REVIEW ARTICLE

Coevolution of Radial Glial Cells and the Cerebral Cortex

Camino De Juan Romero and Víctor Borrell

Abstract: Radial glia cells play fundamental roles in the development of the cerebral cortex, acting both as the primary stem and progenitor cells, as well as the guides for neuronal migration and lamination. These critical functions of radial glia cells in cortical development have been discovered mostly during the last 15 years and, more recently, seminal studies have demonstrated the existence of a remarkable diversity of additional cortical progenitor cell types, including a variety of basal radial glia cells with key roles in cortical expansion and folding, both in ontogeny and phylogeny. In this review, we summarize the main cellular and molecular mechanisms known to be involved in cerebral cortex development in mouse, as the currently preferred animal model, and then compare these with known mechanisms in other vertebrates, both mammal and nonmammal, including human. This allows us to present a global picture of how radial glia cells and the cerebral cortex seem to have coevolved, from reptiles to primates, leading to the remarkable diversity of vertebrate cortical phenotypes.

GLIA 2015;63:1303–1319

Key words: human, ferret, direct neurogenesis, basal progenitor, cortical folding, expansion

Introduction

The mammalian cerebral cortex is by far the most complex organ in the animal kingdom, where information from all sensory modalities is processed and integrated with previously stored information to give rise to all kinds of motor and social behaviors, including those most characteristic of humans such as abstract thinking, art, and sense of humor (Kandel et al., 2000). Importantly, the extraordinary size and organizational–functional complexity of the human cerebral cortex emerges through a similarly extraordinary and complex developmental process. This process involves the extensive proliferation of a limited number of neural stem cells that generate the myriads of neurons and glial cells of the mature cerebral cortex. In addition, as new cortical neurons are formed they must move away from their site of birth to their final location near the brain's surface, in a critical process subject to very tight genetic regulation known as radial migration (Ross and Walsh, 2001; Sidman and Rakic, 1973). Perturbation of any one of these steps commonly results in significant organizational anomalies, leading to severe learning deficits, cognitive disability, and intractable epilepsy (Barkovich et al., 2012). Thus, the cerebral cortex is considered the seat of higher cognitive thinking and, by extension, the most distinctive feature of human uniqueness (Rakic, 2009).

The cerebral cortex of modern humans is the result of intense evolution and positive selection during the last few hundred million years, from our stem vertebrate ancestor (Dorus et al., 2004; Kouprina et al., 2004). During this long process of vertebrate evolution and diversification the brain was indeed subject to intense modifications in size, shape and organization, from which each species selected the best fit for their biological needs and survival (Borrell and Reillo, 2012; Rakic, 2009). Accordingly, the relative size, shape and complexity of the cerebral cortex varies widely between species, from the very small and simple cortex of reptilians, somewhat bigger in birds, to the larger and much more elaborate neocortex of small rodents, and finally to the outstandingly large and folded neocortex of cetaceans, great apes and humans (O'Leary et al., 2013 Welker, 1990). Significantly, *Homo sapiens* has by far the largest brain-to-body weight ratio of all mammals (Purves, 1988). Differences in brain size between species are largely due to variations in the cerebral cortex, which changes several-fold more than any other brain structure (Finlay and Darlington, 1995). Increases in cortical size

View this article online at wileyonlinelibrary.com. DOI: 10.1002/glia.22827

Published online March 23, 2015 in Wiley Online Library (wileyonlinelibrary.com). Received Dec 22, 2014, Accepted for publication Mar 9, 2015.

Address correspondence to Víctor Borrell. E-mail: vborrell@umh.es

From the Instituto De Neurociencias, Consejo Superior De Investigaciones Científicas & Universidad Miguel Hernández, Sant Joan D'alacant, Spain

The Instituto de Neurociencias is a "Centre of Excellence Severo Ochoa."

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2015 The Authors. Glia Published by Wiley Periodicals, Inc.

usually occur in surface area rather than in thickness, resulting in a very extensive sheet of relatively thin cortical tissue (Rakic, 1995). This is usually accompanied by its folding and buckling (gyrification), which allows fitting a very large cortical sheet within a limited cranial volume. Although this general rule leads to the assumption that increased brain size is inevitably bound to cortical folding, numerous exceptions exist where large brains display a smooth cortex, and smaller brains have cortical folds (Welker, 1990). Likewise, although gyrencephaly ("folded brain") is frequently assumed to be a distinctive primate trait, it is a rather common feature occurring in all major families of mammals across phylogeny, including rodents, carnivores, ungulates, cetaceans, marsupials and even a monotreme (Echidna; Borrell and Reillo, 2012; Welker, 1990). Such widespread display of gyrencephaly occurrence has been proposed to reflect that this trait has a common evolutionary origin for mammals (Borrell and Reillo, 2012; Reillo et al., 2011), with lissencephaly ("smooth brain") emerging secondarily (Borrell and Reillo, 2012; Kelava et al., 2012) as supported by recent analyses (Kelava et al., 2013; Lewitus et al., 2014; O'Leary et al., 2013).

All neurons of the mature cerebral cortex derive from the primary progenitor cells lining the embryonic telencephalic ventricle, known as apical radial glial cells (Kriegstein and Alvarez-Buylla, 2009). In mammals, apical radial glial cells generate cortical neurons directly, or indirectly via transit amplifying neurogenic progenitors. The balance between progenitor cell amplification, self-renewal and neurogenesis determines the size and cellular composition of the cerebral cortex, which is characteristic in each species (Kriegstein et al., 2006). Recent studies investigating interspecies differences in cortical progenitor cells and their dynamics are beginning to shed light on the developmental mechanisms underlying cerebral cortex expansion and folding, with important implications in human disease (Florio and Huttner, 2014). In the next sections we extend on the distinctive features of embryonic radial glial cells, their diversity and variation across species, the consequences of this variation in cerebral cortex gyrification, and how changes during evolution in progenitor subtype, abundance, and localization may have accounted for cortical expansion and folding.

Progenitor Cells in the Developing Mouse Cerebral Cortex

The development of the mouse cerebral cortex involves multiple types of progenitor cells, including neuroepithelial cells, apical radial glia cells (aRGCs), intermediate progenitor cells (IPCs), apical intermediate progenitors (aIPs), subapical progenitors (SAPs), and the recently identified basal radial glia cells (bRGCs). In the next sections we provide a summarized view of each of these subtypes, with their similarities and differences. Although much remains to be learned about aRGCs and bRGCs, emerging evidence suggests that both their common and distinctive features are likely relevant for their having distinct roles in cortical development.

Neuroepithelial Cells

At the onset of mouse cortical development the anlage of the cerebral cortex is uniformly composed of a single layer of neuroepithelial cells (NECs), which are self-amplifying progenitor cells committed to the neural lineage that span the entire thickness of the telencephalic wall (Bayer and Altman, 1991; Sidman and Rakic, 1973). Although NECs retain contact with both apical and basal sides of the neural tube (facing the luminal space or the brain's surface, respectively), they always undergo mitosis at the apical side to then undergo interkinetic nuclear migration (INM) during the other phases of the cell cycle: during G1 they move their soma to the basal side, where they undergo S-phase, and during G2 they move back down to the apical side to finally undergo mitosis (Takahashi et al., 1993). The dynamic movement of these cell bodies along the apical–basal axis of the neuroepithelium confers it a false appearance of stratification (pseudostratified; Bayer and Altman, 1991; Sidman and Rakic, 1973; Taverna et al., 2014). NECs display typical epithelial features including high apical-basal polarization, as evidenced from the different composition of their plasma membrane along the apical-basal axis: tight junctions and adherens junctions at the apical domain, and integrin receptors at the basal domain (Aaku-Saraste et al., 1996; Manabe et al., 2002; Zhadanov et al., 1999). At around embryonic day (E) 10.5, with the onset of neurogenesis and first accumulation of neurons in the cortical surface, this pseudostratified neuroepithelium becomes multilayered, and NECs downregulate some epithelial features like tight junctions and start displaying hallmarks of astroglia, thus transforming into apical radial glial cells (aRGCs). The transition from NECs to aRGCs largely depends on the action of the morphogen Fgf10 (Sahara and O'Leary, 2009).

Apical Radial Glia Cells

Apical RGCs first become distinct from NECs by the loss of tight junctions and the display of several astroglial features, such as electrondense glycogen granules, and expression of proteins such as the astrocyte-specific glutamate transporter (GLAST), the calcium-binding protein S100β, the intermediate filament vimentin and brain lipid binding protein (BLBP; Campbell and Gotz, 2002; Hartfuss et al., 2001; Kriegstein and Gotz, 2003; Noctor et al., 2002). But similar to NECs, aRGCs retain expression of nestin, have a strong apical–basal polarity and maintain contact with both the apical and basal sides of the developing cortex (Hartfuss et al., 2001; Weigmann et al., 1997). In their apical side aRGCs extend a single

apical process contacting the ventricular surface. The apical-most segment of the basolateral domain of the plasma membrane forms adherens junctions with neighboring cells, thus integrating within the ventricular adherens junction belt (Gotz and Huttner, 2005). Hallmarks of this apical domain in aRGCs are the accumulation of Prominin-1, Par3/Par6/aPKC, β-catenin, and N-cadherin (Gotz and Huttner, 2005). Expression of ZO1 (zona occludens-1), a tight junction-associated protein, is not affected although the apical domain down regulates tight junctions but not adherens junctions, because in the absence of tight junctions ZO1 localizes to adherens junctions. Also, in this apical side a single cilium protrudes into the ventricular lumen, where it senses signals present in the cerebrospinal fluid that modulate the biology of these progenitor cells (Gotz and Huttner, 2005; Lehtinen and Walsh, 2011; Lehtinen et al., 2011; Paridaen and Huttner, 2014; Paridaen et al., 2013). The idea of a modulatory fine-tuning effect mediated by the CSF has been recently addressed in a study on Otx mutants, showing not only the important role of Otx2 in choroid plexus development, but also the contribution of this structure to alterations in Wnt signaling and telencephalic proliferation (Johansson et al., 2013). In their basal side aRGCs extend a single basal process (radial fiber) that terminates onto the brain's outer surface (pial surface) or, occasionally, onto a blood vessel (Javaherian and Kriegstein, 2009; Sidman and Rakic, 1973; Takahashi et al., 1990). In contrast to the simple basal process of NECs, the basal fiber of aRGCs branches profusely in the vicinity of the cortical surface, within the marginal zone (MZ), and each branch is tipped with bulb-like endings that attach to the basal lamina of the pial membrane (Sidman and Rakic, 1973; Takahashi et al., 1990; Yokota et al., 2010). The basal lamina is rich in extracellular matrix (ECM) proteins, and integrins and G-protein coupled receptors are critical for its molecular interaction with aRGCs and bRGCs (Beggs et al., 2003; Fietz et al., 2010; Hartmann et al., 1998; Hausmann and Sievers, 1985; Jeong et al., 2013; Koirala et al., 2009; Marthiens et al., 2010; Niewmierzycka et al., 2005; Pawlisz and Feng, 2011; Singer et al., 2013). Like NECs, aRGCs display INM, undergoing mitosis at the apical surface of the telencephalon (Noctor et al., 2001). However, from the onset of neurogenesis the basal side of the cortical wall becomes populated by progressively larger numbers of neurons and pioneer axonal fibers, and the cell bodies of aRGCs become confined to the apical region, constituting the primary cortical germinal layer: ventricular zone (VZ; Boulder_Committee, 1970). The establishment of the VZ brings two major differences compared with the neuroepithelium stage of the early cortical anlage: (a) the INM of aRGCs takes place only across the thickness of the VZ, without reaching the basal lamina of the cortex and (b) the basal process of aRGCs becomes progressively longer as the cortical wall becomes thicker (Bayer and Altman, 1991; Sidman and Rakic, 1973).

