fundamental question in neuroscience: how does the neocortex expand in development and evolution?

Development of the neocortex has mostly been studied in rodent animal model systems, that is, mouse and rat. All neurons and macroglial cells of the neocortex originate, in the vast majority of cases indirectly, from neuroepithelial cells (NECs). These cells initially form a pseudostratified epithelium and exhibit apical-basal polarity (Figure 2(a)). The apical surface of the neuroepithelium faces the lumen of the lateral ventricles, and the basal side with its basal lamina constitutes the pial surface.[1,2]

With the onset of cortical neurogenesis, NECs transform into radial glial cells (RGCs), and additional cell types, that is, secondary neural progenitor cells (NPCs) and neurons, are generated. Neurons and most of the secondary NPCs translocate their cell bodies to a location basal to the apical-most germinal zone harboring the RGC bodies, called the ventricular zone (VZ) (Figures 2(b)–(d) and 3). Concomitant with the basal translocation of secondary NPCs and neurons, RGCs elongate and maintain, like their NEC precursors, contact with the basal lamina by developing a basal (radial) process (Figure 2). RGCs are thus highly related to NECs, exhibiting pronounced apical-basal polarity.[1,2]

This unique cell type was first described at the end of the 19th century and the beginning of the 20th century (reviewed in Ref 3), and its name 'radial glial cell' was introduced by Pasko Rakic in the early 1970s.[4] RGCs were initially thought to be a scaffold for neuronal migration[4] and then give rise to astrocytes.[5–8] These functions are true, but an additional, fundamental role of RGCs has recently been uncovered—they act as NPCs. Although the possibility of such a role was raised already in the late 1980s,[9,10] firm evidence for RGCs being NPCs has only been available since the early 2000s,[11–14] being provided independently by four different groups of researchers.

The first line of evidence, obtained in cell culture experiments, was provided by Götz et al.[11] These researchers used transgenic mice that express GFP under the human GFAP promoter to selectively label radial glia cells, and found that GFP-positive cells isolated from embryonic mouse neocortex

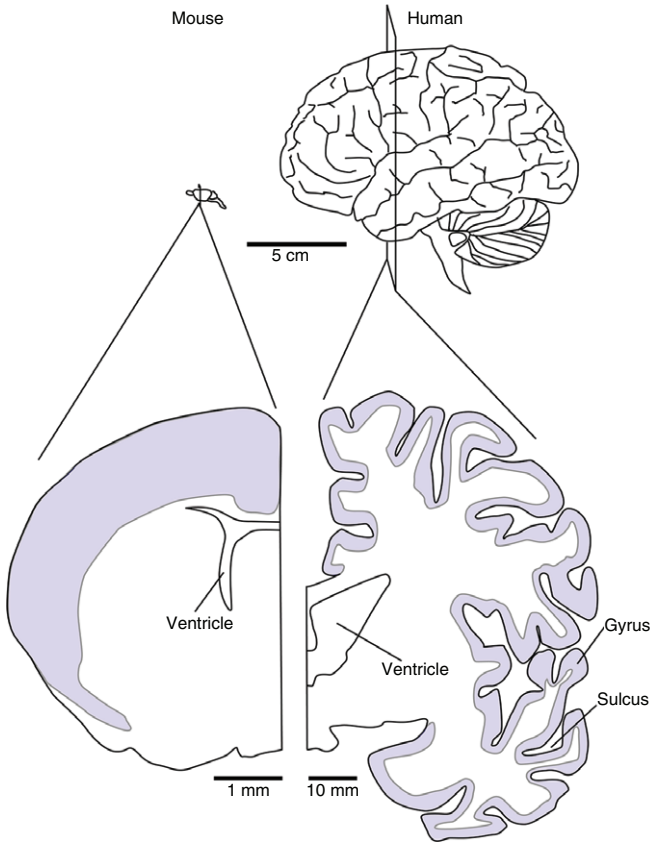

FIGURE 1 | Lissencephalic mouse and gyrencephalic human neocortex. Cartoons of mouse (left) and human (right) brain (top) and of a coronal section (bottom). Blue area indicates the gray matter.

