

The Role of Intermediate Progenitor Cells in the Evolutionary Expansion of the Cerebral Cortex

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The vertebrate cerebral cortex varies from the 3-layered dorsal cortex of reptiles to the 6-layered lissencephalic cortex characteristic of rodents and to the 6-layered gyrencephalic cortex typical of carnivores and primates. Distinct developmental mechanisms may have evolved independently to account for the radial expansion that produced the multilayered cortex of mammals and for the tangential expansion of cortical surface area that resulted in gyrencephalic cortex. Recent evidence shows that during the late stages of cortical development, radial glial cells divide asymmetrically in the ventricular zone to generate radial glial cells and intermediate progenitor (IP) cells and that IP cells subsequently divide symmetrically in the subventricular zone to produce multiple neurons. We propose that the evolution of this two-step pattern of neurogenesis played an important role in the amplification of cell numbers underlying the radial and tangential expansion of the cerebral cortex.

Introduction

The cerebral cortex arises from the dorsal aspect of the telencephalon and is responsible for higher perceptual and cognitive functions. In mammals, cortical structure varies from the smooth lissencephalic cortex of rodents to the highly folded gyrencephalic cortex of primates. Despite differences in the pattern of gyral folding, cortical cytoarchitecture shares important characteristics across mammalian species including 6 basic layers. The mammalian neocortex is thought to have evolved from a common ancestor of reptiles and mammals (reviewed in Zardoya and Meyer 2001).

The dorsal cortex of reptiles is a laminar brain region that shares functional and cytoarchitectonic characteristics with mammalian neocortex (Reiner 1991). Adult turtle cortex is a simplified structure in comparison with the mammalian cortex, consisting of 3 basic layers: a cellular layer that contains densely packed excitatory neurons sandwiched between inner and outer plexiform layers. Turtle dorsal cortex possesses functional areas that share characteristics with mammalian neocortex, such as primary visual and primary somatosensory areas (Reiner 1991). Furthermore, thalamic inputs to cortical neurons and cortical outputs to the striatum and brain stem are segregated in the turtle dorsal cortex as in mammals (Ulinski and Nautiyal 1988; Reiner 1993). However, despite the shared similarities, reptilian cortex does not have an equivalent to the superficial layers (II–IV) of the mammalian neocortex (Reiner 1991). Thus, although the basic outline of mammalian neocortex was derived from an ancestral dorsal cortex, important new cell types and architectonic structures have been added during evolution. Comparative studies suggest that the de-

velopment of cortical layers II–IV accompanied the laminar differentiation of the cortex, as well as the appearance of the inside-out pattern of cortical histogenesis and the development of columnar organization (Reiner 1991).

The radial expansion that generated the superficial cortical layers represented an important step in the evolution of the cerebral cortex, but additional structural changes have occurred, most notably, the tangential expansion of cortical area associated with the transformation of lissencephalic neocortex to the gyrencephalic neocortex that is present in primates and other mammals. The radial and tangential expansion of cortex has occurred independently in different species in different epochs, but it is possible that they represent a developmental mechanism that is observable at embryonic stages of cortical development.

We explored factors that might have contributed to the evolution of gyrencephalic neocortex by comparing the developing cortical architecture in 3 representative species: turtle, rat, and ferret. An increase in proliferation underlies the generation of the increased number of cortical cells that populate larger mammalian cortices. We therefore focused our attention on the 2 proliferative zones that have been identified in mammalian neocortex, the ventricular zone (VZ) and the subventricular zone (SVZ). Radial glial cells in the VZ have been shown to generate neurons (Malatesta and others 2000; Miyata and others 2001; Noctor and others 2001; Tamamaki and others 2001) and appear to do so by asymmetric division (Miyata and others 2001; Noctor and others 2001). In addition, radial glial cells generate a secondary population of progenitor cells called intermediate progenitor (IP) cells (Noctor and others 2004). IP cells migrate to the overlying SVZ where they generate neurons for the upper cortical layers (Tarabykin and others 2001; Letinic and others 2002; Haubensak and others 2004; Miyata and others 2004; Noctor and others 2004) and appear to do so by symmetric division (Haubensak and others 2004; Noctor and others 2004).

