

REVIEW

Neural progenitors, neurogenesis and the evolution of the neocortex

Marta Florio and Wieland B. Huttner*

ABSTRACT

The neocortex is the seat of higher cognitive functions and, in evolutionary terms, is the youngest part of the mammalian brain. Since its origin, the neocortex has expanded in several mammalian lineages, and this is particularly notable in humans. This expansion reflects an increase in the number of neocortical neurons, which is determined during development and primarily reflects the number of neurogenic divisions of distinct classes of neural progenitor cells. Consequently, the evolutionary expansion of the neocortex and the concomitant increase in the numbers of neurons produced during development entail interspecies differences in neural progenitor biology. Here, we review the diversity of neocortical neural progenitors, their interspecies variations and their roles in determining the evolutionary increase in neuron numbers and neocortex size.

KEY WORDS: Basal radial glia, Evolution, Neocortex, Neural progenitors, Neurogenesis, Outer subventricular zone

Introduction

The neocortex – the outer covering of the cerebral hemispheres – is a structure unique to, and a hallmark of, mammals. Since its relatively recent evolutionary origin in the mammalian ancestor ~220 million years ago (Meredith et al., 2011; O’Leary et al., 2013), the neocortex has undergone dramatic expansion, independently, in several mammalian lineages (Borrell and Reillo, 2012), reflecting a positive selection for increased neuron numbers. Neocortical expansion is particularly evident in anthropoid primates, notably humans, in which the neocortex constitutes up to two-thirds of the overall brain mass and contains ~16 billion neurons (Azevedo et al., 2009). In search of the ontogenetic mechanisms underlying neocortex expansion, recent studies have shed light on interspecies differences in neurogenesis – the process by which neurons are generated from different classes of neural progenitor cells (NPCs; which include neural stem cells) during development. These differences primarily concern the proliferative capacity, the abundance, and the lineage relationships of specific types of NPCs.

In this Review, we focus on the diversity of mammalian NPCs, their modes of cell division and their variation across species, which might account for neocortical expansion during evolution. In addition, focusing on the divergence between rodent and primate lineages, we discuss specific cell biological features underlying NPC interspecies variations. Finally, we place existing data into the context of influential theories of neocortex development and evolution.

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, Dresden 01307, Germany.

*Author for correspondence (huttner@mpi-cbg.de)

Cytoarchitecture of the neocortex

A common cytoarchitectural design: columns, layers and areas

The neocortex consists of a thin mantle of gray matter (GM) enclosing the underlying white matter (WM) (Fig. 1). The GM comprises a network of glutamatergic and GABAergic neurons (which contribute ~70-80% and ~20-30% of the total neocortical neurons, respectively), supported by glial cells and blood vessels. The WM consists of myelinated axons stemming from, or directed to, the overlying GM. Here, in reviewing neurogenesis, we confine our discussion to the origin of glutamatergic neurons.

In the radial dimension of the neocortex, glutamatergic neurons are arranged in columns (Mountcastle, 1997), which work modularly as functional microunits, with neurons in a given column stereotypically interconnected (Rakic, 2008). In the lateral dimension, neurons are arrayed in six laminae or layers (I-VI, with I being the outermost and VI the innermost). Within each layer, glutamatergic neurons tend to share similar cell type identities and connectivity patterns: deep layer (VI-V) neurons preferentially connect with subcortical structures, whereas upper layer (IV-II) neurons mostly establish contralateral or ipsilateral cortico-cortical connections (Douglas and Martin, 2004). Regional discontinuities in neuron type, density and connectivity subdivide the GM into a number of distinct areas, each representing a functionally specialized cortical field.

Interspecies variations in neocortex morphology

The columnar, laminar and area design of the neocortex is conserved in all mammalian species (Mountcastle, 1997). Yet, as discussed below, in compliance with these cytoarchitectural principles, the neocortex underwent substantial morphological transformations during mammalian evolution.

Neocortex expansion

Even a cursory anatomical comparison of the human and mouse neocortex reveals two striking differences (Fig. 1). First, the human neocortex is ~1000 times larger than that of the mouse. Second, whereas the surface of the mouse neocortex is smooth (lissencephalic), the surface of the human neocortex is highly convoluted (gyrencephalic), featuring a complex pattern of fissures (sulci) and ridges (gyri). Extending the comparison to other mammalian species, these differences can be generalized. First, the size of the neocortex varies remarkably among species. Notably, such variation primarily concerns the surface area of the neocortex, rather than its thickness, which varies only modestly across species (Rakic, 2000, 2009; Falk and Gibson, 2008), suggesting an evolutionary constraint to growth in the radial dimension. Second, gyration correlates with neocortical enlargement (Borrell and Reillo, 2012; Lewitus et al., 2013): in all mammalian orders, as the surface area of the cortex increases, so does its folding. This folding appears to be an evolutionary solution to the problem of increasing cortical surface area (i.e. neuron numbers) without prohibitively increasing the size of the skull (Kelava et al., 2013; Zilles et al., 2013). In order to fold, the neocortex needs to be

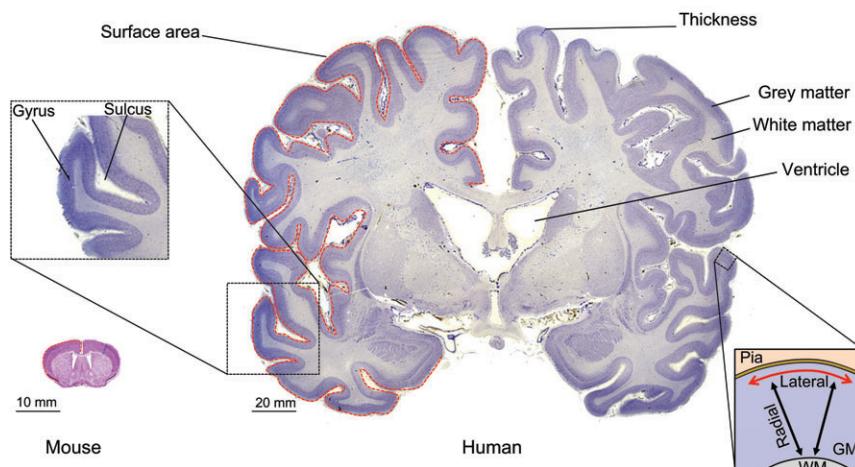


Fig. 1. Neocortex morphology. Coronal sections of the mouse (left) and human (right) adult neocortex are shown. Red dashed lines highlight the contour of the pial surface of the gray matter (GM). Blue dashed lines highlight neocortical thickness. The inset on the left shows an area of the human neocortex at higher magnification, highlighting a neocortical gyrus that is contiguous to a sulcus. The inset on the right outlines the principal dimensions by which the adult neocortical GM is described: (1) radial (black arrows), i.e. along the white matter (WM)-to-pia axis, corresponding to the apical–basal axis in terms of tissue polarity; and (2) lateral (red arrow), i.e. along the axis perpendicular to the radial axis. Mouse neocortex adapted with permission from the High Resolution Mouse Brain Atlas (Sidman, R. L., Kosaras, B., Misra, B. M. and Senft, S. L., 1999), <http://www.hms.harvard.edu/research/brain/>; human neocortex adapted with permission from <http://www.brains.rad.msu.edu> and <http://brainmuseum.org> (supported by the US National Science Foundation).

pliable. Hence, a relatively thin GM mantle might have permitted the evolution of neocortical gyration, which in turn is conducive to neocortical expansion. Finally, it is important to note that, while neocortical layering as such is relatively uniform across species, the proportion of GM devoted to upper versus deep layers differs considerably among orders; upper layers occupy almost half of the GM thickness in primates but only a fifth in rodents (DeFelipe et al., 2002; Kriegstein et al., 2006).

Scaling rules and neuron numbers

Often envisioned as a ‘hallmark of human evolution’, neocortical expansion is in fact characteristic of mammalian evolution as a whole, and the neocortex enlarged several times, independently, in most mammalian orders (Striedter, 2005; Lewitus and Kalinka, 2013; Lewitus et al., 2013). In the primate lineage, however, neocortical expansion occurred at a faster pace and in accordance with species-specific morphometric rules.