Apical RGCs are more fate-restricted progenitors than NECs and become the primary progenitor cells of the cerebral cortex, giving rise directly or indirectly to all cortical glutamatergic neurons (Malatesta et al., 2003, 2000; Miyata et al., 2001; Noctor et al., 2001). Regarding their proliferative and fate potential, aRGCs undergo either symmetric proliferative divisions (one aRGC generates two aRGCs), asymmetric neurogenic divisions (one aRGC and one neuron are generated) or asymmetric proliferative divisions (one aRGC and a different progenitor cell are generated; Gotz and Huttner, 2005). The probability or frequency of each type of division vary between developmental stages, with most divisions being symmetric proliferative at early stages and asymmetric proliferative at later stages (Bayer and Altman, 1991; Takahashi et al., 1994, 1996). Asymmetric neurogenic divisions seem to occur at a low frequency in mouse aRGCs, even at early stages (Attardo et al., 2008; Haubensak et al., 2004; Kowalczyk et al., 2009), whereas asymmetric proliferative divisions are gradually more frequent along development, increasing their amplificative potential (Haubensak et al., 2004; Kowalczyk et al., 2009; Kriegstein et al., 2006; Martinez-Cerdeno et al., 2006; Noctor et al., 2004). These divisions generate new types of progenitor cells that will undergo mitosis at positions immediately basal from the VZ, in a secondary germinal layer named subventricular zone (SVZ), and therefore they are referred to collectively as basal progenitors (Boulder_Committee, 1970; Fish et al., 2008; Haubensak et al., 2004; Kowalczyk et al., 2009; Noctor et al., 2004). Basal progenitors are the major source of cortical neurons in mouse (Haubensak et al., 2004; Kowalczyk et al., 2009). One distinctive feature of aRGCs from basal progenitors is the expression of the paired-box transcription factor 6 (Pax6), absent in the latter (Estivill-Torrus et al., 2002; Gotz et al., 1998; Warren et al., 1999). Conversely, the T-box transcription factor 2 (Tbr2) is typically expressed by basal progenitors and absent in aRGCs (Arai et al., 2011; Englund et al., 2005; Hevner et al., 2006). Pax6 and Tbr2 are currently accepted markers of radial glia and neurogenic progenitors, respectively, and their mutually exclusive expression in mouse seems caused by the repressive activity of Tbr2 over Pax6 expression (Sansom et al., 2009; Sessa et al., 2008). At later stages, during the period of maximum neurogenesis, a second class of RGCs emerges in the developing cerebral cortex: basal radial glia cells (bRGCs; Shitamukai et al., 2011; Wang et al., 2011).

Apical Intermediate Progenitors and Subapical Progenitors

Apical intermediate progenitors (AIPs) are a small population of progenitors located in the VZ that exhibit apical–basal polarity, extend apical and basal processes, and divide apically,

similar to aRGCs. However, the basal process of AIPs tends to be confined to the VZ, and they largely undergo neurogenic self-consuming divisions. These were originally referred to as short neural progenitors (Gal et al., 2006; Tyler and Haydar, 2013). Subapical progenitors (SAPs) have been recently identified as another type of basal progenitor in the embryonic mouse cortex, which also display apical and basal processes like aRGCs, but divide at basal positions within the VZ, unlike the latter (Pilz et al., 2013). Also unlike AIPs, SAPs undergo multiple rounds of fast amplificative divisions, each generating a large progeny of cells (Pilz et al., 2013). The apical process of SAPs is integrated in the apical adherens junction belt at mitosis but it remains to be determined if it is exposed at all to the ventricular lumen, that is, whether or not it contains apical plasma membrane (Taverna et al., 2014). The abundance of AIPs and SAPs in the embryonic mouse cortex is low (Pilz et al., 2013) and their full impact on the development of the cerebral cortex remains to be defined.

Basal Intermediate Progenitor Cells

Basal intermediate progenitor cells, from now on referred to as intermediate progenitor cells (IPCs) are nonepithelial progenitors with multipolar morphology generated by aRGCs in the apical surface of the VZ, from where they migrate basally to coalesce forming a secondary germinal layer: the subventricular zone (SVZ; Attardo et al., 2008; Boulder_Committee, 1970; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Newly generated IPCs undergo a transition phase of a few hours when they translocate their cell soma to the VZ but retain an apical process anchored in the apical adherens junction belt, likely inherited from their mother aRGC (Borrell et al., 2012; Noctor et al., 2008; Wilsch-Bräuniger et al., 2012). The prompt retraction of this apical process and full delamination of IPCs from the VZ is critical for their cell cycle progression and depends on the action of Robo receptors, which in part mediate the downregulation of N-Cadherin (Borrell et al., 2012; Wong et al., 2012). Once in the SVZ, IPCs undergo mostly symmetric neurogenic divisions, that is one self-consuming division to produce two neurons (Attardo et al., 2008; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). As a result, IPCs double the neurogenic potential of aRGCs (Kriegstein et al., 2006). Importantly, the vast majority of neurons in all layers of the mouse cerebral cortex are generated by IPCs (Attardo et al., 2008; Haubensak et al., 2004; Kowalczyk et al., 2009), and hence these are fundamental for cortical neurogenesis. As opposed to aRGCs, mature IPCs typically express the transcription factor Tbr2, but not Pax6 (Arai et al., 2011; Englund et al., 2005). Hence, newborn IPCs downregulate Pax6 and upregulate Tbr2. Although little is known about the relevance of this switch in gene expression, it may play an important role in reducing the amplificative potential of IPCs; that is to switch from the self-amplifying and self-renewing divisions of aRGCs to the self-consuming neurogenic divisions of IPCs. Seen from a different perspective, IPCs may represent a last stage in the life cycle of aRGCs, one last proliferative state before being exhausted as progenitors and becoming postmitotic neurons. Importantly, there are other regions in the developing murine forebrain apart from the cerebral cortex where IPCs can actually self-amplify, particularly in the basal ganglia (Pilz et al., 2013).

Basal radial glia cells

Basal RGCs (bRGCs) were originally identified and characterized in the developing human and ferret cerebral cortex (both gyrencephalic mammals) as basal progenitor cells sharing many features with aRGCs but with their soma located in the outer subventricular zone (OSVZ; see next section), instead of the VZ (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). In fact bRGCs are alternatively named OSVZ radial glia-like cells (oRGs; Hansen et al., 2010). Interestingly, bRGCs share many features with aRGCs, including expression of Pax6 but not Tbr2, expression of vimentin, and extension of a long basal fiber radially to the cortical surface where it contacts its basal lamina in mouse, human and ferret (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011; Shitamukai et al., 2011; Wang et al., 2011). In contrast to aRGCs, however, bRGCs do not extend an apical process to the ventricular surface, and hence are delaminated from the apical adherens junction belt and do not directly receive signals from the cerebrospinal fluid (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). Concomitantly, bRGCs undergo mitosis at basal positions within the developing cortex, where they reside as part of the population of basal progenitors that constitute the SVZ (Shitamukai et al., 2011; Wang et al., 2011). Because of their unique localization and their morphology distinct from IPCs, bRGCs are readily identifiable using antiphosphovimentin antibody stains, which reveal the characteristic basal process of mitotic bRGCs (Fietz et al., 2010; Wang et al., 2011). Intriguingly, although bRGCs share molecular and morphological similarities with aRGCs, in mouse they mostly undergo self-consuming neurogenic divisions, like IPCs (Wang et al., 2011). However, as opposed to IPCs, bRGCs are found at extremely low abundance in the embryonic mouse cortex (3–5% of mitoses depending on the developmental stage), so the relevance of their contribution to mouse cortical development remains undefined (Shitamukai et al., 2011; Wang et al., 2011).

Variations on a Mouse Theme: Toward a Global Understanding

In the previous section we have outlined the progenitor cell types, their properties and abundance as found in the

embryonic mouse cerebral cortex. However, a growing body of evidence demonstrates that this basic plan varies significantly across species at various levels, which ultimately seem to impact significantly on the size and complexity of the mature cerebral cortex.

Abundance of Basal Progenitors

A seminal study of the embryonic macaque cerebral cortex by Smart and colleagues in 2002 demonstrated the extraordinary size and complexity of the SVZ in this species (Smart et al., 2002). Compared with the mouse embryonic cortex, where the SVZ contains a relatively modest number of basal progenitor cells, in macaque the SVZ contains much larger numbers of progenitor cells, in fact becoming the predominant germinal layer. At the peak of neurogenesis SVZ progenitors in mouse account for 15–30% of cortical mitoses, whereas in human these account for up to 85% of all mitoses (Dehay and Kennedy, 2007; Haubensak et al., 2004; Kowalczyk et al., 2009; Reillo et al., 2011). As a result of this remarkably greater accumulation of basal progenitor cells, the SVZ is much thicker in macaque than mouse and, consequently, becomes subdivided in various sublayers: inner SVZ (ISVZ) and outer SVZ (OSVZ; Dehay and Kennedy, 2007; Smart et al., 2002). The ISVZ is immediately basal to the VZ and contains a high density of progenitor cells; the OSVZ is basal to the ISVZ and is much thicker than ISVZ or VZ, but also contains a significantly lower density of progenitor cells. In addition, ISVZ and OSVZ are separated by a distinct fiber-rich layer named inner fiber layer, and the OSVZ is flanked basally by another fiber-rich layer, the outer fiber layer (Smart et al., 2002). At the time when all these subdivisions of the SVZ were first observed in the macaque monkey they had never been observed in the embryonic mouse cortex, and so it was interpreted as being a primate-specific feature (Dehay and Kennedy, 2007; Smart et al., 2002). Later analyses showed the existence of very prominent ISVZ and OSVZ also in the human embryo, demonstrating the conservation of these features in primates (Bayatti et al., 2008; Dehay and Kennedy, 2007). However, subsequent studies demonstrated the existence of distinct and thick ISVZ and OSVZ in nonprimate species, particularly ferret but also prominently in cat and sheep, belonging to two mammalian superfamilies (*Carnivora* and *Cetartiodactyla*) different from primates (Reillo and Borrell, 2012; Reillo et al., 2011). Importantly, the relative abundance of progenitor cells allocated to each of these germinal layers in the developing embryo cortex is also significantly different between species (Reillo et al., 2011), and current evidence supports the notion that this abundance may be relevant for the development of gyrencephaly (see sections subsequently).