The expansion of cerebral cortex in the radial and/or tangential dimensions requires an increase in neuronal production. Increased neurogenesis achieved solely through asymmetric cell production (1 neuron per division) could be achieved by one or more of the following changes in the proliferative zones: 1) an increase in the number of progenitor cells in the neuroepithelium, 2) a shorter cell cycle, or 3) a longer neurogenic period. On the other hand, increased neurogenesis achieved solely through symmetric divisions (2 neurons per division) would occur through symmetric terminal divisions that deplete progenitor cells.

Prolonged neurogenesis requires a sustainable pattern of proliferation with renewal of the progenitor pool but would

also benefit from the symmetric generation of neurons in order to increase neuronal output. A two-step pattern of neurogenesis that fulfills these requirements has been described in the rat (Noctor and others 2004). The first step is an asymmetric radial glial cell division at the VZ surface that maintains the radial glial cell population by self-renewal while also generating an IP cell (Noctor and others 2004). The second step involves the symmetric division of IP cells in the SVZ that either amplifies the SVZ progenitor pool by generating 2 progenitor cells or produces 2 neurons. This two-step pattern of cell division increases the rate of neuron production because each radial glial cell division ultimately produces at least 2 neurons. Furthermore, a greater number of neurons can be generated over a shorter time span than can be achieved through asymmetric neurogenesis. We propose that the evolution of this two-step pattern of neurogenesis played an important role in the amplification of neuronal numbers that resulted in the radial and tangential expansion of cerebral cortex.

Materials and Methods

Tissue Preparation

Cortical tissue was prepared from 12 prehatched and 2 adult turtles; 12 prenatal, 2 postnatal, and 1 adult rat; 12 prenatal, 2 postnatal, and 1 adult ferret. Embryonic turtles were staged according to Yntema (1968). Pregnant animals were deeply anesthetized using ketamine (90 mg/kg) and xylazine (7 mg/kg), and embryos were removed and immersed or perfused intracardially with 4% paraformaldehyde (PF, Sigma, St. Louis, MO). Brains were postfixed 24 h in PF, washed in phosphate-buffered saline (PBS), and cryoprotected in 30% sucrose PBS. Brains were frozen, and coronal slices 5–10 μm thick were cut on a cryostat (Microm, Kalamazoo, MI). Tissue was stained with cresyl violet (Sigma) at 37 $^{\circ}\text{C}$ for 1–10 min, dehydrated with graded alcohols, differentiated with glacial acetic acid, treated with xylene, and coverslipped with DPX (Fluka, San Marcos, CA). Nissl-stained tissue was imaged on a Zeiss microscope equipped with an Axiocam camera (Zeiss, Thornwood, NY). Statistical analysis was performed using Instat (GraphPad Software, San Diego, CA). Animals were treated in accordance with protocols

approved by the Institutional Animal Care and Use Committee at University of California, San Francisco.

Immunohistochemistry

Slide-mounted sections were preblocked for 1 h at room temperature with 10% donkey serum (Chemicon, Waltham, MA), 2% gelatin (Sigma), and 1% triton X-100 (Sigma) in PBS and incubated with the primary antibody overnight at room temperature. Sections were rinsed 3 times for 10 min in PBS and incubated with the secondary antibody for 1 h at room temperature. Sections were rinsed in PBS 3 times for 10 min and coverslipped with Aqua-Mount (Lerner, Pittsburgh, PA). Antibodies were diluted in PBS containing 1% donkey serum, 0.2% gelatin, and 0.1% triton X-100. The primary antibodies used were anti-phosphohistone H3 (1:1000, Upstate, Waltham, MA), anti-phosphorylated vimentin (4A4, 1:1000, MBL, Woburn, MA), and doublecortin (1:200, Santa Cruz Biotechnology, Santa Cruz, CA). Sections were imaged on a Fluoview confocal microscope (Olympus, Melville, NY) and figures composed using Adobe Photoshop and Macromedia Freehand.