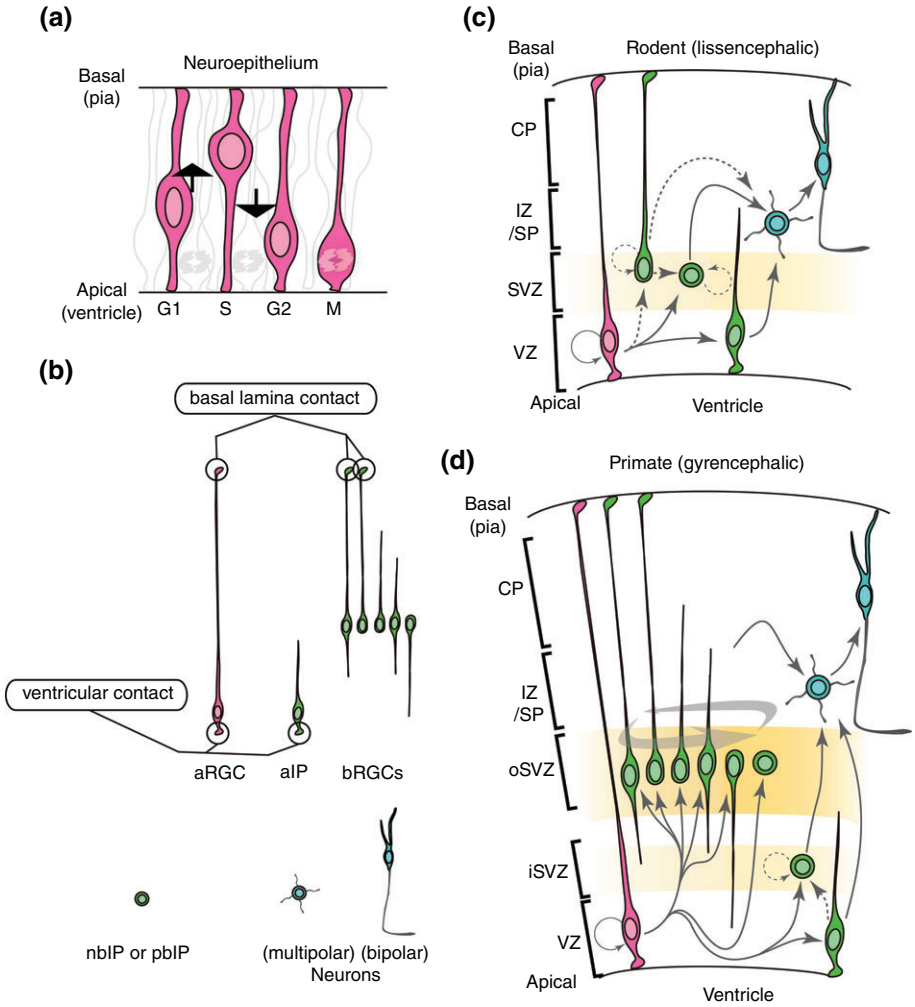

FIGURE 2 | Principal zones and progenitor cell types of developing neocortex. (a) Neuroepithelial cells (red). Arrows indicate the nuclear migration during the cell cycle. (b) Cell types after the onset of neurogenesis. Primary progenitor cells (apical radial glial cell, aRGC), secondary progenitor cells (apical intermediate progenitor, aIP; basal radial glial cells, bRGs; neurogenic and proliferative basal intermediate progenitor cells, nbIP and pbIP) and postmitotic neurons are indicated in red, green and blue, respectively. (c) Major lineages from primary progenitor cells via secondary progenitor cells to neurons (see (b)) in a lissencephalic rodent. Orange band indicates the SVZ. (d) Major lineages from primary progenitor cells via secondary progenitor cells to neurons (see b) in a gyrencephalic primate. Orange bands indicate the oSVZ and iSVZ. SP, subplate.

generated neurons in culture. The first evidence with intact embryonic neocortex tissue was subsequently reported by Kriegstein et al. and Miyata et al., who observed, by time-lapse imaging, the generation of neurons from retrovirus-infected GFP-expressing[12] or from DiI-labeled[13] RGCs in organotypic slice culture. Importantly, the study by Kriegstein et al.,[12] and independently that by Tamamaki et al. who used adenovirus to express GFP in RGCs,[14] provided *in vivo* evidence that the progeny of RGCs includes neurons. Despite the known close relationship of RGCs to NECs,[1,2] these studies were perceived as somewhat like a paradigm shift, and were followed by a significant increase in studies on cortical NPCs that has continued until today.

A major conceptual change occurred with the identification of basal progenitors (BPs) as the major producers of cortical neurons and the characterization of BP subtypes. Again, several research groups independently contributed these findings, in two main steps. First, using essentially the same approaches as in the time-lapse imaging analyses of RGCs,[15,16] or by generating the *Tis21*::GFP knock-in mouse line which specifically reveals NPCs committed to the neurogenic lineage,[17] a novel class of NPC was identified and characterized in embryonic mouse and rat neocortex. These NPCs characteristically

undergo mitosis in a secondary germinal zone located basal to the VZ called the subventricular zone (SVZ), hence the name BPs. The BP type initially characterized in mouse and rat typically undergoes only one cell cycle, lacks apical-basal polarity at mitosis,[18] and divides to generate two neurons, that is, it functions as an intermediate progenitor cell.[15–17,19] In addition, the NPC lineages from RGCs to neurons have recently been determined by an elegant clonal analysis approach in mouse embryos *in vivo*.[20]

Extending the analyses of NPCs in embryonic/fetal neocortex from lissencephalic rodents to gyrencephalic species,[21–23] that is, primates (notably human) and ferret, led to the identification and initial characterization of a second principal type of BP that, in contrast to intermediate progenitors, retains certain RGC features.[24–26] This BP type, in particular, has been implicated in the increase in neuron number and the expansion of the neocortex in development and evolution. Moreover, during the past few years, an increasing complexity of BP subtypes has become apparent.[27] In this review, we summarize the current knowledge about NPCs in the embryonic/fetal neocortex of lissencephalic and gyrencephalic mammals, and discuss their role in neocortex development and evolution. As to the evolution of neurogenesis in the diverse metazoan nervous system, including nonmammalian vertebrates, the reader is referred to a comprehensive recent review[28] and a recent original paper.[29]

CYTOARCHITECTURE OF DEVELOPING NEOCORTEX

The basic principles of the cytoarchitecture of the developing neocortex in lissencephalic rodents and gyrencephalic primates or ferret are similar. In all mammals, a pseudostratified neuroepithelium (Figure 2(a)) is formed after neural tube closure. NECs then produce the first-born neurons, and thus a preplate and a VZ emerge.[21,30] Subsequently, with the appearance of the cortical plate (CP), the preplate is split into a marginal zone and a subplate. At the same time, a SVZ arises basal to the VZ. In between the CP and SVZ, an intermediate zone (IZ) develops[30] (Figure 2(c) and (d)).