In most mammalian orders, the size of the neocortex tends to scale allometrically with brain size. This means that, during evolution, as the volume of the brain increases the neocortex enlarges disproportionately compared with most other parts of the brain, becoming its largest portion (Finlay and Darlington, 1995; Striedter, 2005) – a process known as neocorticalization. Although neocorticalization is a widespread mammalian trait, neocorticalization in primates is characterized by a steeper slope than in other mammals (Barton and Harvey, 2000), making primates, on average, more neocorticalized than any other mammals with equivalent brain size (Jerison, 1973; Striedter, 2005). The extent of neocorticalization is further increased in anthropoid primates, especially humans, in which the neocortex constitutes up to ~80% of the brain mass (Stephan et al., 1981; Striedter, 2005).

Intuitively, a larger neocortex contains more neurons than a smaller one, and, conceivably, a positive selection for increased neuron numbers might underpin the evolutionary success of an expanded neocortex. However, neuron numbers relative to neocortical volume (i.e. neuron density) varies considerably across mammalian orders. In rodents and insectivores, as neocortical size increases, neuron density tends to decrease owing to a corresponding increase in cell size (DeFelipe et al., 2002; Herculano-Houzel et al., 2006; Sarko et al., 2009; Herculano-Houzel, 2012). In primates, by contrast, neuron density remains relatively constant as neocortical size increases (Charvet et al., 2013), with no correlative increase in cell size (Herculano-Houzel et al., 2007; Herculano-Houzel, 2009).

Accordingly, primates appear to follow a different set of scaling rules than rodents, such that neocortical neuron numbers increase at a higher rate along with enlargement of the brain (Herculano-Houzel et al., 2007; Azevedo et al., 2009). Notably, humans conform to these rules.

Neural progenitor cell types

The above mentioned changes in neocortex morphology partially reflect differences in the biology of different classes of NPCs that operate during neocortical neurogenesis. From a cell biological perspective, three principal classes of NPCs exist in the developing mammalian neocortex: apical progenitors, basal progenitors and subapical progenitors (Fig. 2A,B). Below, we provide a brief description of each of these NPC types and subtypes (summarized in Fig. 3), which is crucial for understanding their interspecies variations.

Apical progenitors

Three types of apical progenitors (APs) have been described: neuroepithelial cells, and the derivative apical radial glia and apical intermediate progenitors.

Neuroepithelial cells

All neurons of the mammalian neocortex ultimately originate from neuroepithelial cells (NECs), which are the cells that initially form the columnar monolayer epithelium constituting the neural plate and, subsequently, the pseudostratified epithelium that constitutes the early neural tube. NECs are polarized along their apical–basal axis and span the entire width of the neuroepithelium, resting on the overlying basal lamina with their basal plasma membrane, and facing the lumen of the neural tube with their apical plasma membrane (Huttner and Brand, 1997; Götz and Huttner, 2005). Adherens junctions (AJs) are located at the apicalmost end of the NEC lateral membrane (Aaku-Saraste et al., 1996; Chenn et al., 1998; Marthiens and ffrench-Constant, 2009), where they form AJ belts that link neighboring NECs to each other.

NECs initially undergo symmetric proliferative divisions (Rakic, 1995) (for more details see Box 1). This expands their population, which has two effects on the growth of the neocortex: (1) expansion in the lateral dimension and (2) growth in the radial dimension, i.e. thickening of the neuroepithelium (Fig. 2C). As NECs progress (asynchronously) through the cell cycle, their nucleus migrates through the cytoplasm along the apical–basal axis of the cell, with mitosis taking place at the ventricular surface. As a consequence, at

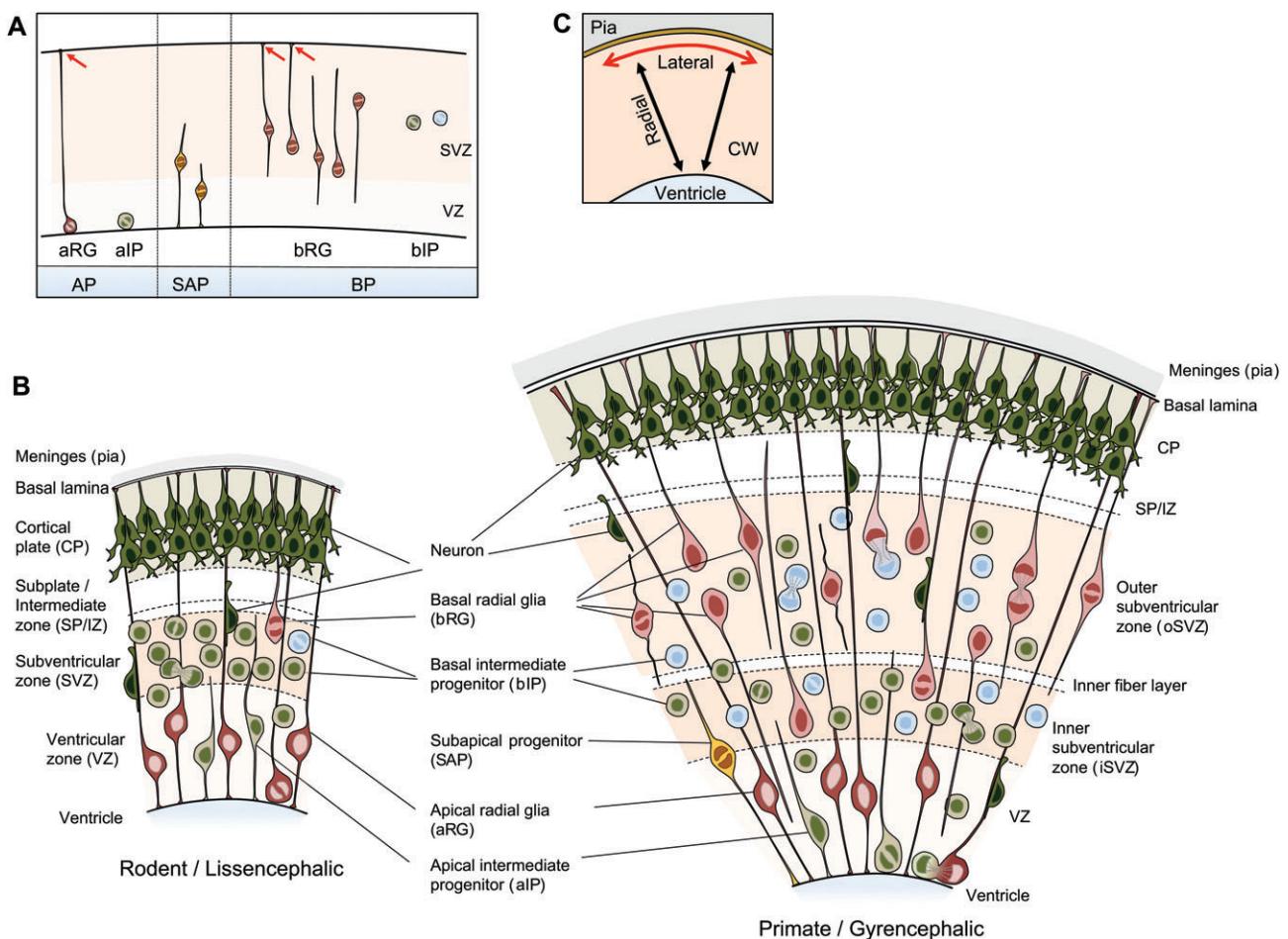


Fig. 2. NPC types in the developing neocortex of representative lissencephalic and gyrencephalic species. (A) NPC types in the mammalian neocortex after the onset of neurogenesis, classified according to cell polarity, the presence of ventricular contact, and the location of mitosis. Red arrows indicate contact of the basal process with the basal lamina. Apical progenitors (APs), which include apical radial glia (aRG) and apical intermediate progenitors (aIPs), are defined by mitosis occurring at the ventricular surface and the presence of ventricular contact. Note that neuroepithelial cells (NECs), the primary APs that give rise to aRG and aIPs, are not depicted, as NECs prevail prior to the onset of neurogenesis. Subapical progenitors (SAPs) are defined by mitosis occurring at an abventricular location and the presence of ventricular contact. Basal progenitors (BPs), which comprise basal radial glia (bRG) and basal intermediate progenitors (bIPs), are defined by mitosis occurring at an abventricular location and the absence of ventricular contact. bRG subtypes (see Box 2 for more information) are also shown: proliferative bIP, blue; neurogenic bIP, green. (B) Coronal section of developing neocortex from a representative lissencephalic species, such as mouse or rat (left), and a representative gyrencephalic species, such as ferret or human (right), depicting the NPC types frequently observed in each of the germinal zones. (C) The major dimensions in which the developing neocortex is described: (1) radial (black arrows), i.e. the ventricle-to-pia axis, corresponding to the apical-basal axis in terms of tissue polarity; and (2) lateral (red arrow), i.e. the axis perpendicular to the radial axis. CW, cortical wall.

any given time NEC nuclei are found at different positions along the apical-basal axis of the neuroepithelium. This process, called interkinetic nuclear migration (INM) (Sauer and Walker, 1959; Taverna and Huttner, 2010), confers the neuroepithelium its characteristic pseudostratification, which allows for increased numbers of NECs to be accommodated and to proliferate per unit of ventricular surface.