Results

Embryonic Cytoarchitecture of Turtle, Rat, and Ferret

The structure of the embryonic turtle cortex is relatively simple in comparison with that of mammals. Embryonic turtle cortex at stage 19 (roughly equivalent to embryonic day [E15] rat) comprised a thin VZ adjacent to the ventricle and an overlying cell-sparse intermediate zone (IZ). Although the turtle dorsal cortex does not possess an SVZ (Blanton and Kriegstein 1991b), we observed a structure in lateral regions of the dorsal cortex that resembles the SVZ (Fig. 1A). This structure, which we label the rudimentary SVZ (rSVZ), extends dorsally but becomes very thin and is indistinguishable from the VZ in dorsal or medial cortical regions. Superficial to the IZ is the relatively cell-dense cortical plate (CP), which contains newly generated excitatory neurons. The most superficial structure in the embryonic turtle cortex is the marginal zone, which will form the outer plexiform layer in the adult (Fig. 1). Interneurons are found

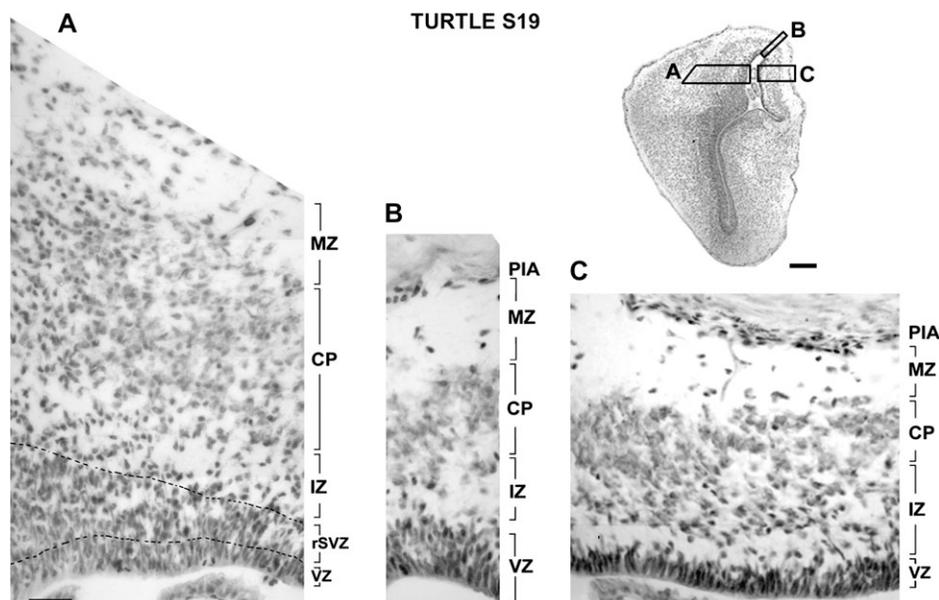


Figure 1. Cytoarchitecture of embryonic turtle dorsal cortex, stage 19 (S19). Low-magnification image of a Nissl-stained coronal section of turtle cortex, with black quadrilaterals (A, B, and C) indicating the location of the higher magnification images shown in panels (A–C). Embryonic turtle cortex has a thin VZ that lines the lateral ventricle. (A) The lateral region of dorsal cortex has a rudimentary SVZ (rSVZ, indicated with dotted lines). The rSVZ becomes indistinguishable in dorsal and medial regions of dorsal cortex. MZ, marginal zone. Scale bar in (A) = 50 μm , and applies to panels B and C, inset = 200 μm .

flanking the CP in the IZ and the molecular layer (Blanton and Kriegstein 1991a).