A major difference between the lissencephalic rodent and gyrencephalic primate or ferret neocortex concerns the SVZ. Although the thickness of the VZ is not massively different between rodents and primates, the primate SVZ is substantially thicker than that of rodents and split into an outer SVZ (oSVZ) and an inner SVZ (iSVZ)[21] (Figure 2(c) and (d)). The thickness of the oSVZ increases massively during the neurogenic period, whereas that of the iSVZ does not change that much. Therefore, the oSVZ, in particular, has been implicated in the expansion of the neocortex in primates.[21–23,31–34]

PROGENITOR CELL CLASSES, TYPES, AND MODES OF DIVISION

NPCs in the embryonic/fetal neocortex fall into two major classes (Figure 3) that can be distinguished by cell biological features, notably cell polarity[32] (see also a recent discussion about NPC nomenclature[35]). NPCs that have apical junctions and thus are inserted into the apical junctional belt and that undergo mitosis at the apical surface are referred to as apical progenitors (APs).[32] The other class of NPCs undergoes mitosis at a nonapical, that is, basal location and are delaminated from the apical junctional belt. These NPCs are referred to as BPs.

Another NPC classification is based on cell lineage (Figure 3). Primary NPCs divide to produce (1) primary NPCs (self-renewal), (2) secondary NPCs, and/or (3) neurons. During this process, they undergo symmetric or asymmetric cell division. Secondary NPCs produce secondary NPCs and/or neurons, undergoing symmetric or asymmetric divisions. Prior to neurogenesis, there is only one type of primary NPC, the NECs, which belong to the APs. With the onset of neurogenesis, the primary NPC type are the aRGCs (which are also APs), and the secondary NPCs comprise both APs, that is, apical intermediate progenitor cells (aIPs), and BPs, that is, basal RGCs (bRGCs) (for their origin in the developing ferret neocortex, see Ref 36) and basal intermediate progenitors (bIPs). Implicit in this classification is that there are no basal primary NPCs. Although bRGCs share some morphological features and marker expression with aRGCs, a recent study showed that

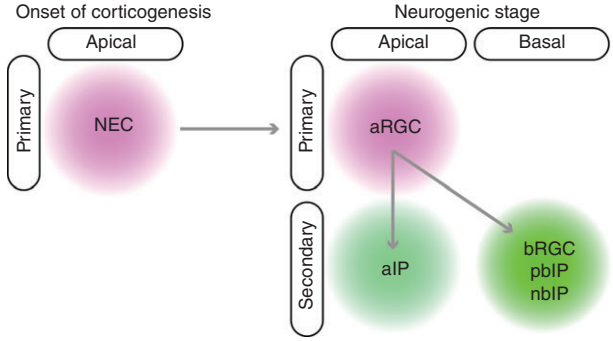

FIGURE 3 | Classification of neural progenitor cells in the developing neocortex (For details, see text).

bRGC morphology is more variable than originally assumed and that there are transitions between bRGCs and bIPs.[27,37] In this section, we review key features of the various types of APs and BPs as well as primary and secondary NPCs.

Apical Progenitors

Neuroepithelial Cells

The initial APs and primary NPCs of the early embryonic brain, the NECs (reviewed in Refs 1,2), constitute a monolayer that forms a pseudostratified epithelium. As already briefly mentioned above, NECs exhibit overt apico-basal polarity, with an apical surface, apical junctions and contact with the basal lamina. Initially, NECs undergo symmetric division to expand their number. Subsequently, they switch to asymmetric division and generate a self-renewed NEC or aRGC and a preplate neuron or BP.

During the cell cycle, NECs exhibit a characteristic movement of their nucleus called interkinetic nuclear migration (INM) (reviewed in Refs 38,39) (Figure 2(a)). During G1, NEC nuclei migrate from the apical (ventricular) surface toward the basal side, where they undergo S phase. During G2, the nuclei return to the apical side where they undergo mitosis. The nuclei of the daughter cells arising from NEC division, irrespective of cell type, migrate again to the basal side. INM is responsible for the pseudostratification of the neuroepithelium. Interestingly, when NECs are experimentally manipulated to lose basal lamina contact and to retract their basally directed process, they no longer undergo proper INM.[40] Instead, they remain at the apical side during all phases of the cell cycle, and thus the apical cell density is increased. As a consequence, there is not enough space for cell division apically, and the NPCs delaminate from the apical surface. These results nicely support the notion[23] that INM ensures that there is sufficient space at the apical side for mitosis. Both actomyosin- and microtubule-dependent mechanisms are known to be involved in INM (reviewed in Ref 41).

Apical Radial Glial Cells

Concomitant with NECs starting to generate preplate neurons, they begin to transform into aRGCs. aRGCs share key features with NECs such as apical-basal polarity (apical plasma membrane, apical junctions and basal lamina contact), INM and PAX6[42], and nestin[9] expression.[2] However, there are also notable differences between NECs and aRGCs. One is the expression of astroglial markers in aRGCs, including brain lipid-binding protein (BLBP),[43,44] glutamate aspartate transporter (GLAST),[45] vimentin,[46] tenascin-C[47] and glial fibrillary acidic protein (GFAP)[6] (not in rodent[48,49]). Even though the significance of the expression of these proteins in aRGCs but not NECs is not fully understood, these proteins serve as useful markers for aRGCs.