Cortical neurogenesis begins with single NECs switching to asymmetric differentiation (Box 1) cell division (Götz and Huttner, 2005; Huttner and Kosodo, 2005). This mode of division results in: (1) self-renewal of the epithelial mother cell, as one of the daughter cells becomes either an NEC or an apical radial glia (i.e. the NPC type that NECs transform into at the onset of neurogenesis) (Hartfuss et al., 2001; Kriegstein and Götz, 2003; Götz and Huttner, 2005); and (2) the generation of a lineage-wise downstream cell type, as the other daughter cell becomes an apical intermediate progenitor, a basal progenitor or a postmitotic neuron. As a consequence, the neuroepithelium transforms into

a hybrid pseudostratified-stratified tissue in which progenitor cell bodies and neurons become spatially segregated: the cell bodies of APs are confined to the apicalmost germinal layer of the cortical wall termed the ventricular zone (VZ); the cell bodies of basal progenitors reside in, and thereby form, a second germinal layer basal to the VZ, referred to as the subventricular zone (SVZ); and newborn neurons migrate from these germinal layers in the basal direction, accumulating beneath the basal lamina in the prospective cortical plate. As the cortical wall progressively thickens, the basolateral plasma membrane of NECs remains in contact with the basal lamina, i.e. it elongates and transforms into a thin radial fiber, the so-called basal process, which serves as a scaffold for neuronal migration (Rakic, 1972).

Apical radial glia

At the onset of neurogenesis, NECs transform into a distinct but highly related NPC type: the apical radial glia (aRG) (Götz and Huttner, 2005). aRG express astrogial markers (Malatesta et al., 2000;

Cell biological properties	Neural progenitor cell types								SAP		
	AP			BP							
	NEC	aRG	aIP	bRG		bIP					
				Basal-P	Apical-P	Both-P	proliferative	neurogenic			
Location of mitosis	apical			basal				basal			
Apical domain at mitosis	yes			no				yes			
Basal lamina contact at mitosis	yes	yes	no	yes/no	no	yes/no	no		no		
Cell polarity at mitosis	apical + basal	apical + basal	apical	basal	pseudo-apical	basal + pseudo-apical	no		apical + basal		
Process retention at mitosis	basal	basal	no	basal	apically directed	basal + apically directed	no		apical + basally directed		
Nuclear movement prior to mitosis	INM	INM	INM	MST (basal direction)	MST (apical direction)	MST (both directions)	no		?		
Pax6/Tbr2 expression	Pax6	Pax6	Pax6	Pax6 (Tbr2)			Tbr2 (Pax6)	Tbr2	?		
Astroglial marker expression	no	yes	yes	yes			no	no	?		
Proliferative potential	yes	yes	no	yes			yes	no	?		

Campbell and Götz, 2002) and upregulate certain transcription factors, notably Pax6 (Götz et al., 1998; Warren et al., 1999; Estivill-Torras et al., 2002; Osumi et al., 2008). Nonetheless, aRG retain a neuroepithelial character: they are held together by, and are integrated into, AJ belts; they possess apical-basal polarity, contacting both the ventricle and the basal lamina; and they undergo INM in the VZ and mitosis at the apical surface (Götz and Huttner, 2005).

Like NECs, aRG can undergo either symmetric proliferative or asymmetric differentiative cell divisions. However, proliferative divisions prevail in NECs, and the switch to differentiative division is accompanied by transformation into aRG. By contrast, as neurogenesis progresses, aRG increasingly switch from proliferative to differentiative divisions (Götz and Huttner, 2005). This gradual switch progressively shifts the growth of the neocortex from a lateral expansion to a mixed form of expansion in both the radial and lateral-pial dimensions (Fig. 2C). In the last decade, several additional NPC types deriving (directly or indirectly) from aRG differentiative divisions have been described (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Gal et al., 2006; Stancik et al., 2010; Tyler and Haydar, 2013; Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011; Betizeau et al., 2013; Pilz et al., 2013). These differ in morphology, location of mitosis, mode of cell division, and intra- and interspecies abundance.

Apical intermediate progenitors

Apical intermediate progenitors (aIPs, also known as short neural precursors) (Gal et al., 2006; Tyler and Haydar, 2013) are the only aRG daughter cell type that exhibits the defining features of APs

(Gal et al., 2006; Tyler and Haydar, 2013): they maintain contact with the ventricular surface; are integrated into the AJ belt; express Pax6; and divide apically after one round of INM (Fig. 2A,B and Fig. 3). However, aIPs lose several typical radial glia features: their basal process detaches from the basal lamina, retracting for mitosis; they downregulate astroglial genes; and they are not endowed with self-renewing potential, but rather undergo only one round of symmetric neurogenic division (Box 1), self-consuming into a pair of neurons (Gal et al., 2006; Stancik et al., 2010; Tyler and Haydar, 2013). Thus, aIPs are a means of doubling the neuron output per apical mitosis.

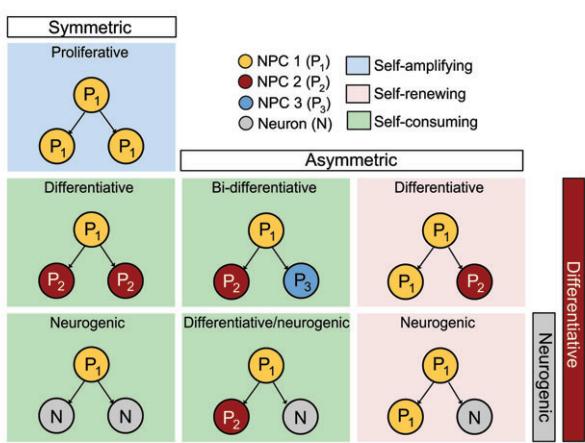
Basal progenitors

Two types of basal progenitor (BPs) can be distinguished: basal intermediate progenitors and basal radial glia (Fig. 2A,B and Fig. 3). Both types can be generated either from APs (specifically, from NECs or aRG) or from BPs themselves.

Basal intermediate progenitors

Basal intermediate progenitors (bIPs) are non-epithelial BPs: following their generation from NECs or aRG, they delaminate from the AJ belt and migrate into the SVZ, retracting their processes and losing apical-basal polarity prior to mitosis (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004) (Fig. 2A,B and Fig. 3). bIPs downregulate astroglial markers and, on their way to the SVZ, start expressing the transcription factor Tbr2 (also known as Eomes) (Englund et al., 2005; Cappello et al., 2006). bIPs can divide in a self-consuming fashion, undergoing (like aIPs) one round of symmetric neurogenic division (Haubensak et al., 2004;

Fig. 3. A summary of the cell biological properties of NPC types in the developing neocortex. The key cell biological features of various NPC subtypes are indicated. The term 'pseudo-apical cell polarity' refers to the presence of an apically directed process but the lack of a canonical apical domain. Modes of nuclear movement prior to mitosis are defined [as interkinetic nuclear migration (INM) or mitotic somal translocation (MST)]. The nuclear movement entailed in NPC delamination from the VZ to the SVZ is not indicated. Pax6 or Tbr2 in parentheses indicates that less than an overwhelming majority of a given NPC type shows this expression. Yes/no indicates that the basal process of the respective bRG subtype may or may not contact the basal lamina.