Diversity of Basal Progenitors

Apical RGCs are the best conserved type of cortical progenitor cell across vertebrates (Molnar et al., 2006; Rakic, 2009; Weissman et al., 2003). As primary progenitor cells of the cerebral cortex, they have been observed from fish to humans. Although different species have a different abundance of aRGCs at the onset of cortical neurogenesis, there is very little variation in their distinctive morphological and molecular features, including apical mitosis, basal process extended radially to the pial surface and Pax6 expression (see previous section; Fietz et al., 2010; Hansen et al., 2010; Noctor et al., 2002; Reillo and Borrell, 2012; Reillo et al., 2011; Weissman et al., 2003). In contrast to the universality of aRGCs, basal progenitors have never been identified in the reptile cortex, and only in some birds at very low frequency or only in the lateral-most aspect of the cortex (i.e., the DVR in chick embryos; Fig. 2), whereas they are very abundant across mammals (Borrell and Calegari, 2014; Borrell and Gotz, 2014; Borrell and Reillo, 2012; Cheung et al., 2007; Gotz and Huttner, 2005; Kriegstein et al., 2006; Lui et al., 2011; Molnar, 2011; Taverna et al., 2014). Thus the developmental relevance of basal progenitors seems to be much greater in mammals than in nonmammalian species. As already mentioned, SVZ basal progenitors come essentially in two flavors: IPCs and bRGCs. The initial detailed descriptions and fate-potential characterization of IPCs were performed in rat and mouse embryos (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Although this was essentially because these species were the most common and malleable animal models for analysis and experimental manipulation, a second critical reason is that IPCs account for over 90% of basal progenitors in these species. In contrast, bRGCs were discovered in mouse (Shitamukai et al., 2011; Wang et al., 2011) only after being identified independently by three laboratories on much more limited and difficult specimens to study: human and ferret embryos (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). Again, the main reason why bRGCs were first discovered in these gyrencephalic species was because of their high abundance, in contrast to mouse (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). Only upon the discovery of the very abundant bRGCs in theses gyrencephalic species, and following a targeted labeling strategy, were they finally identified also in mouse embryos, but at a significantly lower abundance (Shitamukai et al., 2011; Wang et al., 2011). Similar searches have been later performed in a number of other relevant species including a near-lissencephalic primate, the marmoset monkey (*Callithrix jacchus*), and a gyrencephalic rodent, the amazonian Agouti (*Dasyprocta agouti*; Garcia-Moreno et al., 2012; Kelava et al., 2012), and both species have been found to contain both IPCs and bRGCs regardless of their cortical phenotype (size and shape) and

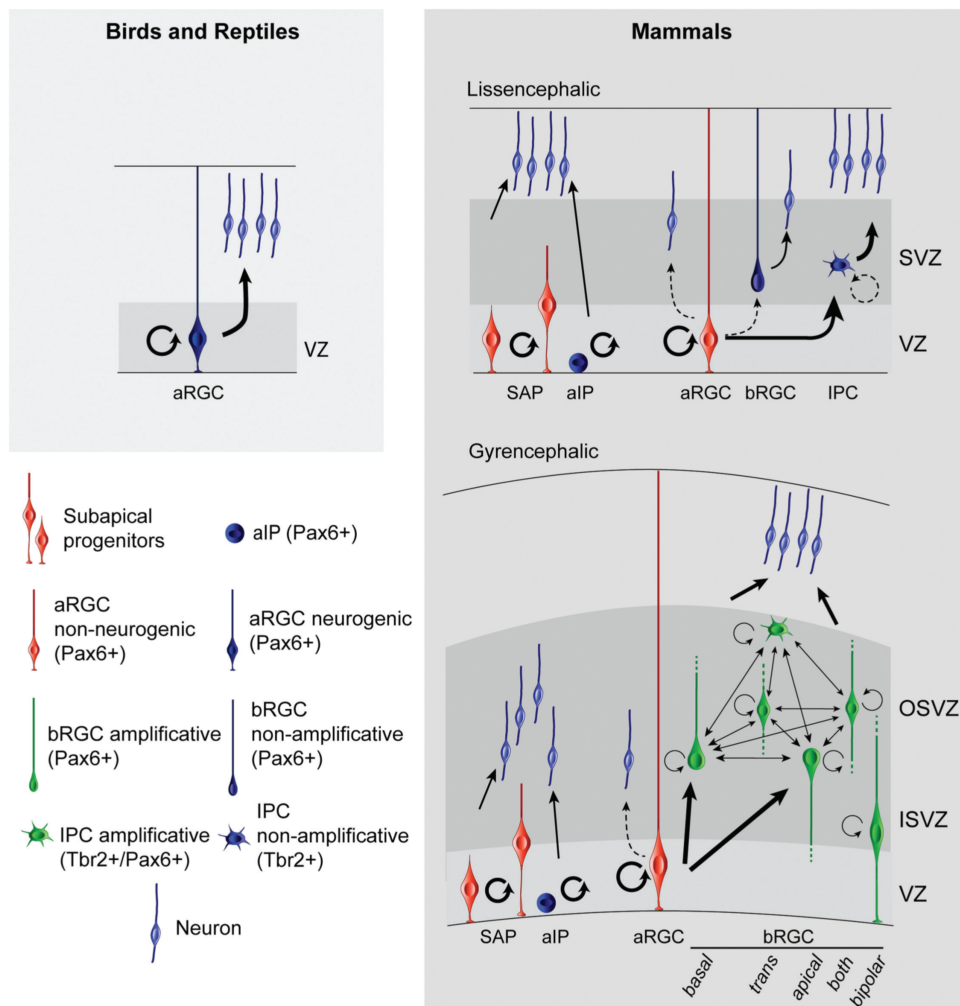

FIGURE 1: Progenitor cell types in the developing cerebral cortex of vertebrates. Schematic drawings illustrating the types of progenitor cells and their lineage relationships in the developing cortex of birds and reptiles (left), and mammals (right). In birds and reptiles, apical radial glia cells (aRGC) in the ventricular zone (VZ) are sole responsible for neuron generation by direct neurogenesis. In lissencephalic mammals (right upper panel), subapical progenitors (SAP) and apical intermediate progenitors (aIPs) coexist with aRGCs largely non-neurogenic. In these species, basal radial glia cells (bRGCs) in the subventricular zone (SVZ) are very scarce whereas the very abundant intermediate progenitors cells (IPCs) are the main source of neuron production by indirect neurogenesis. In gyrencephalic mammals (right lower panel) there is a wide variety of bRGC types (basal, transient, apical, both, and bipolar), indicative of the high complexity of the SVZ, which is subdivided in Inner (ISVZ) and Outer SVZ (OSVZ). In these more complex species, cortical neurons are generated by indirect neurogenesis from all basal progenitors, though most abundantly by bipolar RGCs. Black arrows indicate the output of the cell division, and thickness indicates the frequency of occurrence.

phylogeny. Hence, differences in basal progenitors between mammalian species are not so much in the types and diversity, but in their relative abundance.

In spite of the remarkably conserved presence of IPCs and bRGCs between mammalian species, recent reports have highlighted the existence of an unprecedented diversity of morphotypes among bRGCs in the embryonic cortex of macaque, ferret and sheep, and also in mouse but at extremely low abundance (Betizeau et al., 2013; Borrell and Gotz, 2014; Pilz et al., 2013). These morphotypes range from bRGCs with only a basal process, as originally described in multiple species (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011), to bRGCs with only an apical process, bRGCs with both a basal and an apical process (which may contact the cortical ventricular surface, as in bipolar bRGCs, or not) and even bRGCs that dynamically extend and retract apical and basal processes along the cell cycle (Fig. 1; Betizeau et al., 2013; Pilz et al., 2013). Very importantly, the identification of these morphotypes in macaque was achieved by videomicroscopy in living brain slices, which also allowed demonstrating that any one of these bRGC morphotypes can transform into any other within the same lineage (originally called transitions), a remarkable degree of interclass plasticity (Betizeau et al., 2013). Transitions occur at mitosis but not

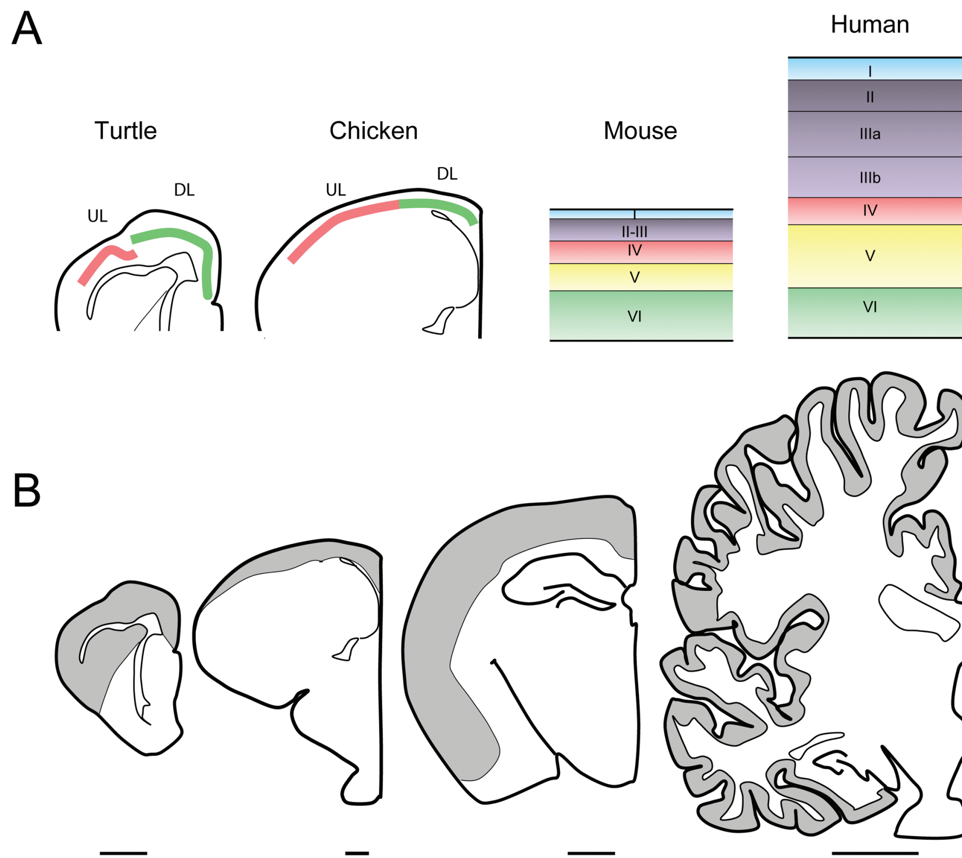

FIGURE 2: Interspecies variations in cerebral cortex morphology. Schematic representations of the turtle, chicken, mouse and human adult cerebral cortex. (A) Representation of the number and distribution of neuronal layers. In turtle and chicken, layer 2/3-type neurons are indicated with a green line, and layer 5-type neurons are represented with a red line. Cortical layers in human and mouse are indicated in roman numerals. (B) Representation of the size and distribution of the cerebral cortex (gray shade) in brain cross sections. Adapted from (Defelipe, 2011; Suzuki and Hirata, 2014). Scale bars: 1 mm for turtle, chicken and mouse; 2 cm for human.

along the cell cycle between mitoses, when bRGCs remain stable as a single morphotype. This remarkable lineage plasticity between bRGCs and IPCs, and its apparent reversibility (bRGC-to-IPC, and IPC-to-bRGC) is in sharp contrast with current observations in mouse where the sequence aRGC-to-IPC or aRGC-to-bRGC is irreversible (Betizeau et al., 2013; Noctor et al., 2004; Shitamukai et al., 2011; Wang et al., 2011). Given that these transitions are a dynamic feature, it will require using similar videomicroscopy approaches to define if they also occur in other species, and at which frequency. In the case of human, although the most parsimonious hypothesis is that the different bRGC morphotypes are also present in the embryonic cortex, they have not yet been identified by live imaging of postmortem human embryonic cortical slices (Hansen et al., 2010; LaMonica et al., 2013; Ostrem et al., 2014). This raises the possibility that this precious material and highly valuable methodology may have some important biases or limitations, such that some progenitor cell subtypes may be less resistant to hypoxia or any other kind of insult resulting from the clinical collection of samples. Future analyses using alternative methods, possibly including single-cell transcriptomics (Pollen et al., 2014), may allow defining if these distinct morphotypes actually correspond to distinct classes of basal progenitors or just transient isoforms of a single type of progenitor, and if they exist in all mammals.