Another notable difference between NECs and aRGCs is the presence, in the latter cells, of a basal process that traverses the layers basal to the VZ (SVZ, IZ, CP). Of note, the aRGC nucleus and the Golgi apparatus are excluded from this basal process.[50] In considering these differences between NECs and aRGCs, it should be noted that the appearance of aRGC-specific features is a gradual process, and that it is not always possible to firmly determine whether a given single cell is still a NEC or already an aRGC.

Finally, NECs and aRGCs are also thought to differ with regard to the mode of cell division and the type of daughter cells produced. Thus, at the onset of neurogenesis, NECs switch to asymmetric division which generate a self-renewing aRGC and either a post-mitotic neuron or a BP.[2] In contrast, aRGCs in the course of neurogenesis undergo, to a variable extent depending on the species, asymmetric self-renewing as well as asymmetric or symmetric consumptive divisions, with the non-aRGC daughter cell(s) being more frequently a BP than a neuron.

Apical Intermediate Progenitors

Apical intermediate progenitors (aIPs), previously known as short neural precursors, constitute an AP-type of aRGC progeny.[51,52] aIPs show INM, undergo mitosis at the apical surface and possess apical plasma membrane and apical junctions. However, the aIP basal process does not reach the basal lamina.[51] aIPs express PAX6 but not astroglial markers such as BLBP and GLAST, and lack t-box brain protein 2 (TBR2) expression.[52] In line with their nature as intermediate progenitors, most aIPs divide symmetrically and generate two neuronal daughter cells.[52,53]

Basal Progenitors

Basal Intermediate Progenitors

bIPs typically reside in the SVZ where they undergo mitosis.[15–17] These NPCs are no longer integrated into the apical junctional belt but have delaminated from ventricular surface. Also, as they lack an overt basal process, bIPs lack apical-basal polarity altogether. Based on differences in their proliferative

capacity, two subtypes of bIPs can be distinguished. One has high proliferative capacity and is referred to as proliferative bIP (pbIP, previously also considered to be the archetype of a transient amplifying progenitor cell). pbIPs amplify in number by symmetric cell divisions. The other bIP subtype, called neurogenic bIP (nbIP), is less proliferative and undergoes symmetric division to generate two neurons. nbIPs are thought to contribute most cortical neurons in rodents[15–17,19] (reviewed in Ref 54). In rodents, most of bIPs express TBR2 and NEUROG2, but not PAX6.[55,56] In contrast, bIPs in developing gyrencephalic neocortex may sustain PAX6 expression, in line with their increased proliferative capacity.[27,57] In species in which an iSVZ and an oSVZ can be distinduished, TBR2+ bIPs are found in both of these germinal zones.[58]

Basal Radial Glial Cells

bRGCs were originally characterized in the developing gyrencephalic neocortex, notably human and ferret,[24–26] and later also in rodents.[59,60] bRGCs[61] are also called outer radial glia[24] or translocating RGC[58] and have previously also been referred to as intermediate radial glia cell[26] (IRGC). bRGCs share certain morphological features with aRGCs. Specifically, most bRGCs characteristically extend a basal process toward the basal lamina and thus possess clear cell polarity. However, bRGCs do not contact the ventricular surface and lack proper apical cell polarity.[24–27]

In terms of marker expression, bRGCs are highly related to aRGCs[24–26], expressing PAX6 and astroglial markers (notably GFAP in primates). In addition, given that a defining feature of bRGCs is the presence of a radial fiber at mitosis, a useful tool to identify bRGCs is immunostaining for phosphorylated vimentin.[25,26] This is so because vimentin is phosphorylated at S55 by CDK1 at prometaphase,[62] and phosphorylated vimentin is found in the radial fiber. Another commonly used marker of rodent bIPs, TBR2, is also expressed in a fraction of primate bRGCs.[24,25,27] Moreover, this marker has revealed differences between mouse and human bRGCs. Specifically, a greater proportion of mouse than human bRGCs express TBR2 protein.[25,37] Consistent with this difference, a recent transcriptome analysis of human and mouse cortical NPCs showed that human aRGCs and bRGCs exhibit more similar gene expression patterns than mouse aRGCs and bRGCs, with mouse bRGCs being more similar to neurons and bIPs.[37]

A recent study[27] has shown that the morphology of bRGCs in the primate oSVZ is more diverse than originally assumed. Thus, in addition to the 'classical' bRGC that is characterized by a basal process at mitosis, bRGCs with a basal and an apical process (also referred to as bipolar RG[63]) and bRGCs with only an apical process at mitosis were also observed.[27] Moreover, the lineage relationships of the various subtypes of bRGCs and bIPs as revealed by live imaging in primate organotypic slice culture have been shown to be diverse.[27] Specifically, all these BP