Box 1. NPC division types


We define the mode of NPC division based on the identity of the two daughter cells (Huttenkrum and Kosodo, 2005) relative to each other, to the parent cell and to the overall cell pool. A cell division is considered symmetric when it leads to two identical daughters, and asymmetric when the two daughters are different from each other. We refer to a division as proliferative when both daughters adopt the same identity as the mother, and differentiative when one or both daughters differ from the mother; bi-differentiative when, in the latter case, the daughters are also different from each other. Neurogenic divisions are differentiative divisions in which at least one daughter withdraws from the cell cycle as a neuron. Depending on whether an NPC gives rise to two, one or no self-renewed daughters, its pool can be amplified, maintained or depleted, respectively. Accordingly, we define a cell division as self-amplifying (i.e. proliferative), self-renewing or self-consuming.

Miyata et al., 2004; Noctor et al., 2004) and thereby doubling the neuron output; it is this feature that led to the original denomination of ‘intermediate’ progenitors. We refer to this ‘canonical’ bIP subtype as the neurogenic bIP. However, bIPs can also undergo one or more rounds of symmetric proliferative divisions before being self-consumed by a symmetric neurogenic division (Noctor et al., 2004; Hansen et al., 2010), hence amplifying their number. These bIPs have been referred to as ‘transit amplifying cells’ (Hansen et al., 2010; Lui et al., 2011) or ‘transit amplifying progenitors’ (Fietz et al., 2012; Stenzel et al., 2014). However, as basal radial glia can also behave like transit amplifying cells, at least in some species (see below), we shall not use the latter term but rather refer to this bIP subtype as a proliferative bIP.

In lissencephalic rodents, bIPs are mostly of the neurogenic subtype, they downregulate Pax6, and they are largely generated in the VZ from APs (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). By contrast, in gyrencephalic primates, the majority of bIPs are of the proliferative subtype, seem to sustain Pax6 expression (Fietz et al., 2010; Betizeau et al., 2013) and are frequently generated in the SVZ not only from proliferative bIPs themselves, but also from basal radial glia. As further discussed below, proliferative bIPs can give rise to one or two basal radial glia daughters.

Basal radial glia

Basal radial glia (bRG) were originally characterized as monopolar BPs, bearing a basal process that contacts the basal lamina but lacking an apical process that contacts the ventricle (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). It was recently shown, however, that in

addition to this ‘classical’ bRG type, now referred to as bRG–basal-P [–P for process (Betizeau et al., 2013)], two additional bRG subtypes can be distinguished, as observed at mitosis: (1) bipolar bRG (Betizeau et al., 2013; Pilz et al., 2013), which exhibit an apically directed process in addition to the basal process and hence are termed bRG–both-P; and (2) monopolar bRG that lack a basal process but exhibit an apically directed process and are referred to as bRG–apical-P (Betizeau et al., 2013). The apically directed process of bRG–both-P and bRG–apical-P does not reach the ventricle, although note that NPCs that do extend a process to the ventricle have been identified and are referred to as bipolar radial glia (Pilz et al., 2013), as discussed below in the section on subapical progenitors. To conceptualize this bRG diversity, it is important to consider their generation and lineage relationships, which allow us to classify bRG as primary or secondary (for more details see Box 2).

Reminiscent of selected aspects of INM, bRG may exhibit nuclear movements preceding mitosis. These movements, termed mitotic somal translocation, were initially observed for bRG–basal-P, in which the cell soma rapidly ascends in the basal direction shortly before mitosis (Hansen et al., 2010; LaMonica et al., 2013; Gertz et al., 2014), but have also recently been observed in the apical direction in bRG–apical-P and in either direction in bRG–both-P (Betizeau et al., 2013). Like aRG, the majority of bRG express astroglial markers and Pax6. However, unlike aRG, almost half of bRG were found to co-express Tbr2 during interphase in the developing macaque neocortex (Betizeau et al., 2013).

The amplification of BPs

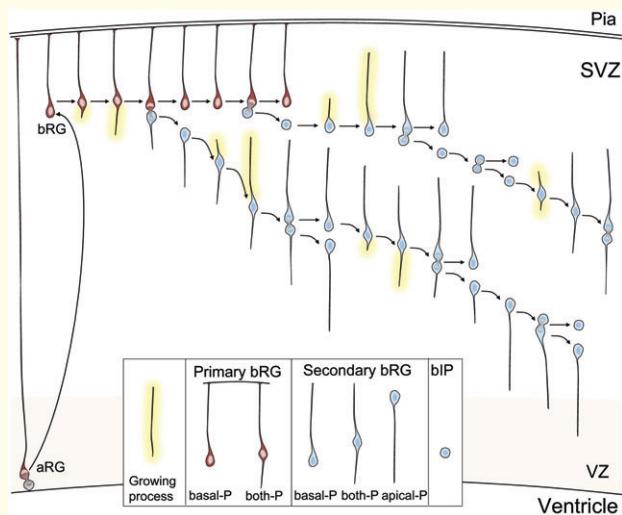
Each BP type and subtype can undergo, albeit to a different extent, symmetric proliferative divisions in the strict sense, which yield two BP daughters of the same (sub)type, as well as proliferative divisions in a broader sense, which yield BPs of different (sub)types, which in turn are able to undergo proliferative divisions (Hansen et al., 2010; Betizeau et al., 2013; LaMonica et al., 2013). As either proliferative division ultimately results in the amplification of the BP pool, we consider any BP division yielding two BPs, irrespective of the BP (sub)type generated, as a symmetric proliferative division. Notably, in addition to these symmetric proliferative divisions, BPs (in particular bRG) can also undergo asymmetric differentiative divisions that generate a neurogenic bIP and/or a neuron.

Subapical progenitors

Subapical progenitors (SAPs) (Fig. 2A,B and Fig. 3), like BPs, undergo mitosis at an abventricular location, that is, in the basal VZ or in the SVZ, but, in contrast to BPs, possess an apical process that traverses the entire VZ and extends to the ventricle, even during mitosis (Pilz et al., 2013). In this context, two subtypes of NPCs undergoing basal mitosis and exhibiting a basally directed process have recently been referred to as bipolar radial glia, one with an apically directed process that does not reach the ventricle, and another with an apical process that contacts the ventricle (Pilz et al., 2013). The former corresponds to bRG–both-P (Betizeau et al., 2013), as described above. The latter, however, considering the defining cell biological features of SAPs, should be regarded as an SAP. At present, little is known about the extent of apical-basal cell polarity of SAPs (as revealed by molecular markers), their expression of astroglial markers and of Pax6 and/or Tbr2, or their mode(s) of cell division in the developing neocortex.

Interspecies variations in NPCs

As mentioned above, the evolutionary enlargement of the neocortex entails a vast increase in the numbers of neurons produced during neocortical development. Neuron output, as defined by the final

Box 2. Primary and secondary bRG genesis, cell divisions and lineage relationships


bRG inherit the basal process from their aRG mother cell, thereby maintaining contact with the basal lamina, but not the parental apical domain. However, in the SVZ, bRG exhibiting a basally directed process have been observed to also extend an apically directed process (that does not reach the ventricle). Moreover, some bRG daughter cells have been observed to regrow a basally directed process (that does not reach the basal lamina). To conceptualize this diversity, we use the term primary bRG to refer to aRG-derived bRG that inherit the parental basal process (i.e. contact with the basal lamina) and to each of their daughters that inherit this basal process and maintain it throughout the subsequent cell cycle, irrespective of whether they extend an apically directed process. Primary bRG include bRG–basal-P and bRG–both-P (if their basal process is aRG-derived), but not bRG–apical-P. By contrast, we use the term secondary bRG to describe bRG born in the SVZ that lack contact with the basal lamina at birth, irrespective of whether they inherit an apically directed process, or grow an apically directed process, a basally directed process, or both. Secondary bRG include all bRG–apical-P, as well as some bRG–basal-P and bRG–both-P (if the basal process is not aRG-derived but regrown).

number of neurons produced, is ultimately determined by the sum, over the entire neurogenic period, of the neurogenic NPC divisions and by their type (asymmetric versus symmetric). Several parameters determine neuron output in a given species: the absolute number of NPCs and the relative abundance of each NPC type; the modes of cell division carried out by each NPC type; the length of the cell cycle of each NPC type; and the duration of the neurogenic period. In the last decade, comparative analyses of these parameters across species have proven extremely valuable for the study of neocortical evolution. Notably, such analyses are also highly relevant for understanding neurodevelopmental disorders that feature a reduction in neuron output (Borrell and Reillo, 2012) (e.g. microcephaly; for a review see Dawe et al., 2007; Gilmore and Walsh, 2013). Below, we discuss interspecies variations in the above parameters.