Amplificative Potential of Basal Progenitors

Since the first descriptions by His, the late-appearing mitoses at basal positions in the developing cortex were viewed as contributing to gliogenesis because these were known to occur mostly at late stages of cortical development. Only with the direct visualization of the cellular product of those divisions by videomicroscopy in slice cultures of the murine cortex it was possible to determine that basal mitoses in the embryonic cerebral cortex are fundamentally neurogenic (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004), prior to becoming gliogenic at the very end of corticogenesis in early postnatal life (Kakita and Goldman, 1999; Suzuki and Goldman, 2003). Time-lapse imaging of murine IPCs also allowed demonstrating that for the most part these progenitors have a very limited capacity for self-renewal, being

mostly dedicated to symmetric self-consuming neurogenic divisions (Attardo et al., 2008; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Likewise, mouse bRGCs essentially lack amplificative potential and rather have direct neurogenic commitment (Wang et al., 2011). However this is not the case for species with bigger and folded brains. Early videomicroscopy studies of postmortem human embryonic tissue showed that both bRGCs and IPCs have the capacity to self-amplify for a few cell cycles, after which bRGCs generate IPCs, and IPCs generate neurons. In fact, IPCs were proposed to be the only and obligatory neurogenic progenitor (Hansen et al., 2010; Lui et al., 2011). The amplificative capacity of bRGCs was also demonstrated in ferret by alternative means (Gertz et al., 2014; Reillo et al., 2011). However, a later videomicroscopy study using macaque embryonic cortex reached somewhat different conclusions regarding which are the neurogenic progenitors (Betizeau et al., 2013). This analysis, that extends the work of Lukaszewicz et al. (2005), clearly demonstrated that cortical neurons are generated not only by IPCs but also by bRGCs, in contrast to previous results in human (Hansen et al., 2010; Lui et al., 2011). Although it is always tempting to speculate about species-specific differences, previous analyses in mouse actually demonstrated that bRGCs are neurogenic progenitors themselves, so the most parsimonious model is the one proposed by Betizeau and colleagues, where transitions between IPCs and all morphotypes of bRGCs are possible, and that all of them have the capacity to be directly neurogenic. Additional analyses from independent laboratories and in larger cell populations will be necessary to decide whether the lineages of cortical progenitors are dramatically different between human and macaque monkey while remarkably similar between human and mouse. Interestingly, Betizeau and colleagues noted that the lineages of IPCs were systematically smaller than those of bRGCs, strongly suggestive of a lower amplificative potential of the former. Taking together observations from mouse, ferret, macaque and human, it seems that in the general sense IPCs represent a less-amplificative and more neurogenically committed type of basal progenitor than bRGCs, and that the former are largely (but not only) below the latter in the cellular lineage.

Mitotic Somal Translocation

Whereas aRGCs undergo interkinetic nuclear migration, which is the movement of the cell nucleus between the apical and basal sides of the VZ during the cell cycle, IPCs and bRGCs remain essentially immobile in their site of residence in the SVZ during cell cycle. Intriguingly, this is the case for the lissencephalic mouse and rat but not for the gyrencephalic ferret, macaque and human, where bRGCs undergo mitotic somal translocation (MST). MST is the radial movement of the cell nucleus, in the basal direction and along the basal process, immediately prior to mitosis and cytokinesis (LaMonica et al., 2012). This translocation of the cell nucleus is preceded by the movement of the cellular centrosome into the basal process of bRGCs, and requires nonmuscle myosin II activation, as previously shown to be important in cortical progenitors (LaMonica et al., 2012; Ostrem et al., 2014; Schenk et al., 2009). MST was first described in human samples and later confirmed in both macaque and ferret (Betizeau et al., 2013; Gertz et al., 2014; Hansen et al., 2010; LaMonica et al., 2013; Ostrem et al., 2014). However, the frequency of these displacements and their extent vary significantly between these species. Whereas in the human embryo 45% of bRGCs undergo MST with an average displacement of 65 μm, in macaque this figures are 24% of bRGCs moving for 10–50 μm, and in ferret only 15% of bRGCs undergo MST with a much smaller displacement of 17 μm on average (Betizeau et al., 2013; Gertz et al., 2014; Ostrem et al., 2014). Because of the basally directed movement of the cell nucleus, and because it has only been observed in species with an OSVZ, it has been proposed that a main role for MST is the expansion of OSVZ thickness while bRGC divisions continue to generate increasing numbers of cortical neurons (LaMonica et al., 2012). Intriguingly, this idea is contradicted by analyses in macaque embryos, where MST has been reported to occur in both the basal and the apical directions. It is also worth highlighting that bRGCs are present in both OSVZ and ISVZ, which are distinctly separated and display very different cytoarchitecture. If MST is important for the radial displacement of bRGCs, it may be finely regulated at the interphase between ISVZ and OSVZ, and may thus contribute to define the thickness of ISVZ and OSVZ.

Molecular Regulation: Evo-Devo Lessons from Transcriptomics

The molecular bases for the similarities and differences highlighted above between cortical progenitor cell types, and between species for the same type of progenitor cell, have only begun to be elucidated. In the cerebral cortex of the mouse embryo aRGCs and bRGCs express the paired-box transcription factor Pax6 and not the T-box transcription factor Tbr2, whereas IPCs express Tbr2 but not Pax6 (a small percentage of mouse bRGCs have been reported to express Tbr2 by some authors, so this point remains controversial; Shitamukai et al., 2011; Wang et al., 2011). Whereas Pax6 has a critical role in the definition of telencephalic territories and multiple aspects of cortical progenitor proliferation (Asami et al., 2011; Estivill-Torrus et al., 2002; Gotz et al., 1998; Kroll and O'Leary, 2005; Mi et al., 2013; O'Leary and Sahara, 2008; Schuurmans and Guillemot, 2002; Sur and Rubenstein, 2005), Tbr2 has an instructive role in defining

the conversion of aRGCs into IPCs (Sessa et al., 2008). In agreement with these roles, Pax6 and Tbr2 expression follows a unidirectional sequence in the cortical progenitor lineage: first Pax6 (aRGCs, bRGCs), second Tbr2 (some bRGCs, IPCs and newborn neurons; Englund et al., 2005; Hevner, 2006). The very small numbers of cells that escape this norm are cells expressing high levels of one gene and very low levels of the other (Arai et al., 2011), which given the linearity of the progenitor cell lineage in mouse cortex, these cells have been interpreted as aRGCs beginning to downregulate Pax6 and upregulate Tbr2, in transition to becoming IPCs (Arai et al., 2011). This is indeed coincident with the brief period when newborn IPCs retract the apical process inherited from their mother aRGC to become multipolar cells in SVZ (Borrell et al., 2012; Noctor et al., 2008). The seemingly mutual exclusivity of Pax6 and Tbr2 expression in mouse suggests a relationship of transcriptional repression (Sansom et al., 2009). This interesting idea was experimentally tested by Broccoli and colleagues using *in utero* electroporation to overexpress Tbr2 in mouse aRGCs, which caused not only an increase in the number of Tbr2+ cells but also a very dramatic decrease in the number of Pax6+ cells, both at the protein and transcript levels (Sessa et al., 2008). A physical interaction of Tbr2 on the Pax6 promoter/enhancer regions has not been yet identified, but such a scenario would explain the loss of Pax6 upon the expression of Tbr2 alone and, more importantly, would be a simple mechanism to explain progenitor lineage irreversibility.

Similar to the interspecies variations in basal progenitor cell types and properties, their gene expression profiles are also remarkably different in larger and gyrencephalic brains compared with mouse. Already at the level of only considering Pax6 and Tbr2, these turn out to be coexpressed by 12–50% of basal progenitor cells in the OSVZ of ferret, macaque and human cortex (up to 80% in macaque ISVZ; Betizeau et al., 2013; Hansen et al., 2010; Reillo and Borrell, 2012; Reillo et al., 2011, Fietz, 2010 #2620). However the initial analyses of human and ferret bRGCs first reported that these expressed Pax6 (or Sox2) but not Tbr2, so the identity of Pax6+/Tbr2+ basal progenitors remained unknown (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). Recent analyses in macaque embryos using nonbiased labeling methods have demonstrated that Pax6 and Tbr2 are actually coexpressed in 35–50% of bRGCs, with most of the remaining bRGCs being Pax6+/Tbr2−. Even more striking, more than 60% of IPCs in macaque express both Pax6 and Tbr2 (Betizeau et al., 2013). This is very intriguing because, on the one hand it strongly suggests that the molecular mechanisms regulating Pax6 and Tbr2 expression may be different between the lissencephalic mouse and gyrencephalic species, including absence of mutual repression potentially due to sequence differences in their promoter-enhancer regions. In contrast, the transcriptomic heterogeneity of bRGCs strongly suggests that there may be yet other previously unrecognized progenitor cell subtypes within bRGCs, or that the expression of some of these hallmark genes may be subject to quite dynamic regulation, similar to their morphotype transitions.

Although Pax6 and Tbr2 have been extensively utilized as markers of apical and basal progenitors, important efforts have been done to unravel gene expression differences between cortical progenitors across species beyond these two transcription factors. As a first approximation, a landmark study reported the content of the whole transcriptome of individual cortical germinal layers in both mouse and human fetuses, and compared their composition (Fietz et al., 2012). Even though any given germinal layer contains a variety of cortical progenitors and other cell types, this first approximation identified thousands of genes expressed in germinal layers at different relative levels between mouse and human. Significantly, this study also led to the intriguing finding that extracellular matrix (ECM) components are highly enriched in the OSVZ of the human fetal cortex compared with mouse (Fietz et al., 2012). Importantly, experiments performed in ferret demonstrate that signaling via ECM components, fibroblast- and platelet-derived growth factors is directly involved in promoting the hyper-proliferation and/or self-renewal of cortical progenitors in gyrencephalic species (Fietz et al., 2010; Lui et al., 2014; Rash et al., 2013; Stenzel et al., 2014). However, neither ECM nor growth factors seem sufficient to cause in mouse the emergence of the remarkable heterogeneity of basal progenitor cells observed in the OSVZ of gyrated species.

If whole tissue transcriptomics has null cellular resolution due to the wide variety of cell types that are analyzed together, and thus it is nearly noninformative on the molecular distinction of progenitor cell types and their lineages (Lui et al., 2014), this is circumvented with the advent of single cell gene-expression profiling. The pioneering work of Matsuzaki and colleagues analyzed for the first time transcriptomics of single cortical cells, and demonstrated that cortical progenitor subclasses (namely apical versus basal progenitors) are cleanly identified by different gene expression fingerprints (Kawaguchi et al., 2008). More recently, combining the use of microfluidics and RNAseq, with sequencing coverage down to the limits of being informative, distinct gene expression profile clusters have been identified that reveal the existence of transcriptionally unique cell types in the developing human cortex (Pollen et al., 2014). This method not only proofs the validity of low coverage sequencing for the identification of specific cell types, but most importantly allows the reliable identification of major classes of progenitor and postmitotic cells of the fetal human cortex from a heterogeneous tissue. Although much promise is held for the future, this blind and

completely unbiased method has not yet been able to distinguish cell types as evidently different as aRGCs from bRGCs, much less any of the particular subtypes and morphotypes of bRGCs. Hopefully, combining this novel technology with the already ample knowledge on the cell biological features that distinguish each class and subtype of cortical progenitor cell, will lead us to identify their specific genetic fingerprint.

Dawn and Expansion of the Neocortex

The mammalian cerebral cortex is characteristically composed of six cellular layers (1 through 6). In contrast, its counterpart in birds and reptiles is much smaller (containing much fewer neurons) and simpler (only composed of three layers; Fig. 2). In addition to the six-layered cortex, the dorsal telencephalon of mammals also includes other parts with features reminiscent of the reptilian cortex, including fewer layers and circuit organization, such as the olfactory bulb, hippocampus and limbic cortex (Molnar et al., 2006; Super and Uylings, 2001). Because birds and reptiles emerged much earlier in evolution than mammals, the smaller, simpler and more ancient parts of the mammalian cortex are known as "archicortex" or "paleocortex," literally meaning "old cortex," whereas the much bigger six-layered counterpart only found in mammals is known as the "neocortex," literally meaning "new cortex." In this review, we have so far addressed which are the mechanisms of neocortical development involved in its expansion in higher mammals compared with mouse and rat. Yet a fundamental question remaining is which might have been the evolutionary mechanisms that led, in the first place, to the expansion of the primitive reptilian cortex into the extraordinarily large mammalian neocortex.