The cytoarchitecture of embryonic mammal cortex at stages of preplate formation resembles reptile cortex (Marin Padilla 2001) but exhibits several differences from that of reptiles, including a substantially thicker VZ (Figs 2, 3, and 4). The VZ in each species comprised neuroepithelial cells that are oriented perpendicular to the ventricular surface and that exhibit interkinetic nuclear migration. However, a well-defined SVZ can be appreciated in both the rat and ferret but not in turtle (Fig. 4). The SVZ consists of cell bodies that are not radially oriented and mitotic cells that do not exhibit interkinetic nuclear migration (Boulder Committee 1970; Takahashi and others 1995). Nissl-stained sections of the ferret and rat brain demonstrate numerous abventricular mitotic figures in the SVZ, whereas turtles, which do not possess an SVZ, had few scattered mitotic figures at a distance from the ventricular surface (Fig. 4).

Embryonic Proliferative Zones

The significant difference in the size of mammalian and reptilian cortex may result from several developmental processes, including differences in embryonic proliferation. We explored the correlation between cellular proliferation and cortical size by comparing the size of the embryonic proliferative zones in turtles, rats, and ferrets. We outlined the area of the VZ and SVZ under the developing CP in coronal sections of the motor/

sensory cortex from rats and ferrets (see Fig. 5A) and averaged the area obtained from 3 alternating sections. In turtles, we measured the area of the VZ in coronal sections taken from the intermediate rostrocaudal level of the turtle dorsal cortex. We also took measurements at other rostrocaudal points to account for differences that might exist along the neural axis. Statistical differences were tested (Mann-Whitney) on the average proliferative area obtained from 3 embryos in each species.

We found that the size of the VZ as measured in coronal sections was similar in the 3 species at the earliest stage of cortical development when cortical neurogenesis begins. In coronal sections of stage 13 developing turtle cortex, the size of the VZ averaged $42\,000 \pm 2500 \mu\text{m}^2$ per coronal section (Fig. 5). In E12 rat, the VZ area was very similar to that of turtle, $45\,000 \pm 1500 \mu\text{m}^2$. In E23 ferret, the VZ area was larger, measuring $61\,000 \pm 3500 \mu\text{m}^2$. However, by midstages of neurogenesis, greater differences were apparent between the turtle and mammalian VZ. The size of the turtle VZ had not changed significantly ($47\,500 \pm 5500 \mu\text{m}^2$). In contrast, rat and ferret VZ area per coronal section had increased 6-fold. The E17 rat VZ had expanded to $274\,000 \pm 22\,600 \mu\text{m}^2$ per section and the E34 ferret VZ to $387\,500 \pm 17\,500 \mu\text{m}^2$. The larger size of the mammalian VZ reflects the presence of more VZ progenitor cells and points to the potential to produce more cortical cells during development. The difference in the VZ area between mammals and turtle may result from a greater number of