Abundance of NPC types

Although the majority of known NPC types have homologous counterparts in most mammalian orders, not all types are equally abundant across species, nor do they contribute equally to the final neuron output. The systematic quantitation of NPC type occurrence in species endowed with an enlarged, gyrencephalic neocortex has

provided valuable information regarding the contribution of each NPC type population to neocortical expansion.

Arguably, the most remarkable interspecies variation concerns expansion of the BP pool, as highlighted by the striking enlargement and remodeling of the SVZ, where BP cell bodies reside (Fig. 2). Indeed, in gyrencephalic species, and most notably in primates, the SVZ develops into two morphologically distinct germinal zones: an inner SVZ (iSVZ), which largely resembles the SVZ of lissencephalic rodents, and an outer SVZ (oSVZ), which is absent in most lissencephalic species (Smart et al., 2002; Reillo et al., 2011). While the thickness of the iSVZ remains relatively constant over the course of neurogenesis, the oSVZ grows progressively thicker (Smart et al., 2002), reflecting a dramatic increase in the number of BPs contained therein. Indeed, at peak stages of neurogenesis the oSVZ harbors up to four times as many progenitors as the VZ and iSVZ combined (Smart et al., 2002). As we discuss later, this increase might reflect an evolutionary advantage of BP-driven neurogenesis.

Importantly, the increase in BPs is accompanied by considerable changes in the cell type composition of the SVZ. In mice, at peak stages of neurogenesis the SVZ mostly contains neurogenic bIPs (~80% of the overall BP pool) and only a small fraction of bRG (~10%) and proliferative bIPs (~10%) (Noctor et al., 2004; Arai et al., 2011; Shitamukai et al., 2011; Wang et al., 2011; Martinez-Cerdeno et al., 2012). In human and macaque, these proportions are drastically different, as bRG become the most abundant BP type (~50–75%), with the remainder of BPs being mostly proliferative bIPs (Hansen et al., 2010; Lui et al., 2011; Betizeau et al., 2013). In this context, it is important to note that bRG abundance per se does not necessarily correlate with neuron output. For example, ferret bRG, which are abundant, have been shown to contribute to the mature cerebral cortex with more astrocytes than neurons (Reillo et al., 2011). The abundance of bipolar radial glia, which include SAPs according to the present cell biological definition (Fig. 2A), has also been analyzed in gyrencephalic and lissencephalic developing neocortices (Pilz et al., 2013). The greater abundance of bipolar radial glia in gyrencephalic species suggests a role of SAPs in neocortical expansion. However, the current lack of markers specific for a given NPC type, notably the BP, hampers a more systematic analysis of NPC diversity. A future challenge in the field will be to determine the specific contribution of each NPC type to the final neuron output.

Modes of cell division

Interspecies differences in the relative abundance of NPC types might reflect differences in their cell division modes. In particular, the ability of a given NPC type to self-amplify (i.e. to undergo symmetric proliferative divisions) is a key determinant of its pool size. Hence, it is not surprising that the most significant evolutionary changes in NPC type abundance concern their proliferative potential. Again, the most remarkable interspecies variation concerns BPs. On the one hand, the majority of bIPs in lissencephalic rodents are neurogenic and divide only once in a self-consuming fashion, whereas bIPs in gyrencephalic primates are endowed with considerable proliferative potential (Hansen et al., 2010; Betizeau et al., 2013). On the other hand, the expansion of the bRG pool in gyrencephalic species is accompanied by the sustained ability of bRG to undergo multiple rounds of self-amplification (Reillo et al., 2011; Betizeau et al., 2013). By contrast, in lissencephalic rodents, in which the bRG pool is inherently small, bRG seem to lack such proliferative capacity and instead undergo asymmetric neurogenic divisions (Shitamukai et al., 2011; Wang et al., 2011). Of note, bRG self-amplification is not a primate-specific trait, as symmetric proliferative divisions of bRG are frequent in the developing neocortex of the ferret (Reillo et al., 2011; Reillo and

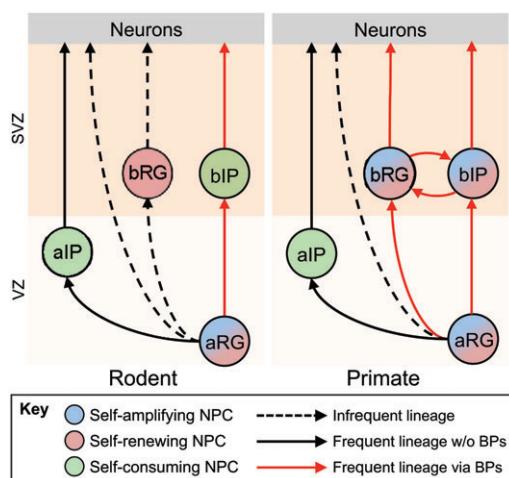


Fig. 4. NPC lineage relationships in the developing neocortex. Lineage relationships of NPCs in the developing neocortex of a representative lissencephalic rodent (left) and a gyrencephalic primate (right). Neurons (gray box) represent the end point of each NPC lineage. Preferential modes of NPC divisions are color-coded. NPCs that are capable of both self-renewing and proliferative divisions are colored both pink and blue. Arrows indicate lineage progression as detailed in the key.

Borrell, 2012; Gertz et al., 2014). By contrast, bRG differentiative (bIP-genic) divisions appear to be characteristic of primate bRG (Hansen et al., 2010; Betizeau et al., 2013), as they occur only rarely in the embryonic neocortex of mouse (Wang et al., 2011) and ferret (Gertz et al., 2014).

NPC lineages

Evolutionary changes in the modes of NPC division contribute to interspecies divergence of NPC lineages and thereby to differences in the final neuron output. Clonal analyses and time-lapse imaging of NPC divisions in slice cultures of the embryonic mouse (Haubensak et al., 2004; Konno et al., 2008; Shitamukai et al., 2011), rat (Noctor et al., 2001, 2004; Wang et al., 2011), macaque (Betizeau et al., 2013) and human (Hansen et al., 2010; LaMonica et al., 2013) neocortex have highlighted important interspecies differences in NPC lineages. In lissencephalic rodents (Fig. 4), the prevailing outputs of aRG differentiative divisions are neurogenic IPs (aIPs, neurogenic bIPs), which divide symmetrically yielding two neurons, i.e. the most frequent lineage path is aRG→neurogenic IP→neuron (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Konno et al., 2008; Stancik et al., 2010; Shitamukai et al., 2011). By contrast, in gyrencephalic primates (notably at mid- to late neurogenesis), aRG characteristically produce bRG, which self-amplify and produce neurons (directly or via bIPs), thereby greatly increasing neuron output. As a result, the majority of neocortical neurons in primates are generated by neurogenic divisions of bRG (Fig. 4), i.e. following an aRG→bRG→neuron lineage path (Hansen et al., 2010; Betizeau et al., 2013; LaMonica et al., 2013). Finally, a recently observed property of primate BPs is their ability to transform into each other. Indeed, as discussed above, all BP subtypes can yield any other BP subtype (Betizeau et al., 2013). Although these findings do not contradict the concept of lineage progression as such – APs give rise to BPs, but not the other way around – they do add an element of NPC subtype flexibility to a given node within a lineage.

Cell cycle length

Cumulative S-phase labeling studies initially showed that, in mouse, an average cell cycle lengthening of NPCs in the VZ accompanies the

progression of neurogenesis (Takahashi et al., 1995). Subsequent analyses distinguished between distinct NPC populations (Calegari et al., 2005; Gal et al., 2006; Arai et al., 2011) and revealed that in mouse: (1) aRG at mid-neurogenesis have a longer cell cycle than at the onset of neurogenesis, which reflects a lengthening of both the G1 phase and S phase (Calegari et al., 2005; Arai et al., 2011); (2) aIPs and neurogenic bIPs have a longer cell cycle than aRG, which is due to a lengthening of the G1 phase in these IPs (Gal et al., 2006; Arai et al., 2011); and (3) bIP-genic/neurogenic aRG and neurogenic bIPs have a shorter cell cycle than proliferative aRG and bIPs, which is due to a shortening of S phase in the former (Arai et al., 2011).