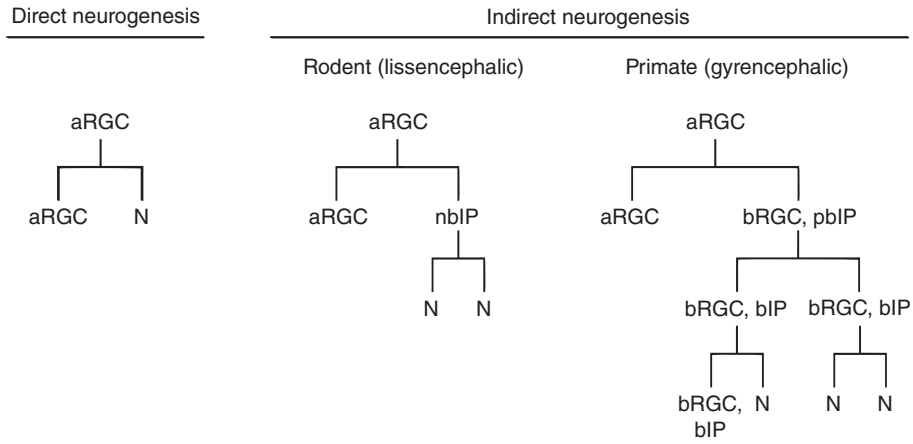

FIGURE 4 | Selected examples of canonical neural progenitor cell lineages to neurons in developing neocortex. Left: direct neurogenesis by asymmetric division of an aRGC. Middle and right: indirect neurogenesis. Middle: in a lissencephalic rodent, an aRGC undergoes asymmetric division to yield an nbIP, which undergoes symmetric consumptive division to yield two neurons (N). Right: in a gyrencephalic primate, an aRGC undergoes asymmetric division to yield a bRGC or pbIP, which undergoes symmetric proliferative division to yield two BPs, bRGCs or bIPs. These then undergo either asymmetric or symmetric division to yield neurons.

subtypes have the potential to generate neurons, and each of them can convert into virtually any of the other subtypes.[27] These crucial observations called for a revision, at least for primates, of the concept in which the major lineage of neurogenesis was thought to be bRG→bIP→neuron.

In addition to these morphological aspects, the expression of molecular markers in bRGCs versus bIPs is less distinct in primates than in rodents. Thus, in the primate oSVZ, the vast majority not only of bRGCs but also of bIPs express PAX6 protein. Conversely, not only most bIPs, but also a significant proportion of bRGCs express TBR2, that is, together with PAX6.[25,27]

During M-phase, bRGCs show a characteristic nuclear movement called mitotic somal translocation (MST).[24] The cell soma rapidly moves in the basal[24]or apical[27] direction just before cytokinesis. Basally directed MST requires non-muscle myosin II, similar to the basally directed INM of aRGCs and the basally directed nuclear migration during BP delamination,[64] and involves the RhoA–Rho-kinase pathway.[65] The biological significance of MST is not yet fully understood. A possible, intriguing role is its involvement in the radial expansion of OSVZ.[66]

INCREASING NEURON PRODUCTION–FROM DIRECT TO INDIRECT NEUROGENESIS

The various NPC lineages underlying neurogenesis crucially determine the number of cortical neurons produced. In rodents, two principal ways of generating neurons in the neocortex have been described, referred to as direct and indirect neurogenesis (Figure 4) (reviewed in Refs 34,54). A typical example of direct neurogenesis has been observed at the onset of neurogenesis, when a NEC (or aRG) undergoes asymmetric cell division to produce an aRG and a neuron. Direct neurogenesis therefore generates only one neuron per AP mitosis, which is sufficient for neurogenesis in, for example, the spinal cord or for the generation of preplate neurons in the neocortex. However, direct neurogenesis is not sufficient to generate the bulk of the neurons in the rodent neocortex, which is achieved via indirect neurogenesis.[18,54,67] Here, an aRG undergoes asymmetric cell division to self-renew and to produce a secondary NPC, which typically is an nbIP that divides in a self-consuming manner to generate two neurons.[15–17]

To produce the massively increased number of neurons in the primate neocortex, which (as is the case for human) can be up to >1000-fold greater than in mouse,[67–69] indirect neurogenesis has evolved to comprise diverse lineages of cortical NPCs that ultimately result in neuron output. Importantly, these lineages include those involving symmetric proliferative divisions of BPs (both bRGCs and pbIPs).

Direct versus indirect neurogenesis and the various lineages of the latter have interesting implications for the cell polarity of newly generated neurons, which after maturation exhibit axon-dendrite polarity.[70] In the case of direct neurogenesis, the newborn neuron can inherit apical and/or basal polarity from the NEC/aRGC mother cell, which may provide cues for the subsequent development of neuronal polarity.[13] By analogy, neurons produced by indirect neurogenesis from bRGCs may inherit certain polarity cues, depending on the morphotype of the mother bRGC. In contrast, neurons produced by indirect neurogenesis from nbIPs, which lack apical-basal polarity,[18] do not inherit cell polarity cues and thus develop neuronal polarity *de novo*[15,71] (Figure 2 (c) and (d)).