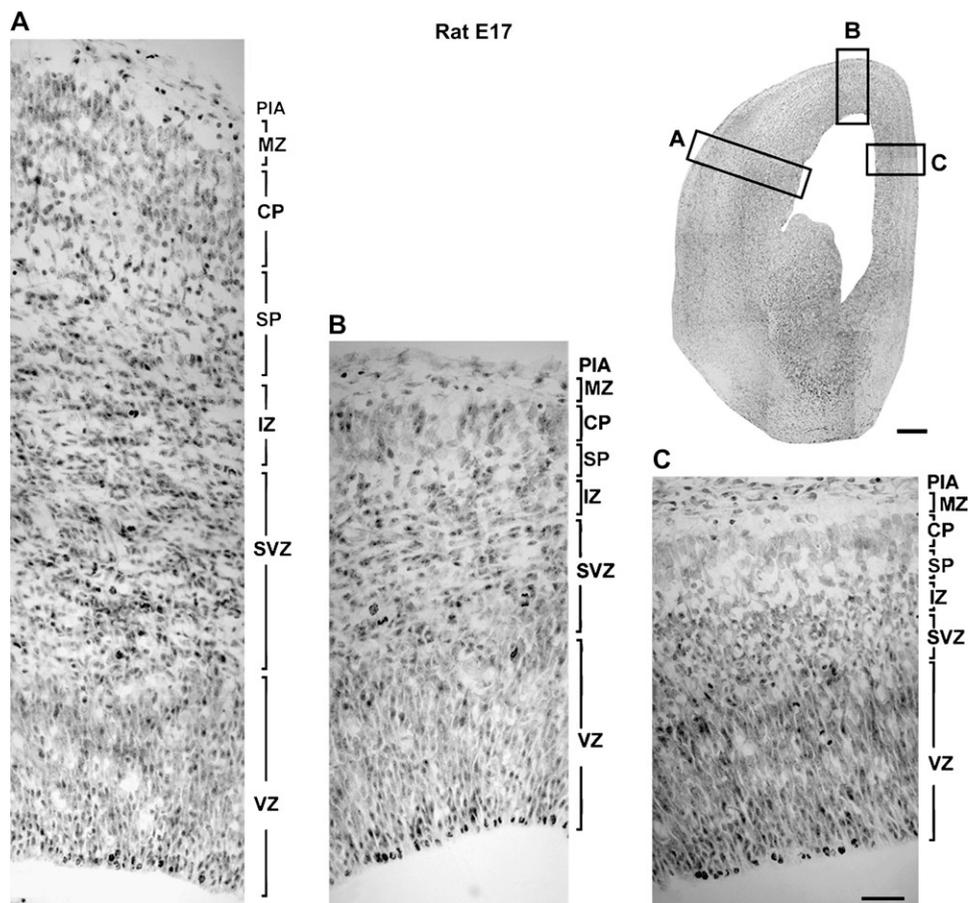


Figure 2. Cytoarchitecture of embryonic rat neocortex, E19. Low-magnification image of a Nissl-stained coronal section of rat cortex, with black quadrilaterals (A, B, and C) indicating the location of the higher magnification images shown in panels (A–C). Embryonic rat neocortex has a thick VZ and SVZ. SP, subplate; MZ, marginal zone. Scale bar in (C) = 50 μm , and applies to panels A and B, inset = 200 μm .