Examination of the developing dorsal telencephalon in reptile embryos demonstrates the complete absence of basal mitoses, hence of basal progenitors (Nomura et al., 2013). Because all mitoses occur at the apical surface, all neurons are produced directly from the primary progenitor cell, aRGCs (Fig. 1), which in mammals populate the ventricular lining of the developing telencephalon and constitute a VZ (Molnar et al., 2006; Nomura et al., 2013). From here on we will refer to the production of neurons directly from aRGCs as "direct neurogenesis." As indicated in a previous section, neurogenic mitoses are either symmetric: generating two neurons and consuming the progenitor mother cell, or asymmetric: generating one neuron and one self-renewed progenitor. In any case, neurogenesis is incompatible with the self-amplification of the progenitor cell at that mitosis, and frequently will result in its complete consumption. Therefore, the development of the reptilian cortex, that can only undergo direct neurogenesis, is prone to generate a very small number of neurons before progenitors are exhausted, and hence to produce a small cortex (Figs. 1 and 2). The situation is largely equivalent in birds, except that a small population of basal progenitors forms at the lateral aspect of the dorsal telencephalon during the neurogenetic period, thus allowing some sparing of aRGCs (Cheung et al., 2007). As in mammals, this small number of basal progenitors duplicates the neurogenic potential of aRGCs to each produce two neurons. Accordingly, the amount of neurons and thickness of the dorsolateral pallium in chicken is much greater than in its dorsomedial counterpart (Fig. 2). Similarly, the amount of neurons constituting the basal telencephalon of chick, which embryonically contains a very large amount of basal progenitors, is vastly greater than its dorsal counterpart, the pallium or "cortex" (Cheung et al., 2007). The fundamental limitation of direct neurogenesis in reptiles and birds is further evidenced by the fact that their period of neurogenesis is rather brief (2–4 days) in comparison to mammals (7 days in mice to 21 weeks in humans, and likely longer in elephants and whales). Altogether, these considerations strongly suggest that direct neurogenesis and a short neurogenetic period finally result in a small cerebral cortex.

Suppressing Direct Neurogenesis

The occurrence of basal progenitors in the dorsal pallium and their coalescence in the SVZ, which allows indirect neurogenesis and increased neuron production, has been widely recognized as the critical milestone in the evolutionary expansion of the cerebral cortex leading to mammals, particularly in its radial dimension and the formation of six layers (Cheung et al., 2010; Cheung et al., 2007; Molnar, 2011; Molnar et al., 2006; Puzzolo and Mallamaci, 2010), as originally proposed by Smart (1972a, 1972b). However, even with the occurrence of indirect neurogenesis, the persistence of aRGCs undergoing direct neurogenesis continues to limit neuron production because each neurogenic aRGC will generate, at most, half as many neurons compared with aRGCs generating basal progenitors; the difference will be even greater if basal progenitors can self-amplify to any extent. Indeed, the lateral pallium of the chicken, containing basal progenitors, is much thicker than its medial counterpart, exclusively limited to direct neurogenesis, but it is also still much thinner than the mouse neocortex (Cheung et al., 2007; Molnar et al., 2006; Fig. 2). In agreement with this notion, the occurrence of direct neurogenesis in the mouse cerebral cortex is remarkably infrequent (Attardo et al., 2008; Haubensak et al., 2004; Kowalczyk et al., 2009; Noctor et al., 2004). Hence, two fundamental changes must have occurred during vertebrate evolution between reptiles/birds and mammals leading to the emergence of the neocortex: (1) abundant generation of a novel type of neurogenic progenitor cell populating and dividing at basal positions in the embryonic cortex and (2) near complete suppression of the direct neurogenic program

from aRGCs (Fig. 1). As discussed in previous sections, the molecular mechanisms responsible for the generation of basal progenitors in mammals are still poorly understood, but some molecules have shown to be of particular importance, such as Tbr2, Dll-Notch, Shh-Smo, Robo receptors, Cux2, Fgfr3, Arx, Magoh, and others (Borrell et al., 2012; Colasante et al., 2009; Cubelos et al., 2008; Komada et al., 2013; Silver et al., 2013; Thomson et al., 2009). Importantly, the process of basal progenitor generation involves their detachment from the apical adherens junction belt and delamination from the ventricular zone. It has been shown that horizontal or oblique cell divisions generate basal radial glia-like cells that lack the apical attachment while keeping the basal process (Shitamukai et al., 2011), highlighting the importance of the mitotic spindle orientation in basal progenitor production and delamination. Similarly, experiments where the gene *Trnp1* was locally knocked-down in mouse embryos showed an alteration in the cleavage plane of apical radial glial cells toward a high occurrence of horizontal divisions, followed by a fast delamination and a very significant increase in the number of bRGCs generated (Stahl et al., 2013). Hence, delamination is a fundamental prerequisite for bRGC generation. In contrast, mechanisms responsible for direct neurogenesis have not been studied as such, but mice mutant for the Notch, Shh, and Wnt pathways strongly suggest their central involvement.

If direct neurogenesis is so detrimental for generating large numbers of neurons, are there any advantages? Saving developmental time is one. Indirect neurogenesis requires that aRGCs first go through one cell cycle to generate basal progenitors, and then these have to cycle once again before producing two neurons. In contrast, with direct neurogenesis the same number of neurons can be produced in half the time, only one cell cycle. Obviously this is not a sustainable strategy because it causes the depletion of the aRGC progenitor pool, as opposed to indirect neurogenesis. However, we have already mentioned that cortical development in nonmammals is very brief, and so this advantage of direct neurogenesis may actually be of critical importance. In the case of the lateral pallium in chicken, the combination of direct and indirect neurogenesis provides the advantages of both strategies, generating a considerable number of neurons in a relatively short period of developmental time.

Neocortical Expansion

Once the evolutionary forces accomplished the daunting task of genetic engineering to produce a completely new type of progenitor cell, the basal progenitor, and also repressed direct neurogenesis to maximize the neurogenic potential of aRGCs, this likely gave rise to the formation of a structure very similar to the modern mouse six-layered neocortex (Figs. 1 and 2). But how did this relatively small neocortex evolve into the gigantic cortices of elephants, whales and humans, with many-fold more neurons and additional laminar subdivisions? One of the simplest scenarios is that changes occurred at the level of cell cycle regulation, particularly in terms of cell-cycle re-entry, whereby basal progenitors would be able to self-renew and self-amplify prior to entering into the self-consuming mode of neurogenic divisions. Consistent with this notion, whereas in mouse most IPCs undergo only one round of division to generate 2 neurons (with few exceptions), in primates most neurogenic basal progenitors have been observed to undergo multiple rounds of amplificative divisions (up to six cycles have been reported; Fig. 1; Betizeau et al., 2013). This kind of developmental mechanism and strategy would result not only in a much larger amount of cortical neurons generated within a given developmental time window, but most importantly in a much greater ratio of neurons per aRGC and therefore in a much greater relative expansion of the neocortex.

Beyond Cortical Expansion: Gyrification

Evolution of the cerebral cortex and its expansion seem to have involved the suppression of direct neurogenesis, the novel emergence of various types of basal progenitors, and conferring these with self-renewing capacity to significantly increase the final neurogenic output. However, a general rule among extant mammals is that bigger brains have a very strong tendency to have a folded cerebral cortex (Welker, 1990). Indeed folding appears to be an evolutionary solution to the problem of increasing cortical surface area while limiting cranial volume. However this is not a universal rule and it has some clear exceptions, so it seems that it takes more than neurogenesis to gyrate. For example, the brains of a manatee and a chimpanzee are remarkably similar in volume, but the former is near lissencephalic and the latter is highly gyrencephalic (www.brainmuseum.org). The same holds true for the brains of the marmoset monkey and ferret (Fig. 3). Similarly, the brains of human patients suffering from lissencephaly (no folds) or polymicrogyria (too many folds) frequently have roughly normal numbers of neurons. So if neuron number is not causally related to cortical folding, what are the driving forces for neocortical gyrification? Several review articles support the radial divergence model (Borrell and Reillo, 2012; Reillo et al., 2011), an original idea proposing that bRGCs may hold the answer to this fundamental question (Fietz and Huttner, 2011; Florio and Huttner, 2014; Lui et al., 2011; Taverna et al., 2014). According to the radial divergence model, the development of gyrencephalic neocortices requires both the production of massive numbers of neurons and the tangential dispersion of these neurons during their radial migration, so that they do not accumulate in very thick layers but rather accommodate along a very large

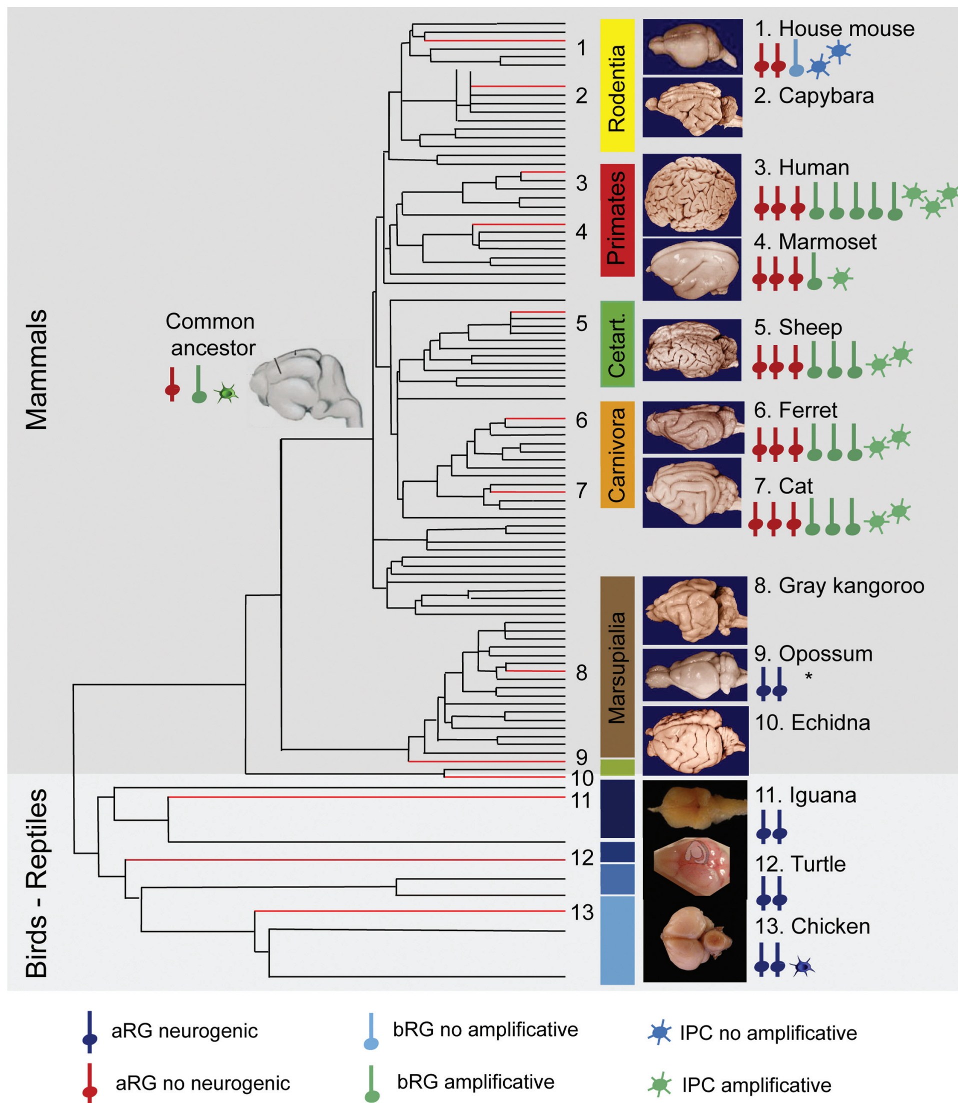

FIGURE 3: Relationships between brain development and progenitor cell type across vertebrate phylogeny. Simplified version of the phylogenetic supertree of vertebrates where a selection of families and their lineage relationships are indicated. Right, external views of the brains of selected species from each of the families highlighted in the tree (red lines and numbers). The number in front of each specimen indicates the position indicated in the phylogeny tree. For each species, the types of progenitor cells present and their gross relative abundance are indicated by the amount of symbols of the same type. Note that both gyrencephalic and lissencephalic species are found within most orders. The ancestor of all placental mammals was probably gyrencephalic, as concluded by (Lewitus et al., 2014; O'Leary et al., 2013). Mammalian brain images are from http://brainmuseum.org. Turtle neocortex image is from Larkum et al. (2007). Iguana and chicken brain images are from www.global.anato.cl.