EVOLUTION OF THE NEOCORTEX – EXPANSION OF BASAL PROGENITORS

When comparing a typical rodent brain such as mouse or rat and a human brain macroscopically, striking differences in two major parameters are immediately apparent—cerebral cortex size and the degree of cerebral cortex folding (i.e., lissencephalic versus gyrencephalic). Thus, the human cerebral cortex is more than 7000 times larger than that of the mouse[67,69] and contains more than 1000 times the number of neurons of the mouse cerebral cortex.[67,69] Before addressing the issue of how the developing human cerebral cortex generates such a large number of neurons, we would like to briefly comment on the topic of areal complexity of the neocortex.[72,73]

In addition to the macroscopical differences (cerebral cortex size and the degree of cerebral cortex folding), there is another significant difference between a rodent brain and the human brain—the number of functional cortical areas. Brodmann divided the human neocortex into 47 areas based on their cytoarchitecture.[74] Also in the neocortex of some rodents, a similar number of cytoarchitecturally distinct areas can be distinguished.[75] However, the number of functional areas is massively increased in primates, especially in great apes and human. For example, there are no areas in rodents equivalent to the language-related areas of Broca and Wernicke.[76] Moreover, different cell cycle kinetics of NPCs have been shown to underlie the distinct cytoarchitecture

of different cortical areas.[22,77–79] In addition, the gene expression patterns are different between prospective gyri and sulci (see below). These results suggest that regional specification is already established at the developmental stage, in line the cortical protomap concept.[72,80]

How, then, does the developing human cerebral cortex generate such a large number of neurons? There are two parameters that, as a matter of principle, determine the number of neurons generated during cortical development. First, the length of the neurogenic period, at least for species that use essentially the same neurogenic program (i.e., similar progenitor-to-neuron lineages, similar progenitor cell cycle length). Second, the number of neuron-generating divisions of cortical NPCs per unit time and their mode of division. Regarding the second parameter, the pool size of neurogenic NPCs is a major determinant of the number of neuron-generating divisions. This pool size, in turn, reflects the overall pool size of all NPCs and their various modes of cell division, as is discussed below.

A recent study suggests that the length of the neurogenic period may be sufficient to explain the increase in cortical neuron number during evolution.[67] In this study, mammals were found to fall into two main groups with regard to the degree of cerebral cortex folding: those with a gyrencephaly index (GI) of >1.5 (high GI group) and those with a GI of ≤1.5 (low GI group). The high GI group contains many monkey and all ape and cetacean species, whereas the low GI group includes all rodents and marsupials. Mathematical modeling showed that, for either the low GI or the high GI group, an increase in the length of the neurogenic period alone can explain the increase in neuron number across species in the respective group. Of note, the conclusion drawn from this modeling is consistent with the results of two experimental studies. One study has proposed that the longer neurogenic period in primates allows for a substantially greater number of rounds of cell divisions as compared to rodents.[81] The other study has shown that prolongation of the neurogenic phase of NPCs by inactivation of the polycomb group complex in embryonic mouse neocortex resulted in an increase in upper-layer neurons and the radial thickness of the upper cortical layers,[82] two hallmarks of the evolutionary expansion of the neocortex.

The pool size of neurogenic NPCs is largely determined by two parameters. One is the number of NECs at the onset of neurogenesis. As estimated in a previous study,[67] this number in human is almost 100 times larger than in the mouse. However, this difference in NEC number alone does not suffice to explain the 1000-fold greater number of neurons in the human neocortex as compared to mouse. An additional, second parameter is required to achieve the neuron number in human, which concerns the lineages from NECs to neurogenic NPCs. Specifically, human and other primates increase the neurogenic NPC pool size by using lineages that involve symmetric proliferative divisions of BPs.[27,31,67] Of note, mathematical modeling has shown that if the mouse were to adopt such human-type combination of lineages, this would be more efficient for generating large number of neurons than increasing NEC number or lengthening the neurogenic period.[67]

The use of symmetric proliferative divisions of BPs reflects the intrinsic advantage that BPs offer over APs to expand the NPC pool. Because of the space limitation for mitosis at the apical surface of the cortical wall, the expansion of the NPC pool occurs in the SVZ rather than that in the VZ[40] and hence rests upon BP proliferation. As described above, at least three BP subtypes with regard to the progeny produced can be distinguished: bRGCs pbIPs, and nbIPs. Of these, pbIPs and bRGCs have proliferative capacity that results not only in their self-renewal but also in their expansion. Indeed, the number of pbIPs and bRGCs have been found to be massively increased in the gyrencephalic species studied so far in detail, notably the ferret and primates such as macaque monkey and human.[24–27]

It should be noted, however, that an expansion of BPs with proliferative capacity does not necessarily result in gyrification. Experimental manipulation of specific genes that regulate BP proliferation has been found to induce gyrification of the mouse neocortex in some,[37,83] but not all,[84] cases. A possible, if not probable, explanation may be that BP expansion will only result in gyrification if bRGCs and not only pbIPs are increased.[85] Consistent with this interpretation, increasing BP proliferation by overexpression of cell cycle regulators in mouse neocortex neither increased bRGC abundance nor resulted in cortical folding,[84] whereas a human-like downregulation of TRNP1[83] or overexpression of human-specific ARHGAP11B,[37] discussed in more detail below, achieved both effects. Furthermore, increasing BP proliferation by overexpression of cell cycle regulators in developing ferret neocortex, which in contrast to mouse contains bRGC at relatively high abundance, did induce extra cortical folding.[84]