Different observations of cell cycle changes in the course of neurogenesis have been made in primates (Kornack and Rakic, 1998; Lukaszewicz et al., 2005; Betizeau et al., 2013). In macaque, the average cell cycle length of NPCs in the VZ was shown to increase until mid-neurogenesis, but then, in contrast to mouse, to decrease from mid-neurogenesis onwards (Kornack and Rakic, 1998). This decrease in cell cycle length at later stages of cortical neurogenesis in monkey has been extended to NPCs in the oSVZ, and was shown to reflect a shortening of both G1 and S phase (Betizeau et al., 2013).

These observations are significant because cell cycle length, in particular the length of the G1 phase, has been shown to be a cell fate determinant (Calegari and Huttner, 2003). Thus, in mice, lengthening the G1 phase in NPCs by pharmacological inhibition of Cdk2/cyclin E (Calegari and Huttner, 2003) or by RNAi-mediated silencing of Cdk4/cyclin D1 (Lange et al., 2009) was shown to trigger premature neurogenesis at the expense of NPC proliferation. Conversely, G1 shortening by overexpression of cyclin D1, cyclin E1 (Pilaz et al., 2009) or Cdk4/cyclin D1 (Lange et al., 2009; Nonaka-Kinoshita et al., 2013) increased NPC proliferation and delayed neurogenesis. In the ferret, the latter form of forced G1 shortening increased cortical folding (Nonaka-Kinoshita et al., 2013). In the macaque, G1 is shorter in cortical areas showing the cytoarchitectural hallmarks of neocortex expansion (Lukaszewicz et al., 2005). Given these findings, it will be a future challenge to determine the extent to which changes in cell cycle length, notably of G1 phase, influence bRG pool size and cell division modes, and contribute to the evolutionary expansion of the neocortex.

Neurogenic period

The length of the neurogenic period is another parameter that can determine neuron output and contribute to interspecies variation, and key evolutionary differences in this parameter have indeed been reported between rodents and primates. First, the onset of neurogenesis is delayed in primates compared with rodents (Rakic, 1995, 2009; Kornack and Rakic, 1998), allowing for greater expansion of the founder NEC pool before neurogenesis begins. Second, neurogenesis in primates is itself protracted for a much longer period of time (up to tenfold longer) compared with rodents (Caviness et al., 1995; Rakic, 1995), allowing for expansion of NPCs, notably bRG, thereby increasing neuron output. Protracting the neurogenic period will only lead to an increase in neuron output if there is no concomitant increase in the length of the cell cycle. Here, a cursory comparison of the cell cycle length of mouse versus macaque NPCs might suggest that the increase in neuron output in primates as compared with rodents is not as large as expected from the longer neurogenic period in primates. Specifically, the average cell cycle at mid-neurogenesis in macaque is about twofold longer than that in mouse (~40–50 h versus ~20–25 h) (Caviness et al., 1995; Takahashi et al., 1995; Kornack and Rakic, 1998; Lukaszewicz et al., 2005; Dehay and

Kennedy, 2007; Arai et al., 2011; Betizeau et al., 2013), which would approximately halve the neuron output per unit time. However, mouse might be an exception, as the cell cycle length of NPCs in ferret, which is more similar to mouse than macaque in the length of the neurogenic period, is in the same range as that of macaque (Reillo and Borrell, 2012; M. Turrero García, PhD Thesis, Technische Universität Dresden, 2013). Thus, the delayed onset of neurogenesis and a protracted neurogenic period might both be key parameters underlying NPC pool expansion and neuron production, and hence neocortical expansion.

Cell biological determinants underlying interspecies variations in NPCs

The cell biology of NPCs has been the subject of several in-depth reviews (e.g. Götz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009) and will not be addressed here as such. Rather, below we discuss only selected aspects of NPC biology that have been shown to be relevant to the interspecies variations in NPCs described above and to actually exhibit differences across mammalian species.

Intrinsic factors

Cleavage plane orientation

As discussed above, a major difference between mouse and human NPCs is the much greater ratio of BPs to APs in the developing human neocortex. This reflects interspecies variation at two levels. One concerns the extent of delamination of the daughter cells arising from aRG divisions, the cell biological basis of which we discuss here. The other concerns the sustained proliferation of human BPs, which we also discuss below.

During cortical neurogenesis in primates, the expansion of the oSVZ is accompanied by substantial shrinkage of the VZ, which has no real counterpart in lissencephalic rodents (Smart et al., 2002; Fietz et al., 2010; Hansen et al., 2010). This VZ shrinkage reflects a decrease in the abundance of APs, notably aRG, suggesting that primate aRG might undergo self-consuming divisions (rather than self-renewing divisions) more frequently than mouse aRG. It is tempting to relate this interspecies variation in the mode of aRG division to the differences in the cleavage plane orientation of mitotic aRG that have been observed between lissencephalic rodents and human (Kosodo et al., 2004; Konno et al., 2008; Noctor et al., 2008; Shitamukai et al., 2011; LaMonica et al., 2013). A cleavage plane oriented perfectly parallel to the apical-basal axis of a mitotic aRG, i.e. a vertical cleavage, will bisect its apical plasma membrane and AJ belt, which are collectively called the apical domain, resulting in apical domain inheritance by both daughter cells (Kosodo et al., 2004; Konno et al., 2008). However, as the apical domain constitutes only a very small portion of the cell body, cleavage planes that deviate only slightly from the apical-basal cell axis – which are usually also scored as vertical – may bypass the apical domain, resulting in it being inherited by only one of the daughter cells (Kosodo et al., 2004). The same is true for non-vertical (i.e. oblique and horizontal) cleavages. Moreover, the basal process, which, at early developmental stages when NECs prevail, may be split into two and inherited by both daughter cells (Kosodo et al., 2008), becomes much longer when NECs transform into aRG and typically does not split upon aRG division, constituting a single object that can only be inherited by one of the daughter cells.

Time-lapse imaging of aRG divisions in the mouse embryonic neocortex at mid-neurogenesis have revealed that upon vertical, apical domain-bisecting cleavage, the daughter cell inheriting both the

apical domain and basal process maintains self-renewing potential, ventricular contact and aRG identity, whereas the daughter cell inheriting only the apical domain but not the basal process delaminates and differentiates into either a neurogenic bIP or a neuron (Konno et al., 2008). By contrast, upon vertical but apical domain-bypassing cleavage, as well as upon non-vertical cleavages, all of which result in the inheritance of the apical domain by one daughter cell and the basal process by the other, both daughter cells were shown to leave the VZ and to differentiate into BPs and/or neurons (Konno et al., 2008; Shitamukai and Matsuzaki, 2012). Interestingly, the basal process-inheriting daughter cell often acquires bRG identity and maintains self-renewal potential (Shitamukai et al., 2011; Shitamukai and Matsuzaki, 2012). However, non-vertical cleavages of mitotic aRG are rare in the embryonic mouse neocortex (Kosodo et al., 2004; Konno et al., 2008; Noctor et al., 2008; Asami et al., 2011; Shitamukai et al., 2011), which presumably accounts for the low abundance of bRG in mouse.

Compared with mouse, non-vertical cleavages of mitotic aRG are more frequently observed in the fetal human neocortex (LaMonica et al., 2013), although vertical cleavages still constitute the majority (Fietz et al., 2010; LaMonica et al., 2013). Time-lapse imaging has shown that horizontal cleavages of human aRG give rise to bRG (LaMonica et al., 2013), similar to previous observations in mouse (Shitamukai et al., 2011). If one extrapolates to human the finding in mouse that, upon non-vertical aRG cleavages, both daughter cells leave the VZ (Konno et al., 2008; Shitamukai et al., 2011), the more frequent occurrence of such aRG cleavages in human than in mouse developing neocortex (LaMonica et al., 2013) would not only provide an explanation for the shrinkage of the human VZ but would also imply an increased generation of bRG in human, which in turn would contribute to expansion of the human oSVZ. Support for this notion has been provided by analysis of cleavage plane orientation in the ferret VZ, where non-vertical aRG cleavages increase concomitant with the formation of the oSVZ (Reillo and Borrell, 2012).