cortical surface area. The cellular mechanism by which the tangential dispersion of radially migrating neurons is achieved is based on bRGCs. Given that radial neuronal migration depends on the fibers of radial glia, neurons faithfully follow the trajectory of these fibers to their final position in the CP. Hence, for migrating neurons to disperse tangentially, perpendicular to the radial fiber scaffold, would require crossing the trajectory of many radial glia fibers, which is never observed in mouse. However, in the embryonic cortex of gyrencephalic species the trajectories of radial fibers are not as parallel as in the lissencephalic mouse, but markedly divergent: the separation between radial fibers increases from VZ to CP (Reillo et al., 2011). As a result of this divergence, radially migrating neurons separate gradually from each other as they approach the CP, resulting in their tangential dispersion and ultimately the growth of the neocortex in surface area (Borrell and Reillo, 2012). In support of the radial divergence model for cortical gyrencephaly, radial fiber divergence is mostly found in areas of the embryonic cortex prospective of significant tangential expansion. Also, the degree of divergence increases dramatically during the last stages of neurogenesis, coincident with the migration of neurons destined to the superficial

cortical layers and undergoing the greatest tangential dispersion (Reillo et al., 2011). An important point of this model is that the divergence of radial glia fibers must be achieved without increasing the number of aRGCs, because otherwise the ventricular surface would also fold, which does not occur in gyrencephalic brains. This is possible thanks to the contribution of the basal process of bRGCs (Fig. 1). Basal RGCs extend a radial fiber from their position in ISVZ or OSVZ to the cortical surface, thereby increasing the number of radial fibers only in the superficial half of the developing cortex. Increasing the relative abundance of bRGCs promotes a significant divergence of the radial fiber scaffold, now composed by full-span radial fibers from aRGCs and the more superficial radial fibers of bRGCs. Moreover, even if bRGCs are present at high abundance, because their soma is not in VZ this does not cause folding of the VZ. Taken together, it is tempting to speculate that the early mammalian neocortex was further expanded by preferentially increasing the generation and amplification of neurogenic basal progenitors, including IPCs and bRGCs, while folding seemingly arose upon the selective expansion of bRGCs.

With the discovery of bRGCs and the postulation of the radial divergence model, significant discoveries have contributed to demonstrate its validity and the molecular mechanisms that may regulate this process. In a first study, bRGC proliferation in the OSVZ was reduced by 30% and this caused a dramatic reduction of cortical surface area and fold size without loss of cortical neurons, which consequently resulted in the thickening of cortical layers (Reillo et al., 2011). Reciprocally, in a gain-of-function approach OSVZ progenitors in the developing ferret cortex were forced to hyper-proliferate and self-renew by overexpression of two cell cycle proteins, Cdk4 and CyclinD1. Expansion of OSVZ progenitors led to the formation of additional cortical folds in ferret while preserving the normal six-layer organization (Nonaka-Kinoshita et al., 2013). Importantly, when the same genetic manipulation was performed in mouse embryos these developed macrocephaly, with a cerebral cortex larger than normal, but not a single fold (Nonaka-Kinoshita et al., 2013). Hence, augmenting the abundance of basal progenitors in the lissencephalic mouse but changing neither their types nor their proportions does not lead to cortical folding, again consistent with observations in human lissencephaly (see above). However, when the abundance of bRGCs was increased disproportionately by the acute downregulation of Trnp1, this led to the formation of cortical folds even in naturally lissencephalic species like mouse (Stahl et al., 2013).

If the above mechanisms operate in naturally gyrencephalic species, they should be observable. Indeed, *Trnp1* expression levels vary significantly during developmental time and along the germinal layers of the human fetal cerebral cortex, in patterns coherent with the prospective expansion of the OSVZ and folding of cortical territories (Stahl et al., 2013). The same may also be true for human disease genes causing regional cortical malformations (Barkovich et al., 2012). For example, mutations in GPR56 were initially identified as causative of regional polymicrogyria, only in the frontoparietal cortex while sparing the rest of the brain (Piao et al., 2004). Recently it has been demonstrated that GPR56 expression is strictly regionalized and regulated in a very complex manner, and new subtle mutations in its enhancer regions have been recently identified as causative of patterned cortical malformations in humans. Most interestingly, there exist species-specific variations in the GPR56 regulatory regions that drive gene expression in distinct patterns in the developing neocortex, strongly suggesting this may be a mechanism contributing to define the species-specific pattern of cortical folding across mammals (Bae et al., 2014).

Is cortical folding the pinnacle of cerebral cortex evolution? On the one hand, folding of the cerebral cortex is not unique to primates but actually a common feature to most mammalian orders (rodents, primates, ungulates, carnivores) including groups evolutionarily very ancient like marsupials and monotremes (Fig. 3; Borrell and Reillo, 2012; Reillo et al., 2011). This indicates that either mechanisms driving gyrencephaly across mammalian phylogeny emerged independently by convergent evolution; or that these mechanisms arose once in mammalian evolution (namely a large OSVZ containing abundant bRGCs), and then were further expanded during speciation leading to gyrencephaly, or lost secondarily leading to lissencephaly. Several sets of experimental evidence argue in favor of the latter. First, the presence of a prominent OSVZ (and hence the distinction between ISVZ and OSVZ) and a high abundance of bRGCs has been observed across a wide variety of gyrencephalic mammals but in very few lisencephalic cases (Garcia-Moreno et al., 2012; Kelava et al., 2012; Martinez-Cerdeno et al., 2012). Second, the relative abundance of OSVZ progenitors in the embryonic cortex has a significant positive correlation with the degree of cortical folding across a variety of species (Fietz et al., 2010; Hansen et al., 2010; Kelava et al., 2012; Pilz et al., 2013; Reillo et al., 2011). Third, models combining phylogeny proximity data with index of gyrencephaly for a wide range of species conclude that lissencephaly emerged from ancestors more gyrencephalic (Kelava et al., 2012). This notion is further substantiated by recent reports concluding that the stem placental mammal displayed some degree of gyrencephaly (Lewitus et al., 2014; O'Leary et al., 2013; Fig. 3).

Conclusions

The cerebral cortex has been subject to an intense process of evolution for the last 200 million years, since the ancestor of reptiles, birds and mammals. At the core of this process we

find radial glial cells and their derivatives, which by being key actors in cortical development have played fundamental roles in its evolution. Starting with the early, small and simple reptilian cortex where all neurons were directly produced by a small population of self-consuming aRGCs, basal progenitors eventually emerged to limit aRGC depletion and expand the neurogenic potential. Later on, evolutionary pressures selected aRGCs with less frequent direct neurogenesis in favor of producing larger numbers of basal progenitors, leading to the emergence of the mammalian neocortex. A subset of these basal progenitors, bRGCs, retained many features of aRGCs, primarily the basal process or radial fiber, which promoted the tangential dispersion of radially migrating neurons. Subsequent evolutionary events led either to the amplification of bRGCs and the elaboration of gyrencephaly, or their reduction in favor of the nonpolar and highly neurogenic IPCs and the simplification of lissencephaly. Within the gyrencephalic species, gene expression patterns were selected to favor regional accumulations of bRGCs and coherent patterns of cortical folding that would maximize cortical volume-to-surface area, and optimize cortical function. Thus, a long journey of coevolution between radial glial cells and the cerebral cortex has resulted in the remarkable diversity and complexity of cortical phenotypes that we can observe today in vertebrates.

Acknowledgment

Grant sponsor: European Union Seventh Framework Programme FP7/2007–2013 under the project DESIRE; Grant number: 602531; Grant sponsor: European Research Council; Grant number: 309633; Grant sponsor: MINECO; Grant number: BFU2012-33473 (to VB)

The authors apologize to many colleagues whose work could not be cited owing to space restrictions. The authors thank M.A. Martínez-Martínez, I. Reillo, A. Cárdenas, F. Calegari, W. Huttner, and M. Götz, for frequent discussions.

References

Aaku-Saraste E, Hellwig A, Huttner WB. 1996. Loss of occludin and functional tight junctions, but not ZO-1, during neural tube closure–remodeling of the neuroepithelium prior to neurogenesis. Dev Biol 180:664–679.

Arai Y, Pulvers JN, Haffner C, Schilling B, Nusslein I, Calegari F, Huttner WB. 2011. Neural stem and progenitor cells shorten S-phase on commitment to neuron production. Nat Commun 2:154.

Asami M, Pilz GA, Ninkovic J, Godinho L, Schroeder T, Huttner WB, Gotz M. 2011. The role of pax6 in regulating the orientation and mode of cell division of progenitors in the mouse cerebral cortex. Development 138:5067–5078.

Attardo A, Calegari F, Haubensak W, Wilsch-Brauninger M, Huttner WB. 2008. Live imaging at the onset of cortical neurogenesis reveals differential appearance of the neuronal phenotype in apical versus basal progenitor progeny. PLoS One 3:e2388.

Bae BI, Tietjen I, Atabay KD, Evrony GD, Johnson MB, Asare E, Wang PP, Murayama AY, Im K, Lisgo SN, Overman L, Šestan N, Chang BS, Barkovich AJ, Grant PE, Topçu M, Politsky J, Okano H, Piao X, Walsh CA. 2014. Evolutionarily dynamic alternative splicing of gpr56 regulates regional cerebral cortical patterning. Science 343:764–768.

Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. 2012. A developmental and genetic classification for malformations of cortical development: Update 2012. Brain 135:1348–1369.

Bayatti N, Moss JA, Sun L, Ambrose P, Ward JF, Lindsay S, Clowry GJ. 2008. A molecular neuroanatomical study of the developing human neocortex from 8 to 17 postconceptional weeks revealing the early differentiation of the subplate and subventricular zone. Cereb Cortex 18:1536–1548.

Bayer SA, Altman J. 1991. Neocortical development. New York: Raven Press.

Beggs HE, Schahin-Reed D, Zang K, Goebbels S, Nave KA, Gorski J, Jones KR, Sretavan D, Reichardt LF. 2003. FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. Neuron 40:501–514.

Betizeau M, Cortay V, Patti D, Pfister S, Gautier E, Bellemin-Ménard A, Afanassieff M, Huissoud C, Douglas RJ, Kennedy H, Dehay C. 2013. Precursor diversity and complexity of lineage relationships in the outer subventricular zone of the primate. Neuron 80:442–457.

Borrell V, Calegari F. 2014. Mechanisms of brain evolution: Regulation of neural progenitor cell diversity and cell cycle length. Neurosci Res

Borrell V, Cárdenas A, Ciceri G, Galcerán J, Flames N, Pla R, Nóbrega-Pereira S, García-Frigola C, Peregrín S, Zhao Z, Ma L, Tessier-Lavigne M, Marín O. 2012. Slit/robo signaling modulates the proliferation of central nervous system progenitors. Neuron 76:338–352.

Borrell V, Gotz M. 2014. Role of radial glial cells in cerebral cortex folding. Curr Opin Neurobiol 27:39–46. C:

Borrell V, Reillo I. 2012. Emerging roles of neural stem cells in cerebral cortex development and evolution. Dev Neurobiol 72:955–971.