In this context, it is interesting to note that NPCs exhibiting bRGC-like morphology and marker expression have been observed in the developing dentate gyrus not only of nonhuman primates[86] but also of rodents.[87–91] Given that the dentate gyrus belongs

to the archicortex, and in light of the fact that the phylogenetic branches leading to the ferret and sheep, in which bRGCs constitute a substantial proportion of BPs,[25,26,63] separated from the phylogenetic branches leading to monkeys, apes and human about 100 million years ago, it appears that bRGCs may well be an ancestral type of NPC. Consistent with this notion, a recent study has shown the presence of NPCs with bRGC-like morphology in the developing pallium of nonmammalian vertebrates such as birds.[29] Together, these findings raise the possibility that for neocortical expansion to occur, the crucial parameter may not be the absence or presence of bRGCs as such, but their abundance.[26]

KEY GENES FOR BP EXPANSION

Several recent studies have identified key genes responsible for BP expansion, by focusing on differences between primate and rodent BPs as revealed by genomics and transcriptomics. Thus, transcriptomic studies have identified genes differentially expressed between mouse and human NPCs or germinal zones.[37,92–94] Here, we concentrate on two such studies, which also comprise in-depth functional analyses of the respective genes in neocortical development.

The first study to be discussed concerns platelet-derived growth factor (PDGF) signaling. Comparative transcriptomics of proliferative aRGCs and nbIPs from mouse embryonic neocortex had pointed to a potential role of PDGF signaling in NPC proliferation.[95] Indeed, a recent study has provided compelling evidence for a role of human PDGFD and PDGF receptor β (PDGFRβ).[92,93] Specifically, in the fetal human neocortex, *PDGFD* and *PDGFRB* are prominently expressed in the VZ and SVZ.[92,93] PDGF was originally identified as a mitogen for fibroblasts and smooth muscle cells (reviewed in Ref 96) and shown to be also involved in tumor growth and metastasis (reviewed in Ref 97). PDGFD is the most recently identified PDGF family member.[98,99] PDGFD binds to the homodimer of PDGFRβ or the heterodimer of PDGFRα/β and activates several downstream signaling pathways (reviewed in Ref 97). In organotypic slice culture of fetal human neocortex, inhibition of PDGFRβ by chemical inhibitors decreased the proliferation of SOX2-positive aRGCs and bRGCs and of TBR2-positive BPs.[93] Conversely, ectopic expression of a constitutively active form of PDGFRβ in mouse embryonic neocortex induced NPC proliferation and an increased appearance of SOX2-positive bRGCs. It remains to be established whether the latter reflected an increased proliferation of bRGCs or an increased delamination of aRGCs. Interestingly, a previous study showed that PDGFD promotes the epithelial-mesenchymal transition (EMT) of cancer cells.[100] Because the generation of BPs, notably their delamination, shares certain features with EMT,[101] the role of PDGFD-PDGFRβ signaling in BP generation and amplification in developing human neocortex may well involve EMT-related processes.

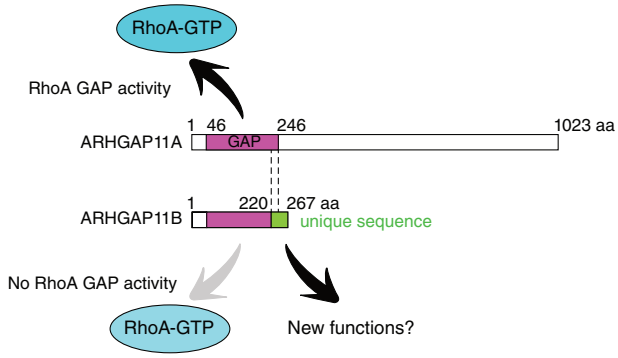

FIGURE 5 | ARHGAP11B. Cartoon showing key differences between ARHGAP11A and ARHGAP11B. ARHGAP11A contains a complete RhoGAP domain (magenta) and exhibits RhoGAP activity toward RhoA. In contrast, ARHGAP11B, due to a frame shift-causing mutation, lacks the C-terminal 26 amino acids of the RhoGAP domain, does not exhibit RhoA-GAP activity, and contains a unique, human-specific 47 amino acid-sequence (green) following residue 220.

The second study to be discussed examined differentially expressed genes in the various cell types isolated from human and mouse developing neocortex.[37] One gene was found to be highly expressed in fetal human aRGCs and bRGCs but not human neurons, nor in any cell type of embryonic mouse neocortex (Figure 5). This gene, *ARHGAP11B*, is human-specific and arose from a partial gene duplication of the ubiquitous gene *ARHGAP11A*.[102] *ARHGAP11B* is not present in the chimpanzee genome but found in the Neanderthal and Denisovan genomes, consistent with the partial gene duplication having occurred just after the divergence of the human lineage from the chimpanzee lineage.[102–105] Ectopic expression of ARHGAP11B in embryonic mouse neocortex increased the number of proliferative BPs and was able to induce gyrification of the neocortex.[37] The molecular function of ARHGAP11B is still unclear. Being a Rho GTPase activating protein (GAP), ARHGAP11A has been implicated in the regulation of cell proliferation through its effects on RhoA activity[106–108] (Figure 5). ARHGAP11B has virtually the same amino acid sequence as ARHGAP11A until residue 220, with this N-terminal region comprising most, but not all,

of the GAP domain. However, because of a frame shift-causing mutation, ARHGAP11B lacks the C-terminal 26 amino acids of the Rho GAP domain and does not exhibit significant GAP activity toward RhoA *in vivo*.[37] Instead, ARHGAP11B contains a unique, human-specific 47 amino acid-sequence following residue 220. In light of the presumptive role of ARHGAP11B in the evolutionary expansion of the human neocortex, a major challenge of future research is to elucidate its molecular function and to determine the role of the human-specific C-terminal 47 amino acid-sequence therein.