The orientation of the cleavage plane is primarily determined by the axis of the mitotic spindle (Lancaster and Knoblich, 2012). Hence, any gene involved in mitotic spindle positioning and maintenance is potentially relevant for the interspecies differences in aRG cleavage plane orientation discussed above. Several studies have focused on mammalian orthologs of genes that govern mitotic spindle positioning in *Drosophila* neuroblasts, such as those encoding LGN (also known as Gpsm2) and inscuteable, and have examined their roles in mouse aRG spindle positioning (Konno et al., 2008; Postiglione et al., 2011). Of particular importance may be genes that carry mutations causing primary microcephaly in humans, as the corresponding gene products are often associated with centrosomes and in some cases have been shown to be involved in aRG mitotic spindle orientation (Fish et al., 2008). However, an in-depth discussion of microcephaly genes is beyond the scope of this article and the reader is instead referred to excellent reviews on this subject (e.g. Manzini and Walsh, 2011).

Extracellular matrix and integrins

In searching for differences in gene expression that may underlie the profound differences in proliferative potential between mouse and human BPs – with most mouse BPs being neurogenic bIPs and most human BPs being bRG – transcriptome analyses have been performed on isolated NPC subpopulations and specific germinal layers. These studies have suggested a role for cell-autonomous extracellular matrix (ECM) production in NPC proliferation. Specifically, mouse NPCs

expressing the antiproliferative/prodifferentiative gene *Tis21* (also known as *Btg2*), the majority of which are neurogenic bIPs, markedly downregulate the endogenous production of ECM constituents as compared with NPCs lacking *Tis21* expression, which comprise mostly proliferating aRG (Arai et al., 2011). Corroborating this finding, the cell-autonomous production of ECM constituents was found to be downregulated in the mouse SVZ compared with the VZ (Fietz et al., 2012). By contrast, in fetal human neocortex, not only the VZ but also the iSVZ and oSVZ were found to sustain the cell-autonomous production of ECM constituents (Fietz et al., 2012). Together, these findings raise the possibility that the considerable proliferative potential of human BPs, which distinguishes them from mouse BPs, might at least in part be due to their ability to form a local ECM-based niche that conveys quasi-autocrine stimulation of their ability to re-enter the cell cycle.

If this were the case, one would expect integrins, which are major receptors for ECM constituents, to have a key role in NPC proliferation, notably with regard to the BPs in the human oSVZ. Triggered by the previous observations that interfering with integrin $\alpha v\beta 3$ signaling reduces the bRG pool size in ferret (Fietz et al., 2010) and that expression of this integrin is sustained in the human oSVZ as compared with human VZ but is reduced in the mouse SVZ as compared with mouse VZ (Fietz et al., 2012), a recent study has explored the consequences of integrin activation in mouse neurogenic bIPs. Indeed, activation of integrin $\alpha v\beta 3$ was found to stimulate mouse bIP cell cycle re-entry, that is, to induce their proliferation (Stenzel et al., 2014). Moreover, consistent with the fact that integrin $\alpha v\beta 3$ is the only known cell surface receptor for thyroid hormones (Bergh et al., 2005), preventing thyroid hormone binding to integrin $\alpha v\beta 3$ was found to abrogate the BP proliferation induced by integrin $\alpha v\beta 3$ activation (Stenzel et al., 2014). These findings raise the possibility that integrin $\alpha v\beta 3$ may be a major player in human BP proliferation and that the defects in the cortical development of human fetuses resulting from a lack of thyroid hormones during pregnancy might be partially due to the reduction in integrin $\alpha v\beta 3$ -dependent BP proliferation.

Extrinsic factors

During cortical neurogenesis in all mammalian species, axons from the thalamus grow into the neocortex to eventually form synapses with neurons in the cortical plate (Molnar and Blakemore, 1995). During this process, when traversing the intermediate zone, these axons secrete mitogenic factors that could potentially affect the proliferation of NPCs within the SVZ (Dehay et al., 2001; Dehay and Kennedy, 2007). Importantly, in gyrencephalic mammals, notably primates, these thalamocortical axons are much more abundant, and their growth cones reside in the intermediate zone for much longer than is the case in lissencephalic rodents. This led to the hypothesis that BP proliferation in the SVZ, and in particular the oSVZ, might be more strongly promoted by thalamocortical axon-derived mitogens in gyrencephalic as compared with lissencephalic species (Dehay and Kennedy, 2007). Indeed, using a binocular enucleation approach in the developing ferret, whereby the thalamocortical axons directed towards the visual cortex are reduced, the proliferation of NPCs, specifically bRG, was markedly decreased in the oSVZ but not in the iSVZ and VZ, eventually resulting in a smaller visual cortex (Reillo et al., 2011). The general concept emerging from these findings is that, during cortical neurogenesis, neuronal input from non-cortical regions contributes to sustaining and promoting cortical NPC proliferation and that interspecies variation in this input might affect neuron output.

NPCs and neocortical expansion: hypotheses and tests

Several influential theories have specifically addressed the question of how the expansion of the neocortex (and the increase in neuron numbers) might entail variations in NPC divisions during neocortical development (Fig. 5). All these theories agree on one main point: neocortical enlargement in the lateral dimension is due to increased numbers of NPCs during development, which in turn is mostly achieved by an increase in the number of their self-amplifying divisions, i.e. a greater proliferative capacity. However, these theories differ as to which proliferating NPC type is the main determinant of neocortical expansion. Within each theoretical framework, several experiments have been designed. As we discuss below, the results of these studies provide direct tests of the predictions of each of these theories, converging onto a unifying model of neocortical expansion.

Increasing the AP pool: the radial unit hypothesis

The radial unit hypothesis (Rakic, 1988, 2009) posits that neocortical surface area and thickness are determined by two subsequent phases of neocorticogenesis. Surface area is set first, during the ‘proliferative phase’, when NECs undergo symmetric proliferative divisions to amplify the founder cell pool. Neocortical thickness is established later on, during the ‘differentiative phase’, when each founder aRG starts generating neurons by sequential rounds of asymmetric division (Rakic, 1995). During the latter phase, clonally related neurons migrate in order of birth along the radial fiber scaffold of their parent cell to finally settle within the cortical plate in a columnar array (Rakic, 1995; Noctor et al., 2001). A radial unit is then defined as an ‘ontogenetic column’ of radially aligned, clonally related neurons originating from a common founder cell.

According to this model of neurogenesis, the evolutionary expansion of neocortical surface area, with little variation in thickness, would be accomplished by increasing the number of radial units before the onset of neurogenesis, with small changes in the numbers of neurons produced within each unit. An implicit prediction of the radial unit hypothesis is that, since NEC abundance determines the ventricular surface area of the neuroepithelium, which in turn sets the size of the ventricles, differences in neocortical surface area between mammalian species should be mirrored, before the onset of neurogenesis, by the size of their lateral ventricles. However, the lateral expansion of the neocortex mostly entails an increase in the pial, rather than ventricular, surface area (Kriegstein et al., 2006; Fietz and Huttner, 2011; Lui et al., 2011).

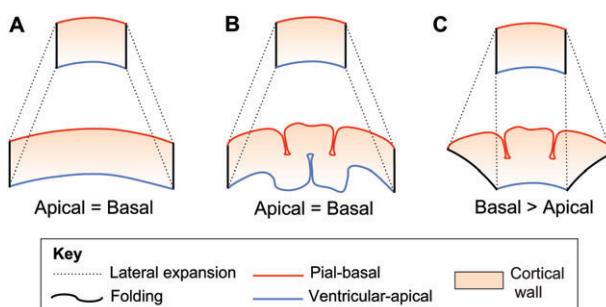


Fig. 5. Principal types of neocortex lateral expansion. (A,B) Equal expansion of the ventricular (apical) and pial (basal) surfaces of the cortical wall could occur without (A) and with (B) the folding of both surfaces. (C) Expansion of the pial (basal), but not the ventricular (apical), surface of the cortical wall results in folding of the pial (basal) surface.