Boulder_Committee 1970. Embryonic vertebrate central nervous system: Revised terminology. Anat Rec 166:257–261.

Campbell K, Gotz M. 2002. Radial glia: Multi-purpose cells for vertebrate brain development. Trends Neurosci 25:235–238.

Colasante G, Sessa A, Crispi S, Calogero R, Mansouri A, Collombat P, Broccoli V. 2009. Arx acts as a regional key selector gene in the ventral telencephalon mainly through its transcriptional repression activity. Dev Biol 334:59–71.

Cubelos B, Sebastian-Serrano A, Kim S, Moreno-Ortiz C, Redondo JM, Walsh CA, Nieto M. 2008. Cux-2 controls the proliferation of neuronal intermediate precursors of the cortical subventricular zone. Cereb Cortex 18:1758–1770.

Cheung AF, Kondo S, Abdel-Mannan O, Chodroff RA, Sirey TM, Bluy LE, Webber N, DeProto J, Karlen SJ, Krubitzer L, Stolp HB, Saunders NR, Molnár Z. 2010. The subventricular zone is the developmental milestone of a 6-layered neocortex: Comparisons in metatherian and eutherian mammals. Cereb Cortex 20:1071–1081.

Cheung AF, Pollen AA, Tavare A, DeProto J, Molnar Z. 2007. Comparative aspects of cortical neurogenesis in vertebrates. J Anat 211:164–176.

Defelipe J. 2011. The evolution of the brain, the human nature of cortical circuits, and intellectual creativity. Front Neuroanat 5:29.

Dehay C, Kennedy H. 2007. Cell-cycle control and cortical development. Nat Rev Neurosci 8:438–450.

Dorus S, Vallender EJ, Evans PD, Anderson JR, Gilbert SL, Mahowald M, Wyckoff GJ, Malcom CM, Lahn BT. 2004. Accelerated evolution of nervous system genes in the origin of *Homo sapiens*. Cell 119:1027–1040.

Englund C, Fink A, Lau C, Pham D, Daza RA, Bulfone A, Kowalczyk T, Hevner RF. 2005. Pax6, tbr2, and tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 25:247–251.

Estivill-Torrus G, Pearson H, van Heyningen V, Price DJ, Rashbass P. 2002. Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. Development 129:455–466.

Fietz SA, Huttner WB. 2011. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. Curr Opin Neurobiol 21:23–35.

Fietz SA, Kelava I, Vogt J, Wilsch-Bräuninger M, Stenzel D, Fish JL, Corbeil D, Riehn A, Distler W, Nitsch R, Huttner WB. 2010. OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. Nat Neurosci 13:690–699.

Fietz SA, Lachmann R, Brandl H, Kircher M, Samusik N, Schröder R, Lakshmanaperumal N, Henry I, Vogt J, Riehn A, Distler W, Nitsch R, Enard W, Pääbo S, Huttner WB. 2012. Transcriptomes of germinal zones of human and mouse fetal neocortex suggest a role of extracellular matrix in progenitor self-renewal. Proc Natl Acad Sci USA 109:11836–11841.

Finlay BL, Darlington RB. 1995. Linked regularities in the development and evolution of mammalian brains. Science 268:1578–1584.

Fish JL, Dehay C, Kennedy H, Huttner WB. 2008. Making bigger brains-the evolution of neural-progenitor-cell division. J Cell Sci 121:2783–2793.

Florio M, Huttner WB. 2014. Neural progenitors, neurogenesis and the evolution of the neocortex. Development 141:2182–2194.

Gal JS, Morozov YM, Ayoub AE, Chatterjee M, Rakic P, Haydar TF. 2006. Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. J Neurosci 26:1045–1056.

Garcia-Moreno F, Vasistha NA, Trevia N, Bourne JA, Molnar Z. 2012. Compartmentalization of cerebral cortical germinal zones in a lissencephalic primate and gyrencephalic rodent. Cereb Cortex 22:482–492.

Gertz CC, Lui JH, LaMonica BE, Wang X, Kriegstein AR. 2014. Diverse behaviors of outer radial glia in developing ferret and human cortex. J Neurosci 34:2559–2570.

Gotz M, Huttner WB. 2005. The cell biology of neurogenesis. Nature Rev Mol Cell Biol 6:777–788.

Gotz M, Stoykova A, Gruss P. 1998. Pax6 controls radial glia differentiation in the cerebral cortex. Neuron 21:1031–1044.

Hansen DV, Lui JH, Parker PR, Kriegstein AR. 2010. Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464:554–561.

Hartfuss E, Galli R, Heins N, Gotz M. 2001. Characterization of CNS precursor subtypes and radial glia. Dev Biol 229:15–30.

Hartmann D, Ziegenhagen MW, Sievers J. 1998. Meningeal cells stimulate neuronal migration and the formation of radial glial fascicles from the cerebellar external granular layer. Neurosci Lett 244:129–132.

Haubensak W, Attardo A, Denk W, Huttner WB. 2004. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: A major site of neurogenesis. Proc Natl Acad Sci US A 101:3196–3201.

Hausmann B, Sievers J. 1985. Cerebellar external granule cells are attached to the basal lamina from the onset of migration up to the end of their proliferative activity. J Comp Neurol 241:50–62.

Hevner RF. 2006. From radial glia to pyramidal-projection neuron: Transcription factor cascades in cerebral cortex development. Mol Neurobiol 33:33–50.

Hevner RF, Hodge RD, Daza RA, Englund C. 2006. Transcription factors in glutamatergic neurogenesis: Conserved programs in neocortex, cerebellum, and adult hippocampus. Neurosci Res 55:223–233.

Javaherian A, Kriegstein A. 2009. A stem cell niche for intermediate progenitor cells of the embryonic cortex. Cereb Cortex 19:i70–i77.

Jeong SJ, Luo R, Singer K, Giera S, Kreidberg J, Kiyozumi D, Shimono C, Sekiguchi K, Piao X. 2013. Gpr56 functions together with alpha3beta1 integrin in regulating cerebral cortical development. PLoS One 8:e68781.

Johansson PA, Irmler M, Acampora D, Beckers J, Simeone A, Gotz M. 2013. The transcription factor otx2 regulates choroid plexus development and function. Development 140:1055–1066.

Kakita A, Goldman JE. 1999. Patterns and dynamics of SVZ cell migration in the postnatal forebrain: Monitoring living progenitors in slice preparations. Neuron 23:461–472.

Kandel ER, Schwartz JH, Jessell TM. 2000. Principles of neural science. New York: McGraw-Hill.

Kawaguchi A, Ikawa T, Kasukawa T, Ueda HR, Kurimoto K, Saitou M, Matsuzaki F. 2008. Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. Development 135:3113–3124.

Kelava I, Lewitus E, Huttner WB. 2013. The secondary loss of gyrencephaly as an example of evolutionary phenotypical reversal. Front Neuroanat 7:16.

Kelava I, Reillo I, Murayama AY, Kalinka AT, Stenzel D, Tomancak P, Matsuzaki F, Lebrand C, Sasaki E, Schwamborn JC, Okano H, Huttner WB, Borrell V. 2012. Abundant occurrence of basal radial glia in the subventricular zone of embryonic neocortex of a lissencephalic primate, the common marmoset callithrix jacchus. Cereb Cortex 22:469–481.

Koirala S, Jin Z, Piao X, Corfas G. 2009. GPR56-regulated granule cell adhesion is essential for rostral cerebellar development. J Neurosci 29:7439–7449.

Komada M, Iguchi T, Takeda T, Ishibashi M, Sato M. 2013. Smoothened controls cyclin d2 expression and regulates the generation of intermediate progenitors in the developing cortex. Neurosci Lett 547:87–91.

Kouprina N, Pavlicek A, Mochida GH, Solomon G, Gersch W, Yoon YH, Collura R, Ruvolo M, Barrett JC, Woods CG, Walsh CA, Jurka J, Larionov V. 2004. Accelerated evolution of the ASPM gene controlling brain size begins prior to human brain expansion. PLoS Biol 2:e126.

Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, Hodge R, Attardo A, Bell C, Huttner WB, Hevner RF. 2009. Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. Cereb Cortex 19:2439–2450.

Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149–184.

Kriegstein A, Noctor S, Martinez-Cerdeno V. 2006. Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. Nat Rev Neurosci 7:883–890.

Kriegstein AR, Gotz M. 2003. Radial glia diversity: A matter of cell fate. Glia 43:37–43.

Kroll TT, O'Leary DD. 2005. Ventralized dorsal telencephalic progenitors in pax6 mutant mice generate GABA interneurons of a lateral ganglionic eminence fate. Proc Natl Acad Sci USA 102:7374–7379.

LaMonica BE, Lui JH, Hansen DV, Kriegstein AR. 2013. Mitotic spindle orientation predicts outer radial glial cell generation in human neocortex. Nat Commun 4:1665.

LaMonica BE, Lui JH, Wang X, Kriegstein AR. 2012. OSVZ progenitors in the human cortex: An updated perspective on neurodevelopmental disease. Curr Opin Neurobiol 22:747–753.

Lehtinen MK, Walsh CA. 2011. Neurogenesis at the brain-cerebrospinal fluid interface. Annu Rev Cell Dev Biol 27:653–679.

Lehtinen MK, Zappaterra MW, Chen X, Yang YJ, Hill AD, Lun M, Maynard T, Gonzalez D, Kim S, Ye P, D'Ercole AJ, Wong ET, LaMantia AS, Walsh CA. 2011. The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. Neuron 69:893–905.

Lewitus E, Kelava I, Kalinka AT, Tomancak P, Huttner WB. 2014. An adaptive threshold in mammalian neocortical evolution. PLoS Biol 12:e1002000.

Lui JH, Hansen DV, Kriegstein AR. 2011. Development and evolution of the human neocortex. Cell 146:18–36.

Lui JH, Nowakowski TJ, Pollen AA, Javaherian A, Kriegstein AR, Oldham MC. 2014. Radial glia require PDGFD-PDGFRbeta signalling in human but not mouse neocortex. Nature 515:264–268.

Lukaszewicz A, Savatier P, Cortay V, Giroud P, Huissoud C, Berland M, Kennedy H, Dehay C. 2005. G1 phase regulation, area-specific cell cycle control, and cytoarchitectonics in the primate cortex. Neuron 47:353–364.

Malatesta P, Hack MA, Hartfuss E, Kettenmann H, Klinkert W, Kirchhoff F, Gotz M. 2003. Neuronal or glial progeny: Regional differences in radial glia fate. Neuron 37:751–764.

Malatesta P, Hartfuss E, Gotz M. 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127:5253–5263.

Manabe N, Hirai S, Imai F, Nakanishi H, Takai Y, Ohno S. 2002. Association of ASIP/mPAR-3 with adherens junctions of mouse neuroepithelial cells. Dev Dyn 225:61–69.

Marthiens V, Kazanis I, Moss L, Long K, Ffrench-Constant C. 2010. Adhesion molecules in the stem cell niche—More than just staying in shape? J Cell Sci 123:1613–1622.

Martinez-Cerdeno V, Cunningham CL, Camacho J, Antczak JL, Prakash AN, Cziep ME, Walker AI, Noctor SC. 2012. Comparative analysis of the subventricular zone in rat, ferret and macaque: Evidence for an outer subventricular zone in rodents. PLoS One 7:e30178.

Martinez-Cerdeno V, Noctor SC, Kriegstein AR. 2006. The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. Cereb Cortex 16:i152–i161. [16766701]

Mi D, Carr CB, Georgala PA, Huang YT, Manuel MN, Jeanes E, Niisato E, Sansom SN, Livesey FJ, Theil T, Hasenpusch-Theil K, Simpson TI, Mason JO, Price DJ. 2013. Pax6 exerts regional control of cortical progenitor proliferation via direct repression of cdk6 and hypophosphorylation of pRb. Neuron 78: 269–284.