Changes in noncoding genomic regions are also important for brain evolution.[109,110] A recent study has identified a novel human-accelerated regulatory enhancer called *HARE5*.[110] *HARE5* is an enhancer of the gene encoding the human Wnt receptor Frizzled 8 (*FZD8*). Compared with the chimpanzee's corresponding enhancer region, *HARE5* has stronger enhancer activity for *FZD8*. Thus, transgenic mice expressing mouse *Fzd8* under the human (i.e., *HARE5*) enhancer exhibit a shorter cell cycle of NPCs and an increased number of BPs as compared to transgenic mice expressing *Fzd8* under the chimpanzee enhancer or wildtype mice. At a later stage of corticogenesis, *HARE5-Fzd8* transgenic mice show enlargement of the neocortex and increased upper layer neurons. These findings not only support the notion of a role of Wnt signaling in brain size,[111] but also implicate *HARE5* as an important regulatory element in the evolutionary expansion of the human neocortex. Moreover, the increase in BPs in the *HARE5-Fzd8* transgenic mice is consistent with two previous lines of investigation. First, that mouse nbIPs downregulate wnt signaling as compared to proliferative aRGCs.[95] Second, that *Fzd8* is expressed in the mouse VZ but barely in the mouse SVZ, whereas *FZD8* expression is not only high in the human VZ but sustained in the human iSVZ and oSVZ.[92]

Complementing the analyses dissecting differential gene expression between human and rodents, a recent study of developing ferret neocortex has screened for genes differentially expressed between VZ and SVZ and between a prospective gyrus and sulcus.[112] This revealed several prospective gyrus-enriched genes (*Fgfr2*, *Fgfr3*, *Lhx2* and *Eomes/Tbr2*) and prospective sulcus-enriched genes (*Cdh8* and *Trnp1*), that exhibited local differential expression patterns also in developing human neocortex. In contrast to ferret and human, these genes showed either a uniform expression or a simple rostral-caudal gradient in embryonic mouse brain. These results indicate the existence of 'cortical protomap' before gyrification takes place. Of note, experimental inhibition (expression of dominant-negative TBR2) or activation (administration of the FGFR3 ligand FGF8) of these prospective gyrus-enriched molecules in the developing ferret neocortex resulted in reduced or increased gyrification, respectively.[113,114]

Of note, a previous study has addressed the role of TRNP1, a regulator of transcription, in mouse cortical development.[83] Overexpression of TRNP1 increased aRGCs and decreased BPs. In contrast, knockdown of TRNP1 increased BPs, notably bRGCs, and decreased aRGCs. Importantly, the TRNP1 knockdown region showed gyrification. These results suggest that differential TRNP1 expression may have a key role in human neocortex folding.

CONCLUSION

The past one and a half decades have witnessed an unsurpassed finesse in the dissection of the major classes and various types of cortical NPCs, both in terms of their cell biology and molecular signatures. It has become clear that cortical expansion is linked to an increase in the abundance and proliferative capacity of BPs, notably bRGCs. By virtue of BPs being delaminated from the ventricular surface, this class of NPCs overcomes the constraint of undergoing apical mitosis at the rather limited space provided by the ventricular surface, as originally proposed by Smart.[115,116] Two principal parameters have been identified as being crucial for cortical expansion, that is, lengthening the neurogenic period and increasing the efficacy of neuron output per unit time. The latter is largely achieved by introducing symmetric proliferative BP divisions into the various lineages from the primary cortical progenitors, that is, NECs and aRGCs, to cortical neurons. Importantly, although cortical expansion tends to be accompanied by an increase in gyrification, these two parameters are not correlated to each other in a simple, linear manner. Thus, the human neocortex has tripled in size compared to that of the chimpanzee, without a corresponding increase in the gyrification index. In line with this consideration, not only specific gene expression programs[112] but also physical forces[117] have been implicated in cortical folding.

Comparative genomic and transcriptomic approaches have led to the identification of human-specific gene expression features, or genes or genomic sequences, that underlie cortical expansion. The former include *Trnp1*, *PDGFD/PDGFRB*, and *PAX6*,[118] the latter *ARHGAP11B* and *HARE5*. Several technological advances will further our efforts to

gain insight into the molecular basis underlying cortical expansion. First, the establishment of 3D *in vitro* systems derived from iPS cells (cerebral organoids)[119] will allow us to identify differences in NPC behavior between the great apes and human,[120,121] and to dissect NPC defects causing disorders of cortical development such as microcephaly.[122] Second, complementing experimental data sets based on transcriptomics, live imaging, histology and cell cycle analyses with mathematical modeling is likely to contribute further conceptual insight into the principles underlying cortical expansion.[24,37,57,67,92–94,123–125] Third, the ability to examine, with regard to their functional effects on NPCs, not only a few selected genes, but complex mixtures of them by microinjection into aRGCs in organotypic slice culture[126,127] (despite the limitations inherent in such in vitro approaches) should help us to overcome the limitations that have been intrinsic to canonical approchaes (e.g., transgenesis) in uncovering additive or synergistic effects of multiple factors. Thus, a promising platform has been established to gain a comprehensive and integrative understanding of the molecular basis that underlies the evolutionary expansion of the human neocortex and that provides a framework for our cognitive abilities.

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