This issue has been addressed by an experimental test of the radial unit hypothesis in mouse (Chenn and Walsh, 2002), whereby NECs were forced to re-enter the cell cycle, and hence increase their pool size, by the expression of a constitutively stabilized form of β -catenin. While these mice indeed developed a larger neocortex, proportional to the increased size of the founder cell population, the surface area of the cortical wall expanded laterally on both the pial and the ventricular side, resulting in both outward and aberrant inward folds, respectively (Fig. 5). Similar phenotypes have been obtained by attenuating cell death during forebrain development (reviewed by Haydar et al., 1999). For example, deletion of caspase 9 led to increased numbers of NECs, resulting in the development of an expanded, but exencephalic, neocortex, with aberrant invaginations of the neocortical wall (Kuida et al., 1998). Caspase 3 deficiency also resulted in neocortical enlargement, along with heterotopic cell masses, a thicker cerebral wall and ventriculomegaly (Kuida et al., 1996). Therefore, increasing the founder pool alone is not sufficient to explain neocortical expansion and, without the counterbalance of other developmental mechanisms, it may not even be compatible with normal neocortical development.

Increasing the BP pool

As early as the 1970s, Smart and colleagues had proposed that, above an optimum threshold, pseudostratification of the VZ acts as a constraint on cell proliferation; in order to achieve any further increase in neuron output, NPCs must be free to undergo mitosis away from the ventricular surface (Smart, 1972a,b). The congestion of nuclei in the VZ is indeed relieved by certain NPCs dividing basally, and the occurrence of such basal mitoses is more frequent in areas of higher neuron production (Smart, 1972a,b). Moreover, the SVZ, where BP cell bodies reside, was observed to be larger in species with an expanded, gyrencephalic neocortex (Smart et al., 2002; Martinez-Cerdeno et al., 2006), especially primates (Smart et al., 2002), and is largest in humans (Kriegstein et al., 2006). Different theories conveyed these observations into an evolutionary framework, and shifted the debate concerning the role of BPs in neocortical expansion.

bIPs and the intermediate progenitor hypothesis

The intermediate progenitor hypothesis (Kriegstein et al., 2006) proposes that evolutionary neocortical expansion may be due to an increase in the genesis of bIPs, mostly owing to a substantial change in their mode of division from symmetric neurogenic to symmetric proliferative. At each round of proliferative division, bIPs would exponentially amplify the neuron output of the founder aRG without increasing the VZ surface. Furthermore, based on the evidence that the SVZ in gyrencephalic species is not uniform but is thicker in areas underlying the formation of gyri and thinner in areas underlying the formation of sulci (Kriegstein et al., 2006), the intermediate progenitor hypothesis provides an explanation of how, by locally modulating neuron density and numbers, changes in patterns of bIP proliferation across different regions of gyrencephalic neocortices may contribute to determining specific patterns of neocortical folding.

Recently, the intermediate progenitor hypothesis has been tested by means of a transgenic mouse line (Nonaka-Kinoshita et al., 2013) in which neurogenic bIPs have been forced to re-enter the cell cycle during mid-neurogenesis (by overexpression of the Cdk4/cyclin D1 complex). Compared with controls, these mice, which were viable until adult stages, displayed a laterally expanded neocortex (both on the ventricular and pial sides) with no significant change in thickness. However, despite this lateral expansion, the

neocortex did not fold (Fig. 5), suggesting that the proliferation of bIPs in the SVZ, although contributing to an increase in neuron numbers and neocortex size, might not be sufficient to induce gyration. These results may be interpreted in light of the recent characterization of bRG in gyrencephalic species (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011; Betizeau et al., 2013), which revealed a pattern of neurogenesis more complex than that proposed by the intermediate progenitor hypothesis.

bRG and the epithelial progenitor hypothesis

Smart and colleagues (Smart et al., 2002) first noted that the primate SVZ is heterogeneous in terms of its pattern of cell nuclei, such that two histologically distinct areas can be distinguished. The iSVZ is densely packed with randomly arranged nuclei, resembling those of bIPs in the rodent SVZ. By contrast, the oSVZ contains nuclei that are radially aligned along the apical-basal axis, similar to those of aRG in the VZ.

A radial-glial morphology of oSVZ progenitors was first demonstrated by GFP lipofection of NPCs in monkey tissue slices, which highlighted apically and basally directed processes extending from some oSVZ nuclei (Lukaszewicz et al., 2005). In the same study, analyses of cell cycle kinetics revealed that primate oSVZ progenitors have a substantial capacity for cell cycle re-entry, in striking contrast to self-consuming bIPs in the rodent SVZ (Lukaszewicz et al., 2005; Dehay and Kennedy, 2007). Subsequently, mitotic BPs with a basally or apically directed process were also observed in the developing human neocortex (Howard et al., 2006).

In light of these observations, the epithelial progenitor hypothesis (Fish et al., 2008) proposes that, in primates, some aRG may overcome the apical constraint by translocating the site of mitosis to the oSVZ while maintaining an epithelial nature and radial-glial-specific features, including cell polarity. The retention of radial-glial identity would confer a specific advantage to oSVZ progenitors: first, similar to aRG, their radial fiber may provide scaffolding for neuron migration and allocation, in agreement with the radial unit hypothesis; second, their epithelial nature may allow better control of proliferation. This hypothesis has recently met its prediction, with the identification and detailed characterization of bRG in the oSVZ of gyrencephalic carnivores and primates, including humans (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011; Betizeau et al., 2013).

bRG and the radial cone hypothesis

As discussed above, a hallmark of neocortical expansion is the much greater increase in pial surface area than ventricular surface area (Fietz and Huttner, 2011; Lui et al., 2011). It has been proposed that this might reflect a principal difference in the shape of radial units during neurogenesis. In lissencephalic rodents, radial units would be of near-cylindrical shape, with the founder aRG at their apical tips. Conversely, in species with an enlarged and gyrencephalic neocortex, radial units would resemble a cone, with a broader base relative to its apical tip. Following on from this hypothesis, which is referred to as the radial cone hypothesis, neocortical expansion could be explained by the addition, within each radial unit, of radial subunits. The founder of each subunit would be a bRG that, by virtue of its basal process, could guide neuron migration, and by virtue of its proliferative capacity could increase neuron output, and eventually pial surface, without increasing ventricular surface (Fietz and Huttner, 2011).

This concept has been broadened by the observation that the scaffolding basal processes of ferret and human aRG and bRG diverge like a fan (Fig. 2B), hence allowing migrating neurons to

spread in the lateral dimension (Lui et al., 2011; Reillo et al., 2011; Borrell and Reillo, 2012; Reillo and Borrell, 2012; Kelava et al., 2013; Lewitus et al., 2013). Interestingly, this fan is wider in prospective gyral regions than in prospective sulcal regions, which resemble the developing lissencephalic rodent neocortex with its largely parallel arrangement of radial fibers (Lui et al., 2011; Reillo and Borrell, 2012; Kelava et al., 2013; Lewitus et al., 2013).

In line with the radial cone hypothesis, two recent studies have provided evidence that an increase in bRG proliferation results in increased cortical folding. First, in the developing mouse neocortex, which is normally scarce in bRG and is lissencephalic, the increased production of bRG (induced by knockdown of the DNA-associated protein Trnp1) led to gyration of the cerebral cortex (Stahl et al., 2013) (Fig. 5). Second, in the developing ferret neocortex, which contains bRG at high abundance and is gyrencephalic, increasing BP proliferation by overexpression of Cdk4/cyclin D1 further increased gyration (Nonaka-Kinoshita et al., 2013).

Conclusions

In summary, the evolutionary expansion of the neocortex is based on differences in NPC biology. During the last decade, substantial progress has been made with regard to uncovering the diversity of NPC types, delineating their lineage relationships and determining their modes of cell division. Key interspecies differences have been revealed, notably regarding the abundance of certain NPC types, the complexity of their lineages and their proliferative potential. Importantly, insight into these topics is increasingly being obtained at the cell biological and molecular level. Together, this increase in our knowledge has made it possible to refine NPC-based concepts of neocortex evolution. Yet, the grand challenge remains of identifying the genomic differences that are ultimately responsible for the greater neuron output of NPCs during the development of the human neocortex. To this end, a further mechanistic dissection of how the numbers and modes of division of the various types of NPCs are controlled, across a range of species, might prove to be a rewarding strategy.

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Competing interests

The authors declare no competing financial interests.

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