Miyata T, Kawaguchi A, Okano H, Ogawa M. 2001. Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron 31:727–741.

Miyata T, Kawaguchi A, Saito K, Kawano M, Muto T, Ogawa M. 2004. Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. Development 131:3133–3145.

Molnar Z. 2011. Evolution of cerebral cortical development. Brain Behav Evol 78:94–107.

Molnar Z, Metin C, Stoykova A, Tarabykin V, Price DJ, Francis F, Meyer G, Dehay C, Kennedy H. 2006. Comparative aspects of cerebral cortical development. Eur J Neurosci 23:921–934.

Niewmierzycka A, Mills J, St-Arnaud R, Dedhar S, Reichardt LF. 2005. Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. J Neurosci 25:7022–7031.

Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR. 2001. Neurons derived from radial glial cells establish radial units in neocortex. Nature 409:714–720.

Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR. 2002. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. J Neurosci 22: 3161–3173.

Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 7:136–144.

Noctor SC, Martinez-Cerdeno V, Kriegstein AR. 2008. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. J Comp Neurol 508:28–44.

Nomura T, Gotoh H, Ono K. 2013. Changes in the regulation of cortical neurogenesis contribute to encephalization during amniote brain evolution. Nat Commun 4:2206.

Nonaka-Kinoshita M, Reillo I, Artegiani B, Martinez-Martinez MA, Nelson M, Borrell V, Calegari F. 2013. Regulation of cerebral cortex size and folding by expansion of basal progenitors. EMBO J 32:1817–1828.

O'Leary DD, Sahara S. 2008. Genetic regulation of arealization of the neocortex. Curr Opin Neurobiol 18:90–100.

O'Leary MA, Bloch JI, Flynn JJ, Gaudin TJ, Giallombardo A, Giannini NP, Goldberg SL, Kraatz BP, Luo ZX, Meng J, Ni X, Novacek MJ, Perini FA, Randall ZS, Rougier GW, Sargis EJ, Silcox MT, Simmons NB, Spaulding M, Velazco PM, Weksler M, Wible JR, Cirranello AL. 2013. The placental mammal ancestor and the post-K-pg radiation of placentals. Science 339:662–667.

Ostrem BE, Lui JH, Gertz CC, Kriegstein AR. 2014. Control of outer radial glial stem cell mitosis in the human brain. Cell Rep 8:656–664.

Paridaen JT, Huttner WB. 2014. Neurogenesis during development of the vertebrate central nervous system. EMBO Rep 15:351–364.

Paridaen JT, Wilsch-Brauninger M, Huttner WB. 2013. Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division. Cell 155:333–344.

Pawlisz AS, Feng Y. 2011. Three-dimensional regulation of radial glial functions by Lis1-nde1 and dystrophin glycoprotein complexes. PLoS Biol 9: e1001172.

Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Straussberg R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, Voit T, Ross ME, Michaud JL, Déscarie JC, Barkovich AJ, Walsh CA. 2004. G protein-coupled receptor-dependent development of human frontal cortex. Science 303:2033–2036.

Pilz GA, Shitamukai A, Reillo I, Pacary E, Schwausch J, Stahl R, Ninkovic J, Snippert HJ, Clevers H, Godinho L, Guillemot F, Borrell V, Matsuzaki F, Götz M. 2013. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. Nat Commun 4:2125.

Pollen AA, Nowakowski TJ, Shuga J, Wang X, Leyrat AA, Lui JH, Li N, Szpankowski L, Fowler B, Chen P, Ramalingam N, Sun G, Thu M, Norris M, Lebofsky R, Toppani D, Kemp DW 2nd, Wong M, Clerkson B, Jones BN, Wu S, Knutsson L, Alvarado B, Wang J, Weaver LS, May AP, Jones RC, Unger MA, Kriegstein AR, West JA. 2014. Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. Nat Biotechnol 32:1053–1058.

Purves D. 1988. Body and brain: A trophic theory of neural connections. Cambridge, MA: Harvard University Press.

Puzzolo E, Mallamaci A. 2010. Cortico-cerebral histogenesis in the opossum monodelphis domestica: Generation of a hexalaminar neocortex in the absence of a basal proliferative compartment. Neural Dev 5:8.

Rakic P. 1995. A small step for the cell, a giant leap for mankind: A hypothesis of neocortical expansion during evolution. Trends Neurosci 18:383–388.

Rakic P. 2009. Evolution of the neocortex: A perspective from developmental biology. Nat Rev Neurosci 10:724–735.

Rash BG, Tomasi S, Lim HD, Suh CY, Vaccarino FM. 2013. Cortical gyrification induced by fibroblast growth factor 2 in the mouse brain. J Neurosci 33: 10802–10814.

Reillo I, Borrell V. 2012. Germinal zones in the developing cerebral cortex of ferret: Ontogeny, cell cycle kinetics, and diversity of progenitors. Cereb Cortex 22:2039–2054.

Reillo I, de Juan Romero C, Garcia-Cabezas MA, Borrell V. 2011. A role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex. Cereb Cortex 21:1674–1694.

Ross ME, Walsh CA. 2001. Human brain malformations and their lessons for neuronal migration. Annu Rev Neurosci 24:1041–1070.

Sahara S, O'Leary DD. 2009. Fgf10 regulates transition period of cortical stem cell differentiation to radial glia controlling generation of neurons and basal progenitors. Neuron 63:48–62.

Sansom SN, Griffiths DS, Faedo A, Kleinjan DJ, Ruan Y, Smith J, van Heyningen V, Rubenstein JL, Livesey FJ. 2009. The level of the transcription factor pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis. PLoS Genet 5:e1000511.

Schenk J, Wilsch-Brauninger M, Calegari F, Huttner WB. 2009. Myosin II is required for interkinetic nuclear migration of neural progenitors. Proc Natl Acad Sci USA 106:16487-16492.

Schuurmans C, Guillemot F. 2002. Molecular mechanisms underlying cell fate specification in the developing telencephalon. Curr Opin Neurobiol 12:26–34.

Sessa A, Mao CA, Hadjantonakis AK, Klein WH, Broccoli V. 2008. Tbr2 directs conversion of radial glia into basal precursors and guides neuronal amplification by indirect neurogenesis in the developing neocortex. Neuron 60:56–69.

Shitamukai A, Konno D, Matsuzaki F. 2011. Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer subventricular zone progenitors. J Neurosci 31:3683–3695.

Sidman RL, Rakic P. 1973. Neuronal migration, with special reference to developing human brain: A review. Brain Res 62:1–35.

Silver DL, Leeds KE, Hwang HW, Miller EE, Pavan WJ. 2013. The EJC component magoh regulates proliferation and expansion of neural crest-derived melanocytes. Dev Biol 375:172–181.

Singer K, Luo R, Jeong SJ, Piao X. 2013. Gpr56 and the developing cerebral cortex: Cells, matrix, and neuronal migration. Mol Neurobiol 47:186–196.

Smart IH. 1972a. Proliferative characteristics of the ependymal layer during the early development of the mouse diencephalon, as revealed by recording the number, location, and plane of cleavage of mitotic figures. J Anat 113: 109–129.

Smart IH. 1972b. Proliferative characteristics of the ependymal layer during the early development of the spinal cord in the mouse. J Anat 111:365–380.

Smart IH, Dehay C, Giroud P, Berland M, Kennedy H. 2002. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. Cereb Cortex 12:37–53.

Stahl R, Walcher T, De Juan Romero C, Pilz GA, Cappello S, Irmler M, Sanz-Aquela JM, Beckers J, Blum R, Borrell V, Götz M. 2013. Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. Cell 153:535–549.

Stenzel D, Wilsch-Brauninger M, Wong FK, Heuer H, Huttner WB. 2014. Integrin alphavbeta3 and thyroid hormones promote expansion of progenitors in embryonic neocortex. Development 141:795–806.

Super H, Uylings HB. 2001. The early differentiation of the neocortex: A hypothesis on neocortical evolution. Cereb Cortex 11:1101–1109.

Sur M, Rubenstein JL. 2005. Patterning and plasticity of the cerebral cortex. Science 310:805–810.

Suzuki IK, Hirata T. 2014. A common developmental plan for neocortical gene-expressing neurons in the pallium of the domestic chicken gallus gallus domesticus and the Chinese softshell turtle pelodiscus sinensis. Front Neuroanat 8:20.

Suzuki SO, Goldman JE. 2003. Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: A dynamic study of glial and neuronal progenitor migration. J Neurosci 23:4240–4250.

Takahashi T, Misson JP, Caviness VS Jr. 1990. Glial process elongation and branching in the developing murine neocortex: A qualitative and quantitative immunohistochemical analysis. J Comp Neurol 302:15–28.

Takahashi T, Nowakowski RS, Caviness VS Jr. 1993. Cell cycle parameters and patterns of nuclear movement in the neocortical proliferative zone of the fetal mouse. J Neurosci 13:820–833.

Takahashi T, Nowakowski RS, Caviness VS Jr. 1994. Mode of cell proliferation in the developing mouse neocortex. Proc Natl Acad Sci USA 91:375–379.

Takahashi T, Nowakowski RS, Caviness VS Jr. 1996. The leaving or Q fraction of the murine cerebral proliferative epithelium: A general model of neocortical neuronogenesis. J Neurosci 16:6183–6196.

Taverna E, Gotz M, Huttner WB. 2014. The cell biology of neurogenesis: Toward an understanding of the development and evolution of the neocortex. Annu Rev Cell Dev Biol 30:465–502.

Thomson RE, Kind PC, Graham NA, Etherson ML, Kennedy J, Fernandes AC, Marques CS, Hevner RF, Iwata T. 2009. Fgf receptor 3 activation promotes selective growth and expansion of occipitotemporal cortex. Neural Dev 4:4.

Tyler WA, Haydar TF. 2013. Multiplex genetic fate mapping reveals a novel route of neocortical neurogenesis, which is altered in the Ts65Dn mouse model of down syndrome. J Neurosci 33:5106–5119.

Wang X, Tsai JW, Lamonica B, Kriegstein AR. 2011. A new subtype of progenitor cell in the mouse embryonic neocortex. Nat Neurosci 14:555–561.

Warren N, Caric D, Pratt T, Clausen JA, Asavaritikrai P, Mason JO, Hill RE, Price DJ. 1999. The transcription factor, pax6, is required for cell proliferation and differentiation in the developing cerebral cortex. Cereb Cortex 9:627–635.

Weigmann A, Corbeil D, Hellwig A, Huttner WB. 1997. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. Proc Natl Acad Sci USA 94:12425–12430.

Weissman T, Noctor SC, Clinton BK, Honig LS, Kriegstein AR. 2003. Neurogenic radial glial cells in reptile, rodent and human: From mitosis to migration. Cereb Cortex 13:550–559.

Welker W. 1990. Why does cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In: Peters A, Jones EG, editors. Cerebral cortex. New York and London: Plenum Press. pp 3–136.

Wilsch-Brauninger M, Peters J, Paridaen JT, Huttner WB. 2012. Basolateral rather than apical primary cilia on neuroepithelial cells committed to delamination. Development 139:95–105.

Wong GK, Baudet ML, Norden C, Leung L, Harris WA. 2012. Slit1b-robo3 signaling and N-cadherin regulate apical process retraction in developing retinal ganglion cells. J Neurosci 32:223–228.

Yokota Y, Eom TY, Stanco A, Kim WY, Rao S, Snider WD, Anton ES. 2010. Cdc42 and gsk3 modulate the dynamics of radial glial growth, inter-radial glial interactions and polarity in the developing cerebral cortex. Development 137:4101–4110.

Zhadanov AB, Provance DW Jr. 1999. Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. Curr Biol 9:880–888.