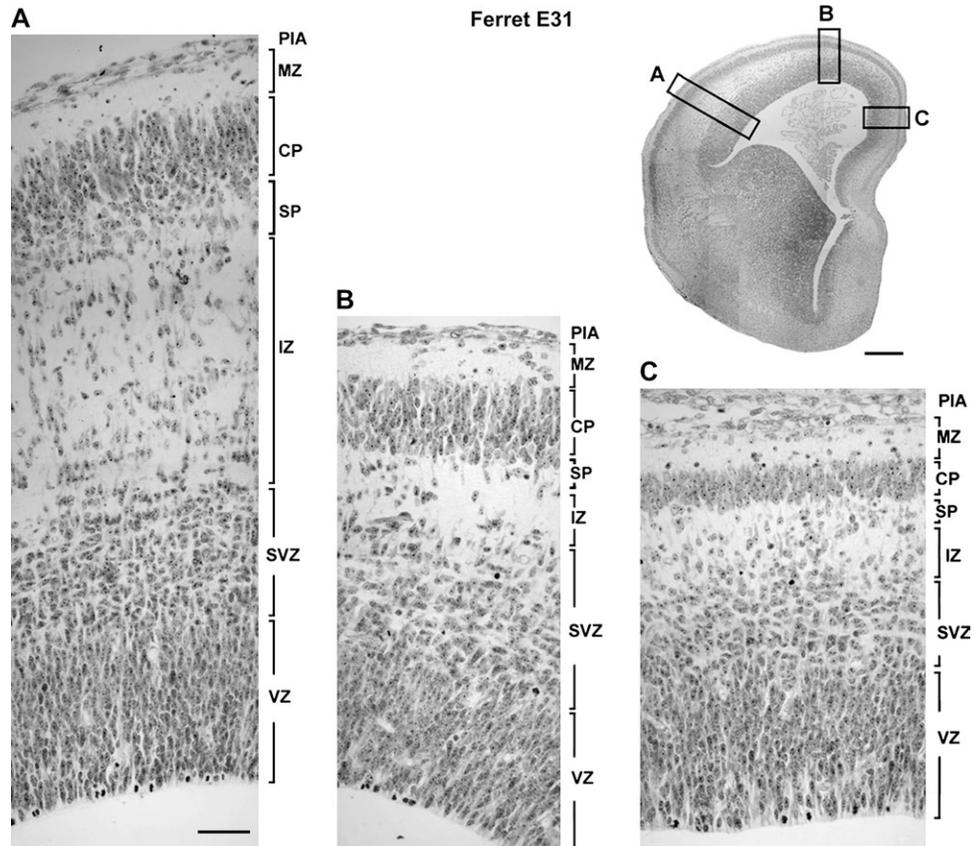


Figure 3. Cytoarchitecture of embryonic ferret neocortex, E31. Low-magnification image of a Nissl-stained coronal section of ferret cortex, with black quadrilaterals (A, B, and C) indicating the location of the higher magnification images shown in panels (A–C). Embryonic ferret neocortex has a thick VZ and SVZ. SP, subplate; MZ, marginal zone. Scale bar in (A) = 50 μm , and applies to panels B and C, inset = 250 μm .

symmetric proliferative divisions in the mammalian neuroepithelium at early stages, which would increase the number of neuroepithelial cells and thus the size of the progenitor pool. We also compared the size of the SVZ in rat and ferret but not in turtles since they lack this structure. The SVZ is not present in either E12 rat or E23 ferret; therefore, we measured the size of the SVZ in embryos at later stages of cortical development. We found that the SVZ area in E17 rats averaged $118\,500 \pm 12\,500 \mu\text{m}^2$ per coronal section and was nearly double in size in E34 ferret ($365\,500 \pm 9700 \mu\text{m}^2$).

Embryonic Progenitor Cells

We next compared basic characteristics of the progenitor cell populations in each species. We immunolabeled coronal sections of cortex with markers such as phosphorylated vimentin (4A4) and phosphohistone H3 and found that these markers labeled mitotic cells at the surface of the VZ in each species (Fig. 6). In addition, we noted that these markers also labeled mitoses away from the ventricle in turtle as in mammals (Noctor and others 2004). There are a small number of adventricular mitoses in the developing turtle. Measurements taken in Nissl-stained tissue showed that the adventricular cells in turtle did not differ in size from adventricular mitotic cells (IP cells) observed in mammals, suggesting that turtle adventricular cells are not endothelial or microglia cells, which are smaller. To determine if the adventricular cells in turtle represent neuronal or glial progenitor cells, we colabeled embryonic turtle tissue

with 4A4 and the neuronal-specific marker doublecortin (Gleason and others 1998) and found double-labeled cells (Fig. 6A, inset). This suggests that some of the 4A4+ adventricular mitoses we observed in the embryonic turtle are in the neuronal lineage.

To examine the contribution of VZ and SVZ progenitor cells to cortical histogenesis, we quantified the proportion of progenitor cells undergoing mitosis at the ventricle versus away from the ventricle. We found that throughout turtle development the majority of divisions occurred at the ventricle, whereas only a small proportion occurred away from the ventricle (Fig. 7). During early stages of mammalian cortical development, we observed a similar pattern; the majority of divisions occurred at the ventricular surface. However, after neurogenesis began, the proportion of adventricular divisions increased steadily and by the final stages of neurogenesis represented the majority of mitoses in the developing cortex. In E20 rat, $61.7 \pm 0.85\%$ of all mitoses was located away from the ventricle and in postnatal day 3 (P3) ferret, an even higher percentage, $78.3 \pm 1.84\%$, was located away from the ventricle (Fig. 7).

In mammals, adventricular mitoses are concentrated in the SVZ but are also found in the VZ and IZ (Smart 1973; Bayer and Altman 1991; Takahashi and others 1995). We next compared the distribution of adventricular mitoses in the 3 species. We found that adventricular mitotic cells were distributed equally across the 3 layers of developing turtle cortex ($n = 38$ cells). In

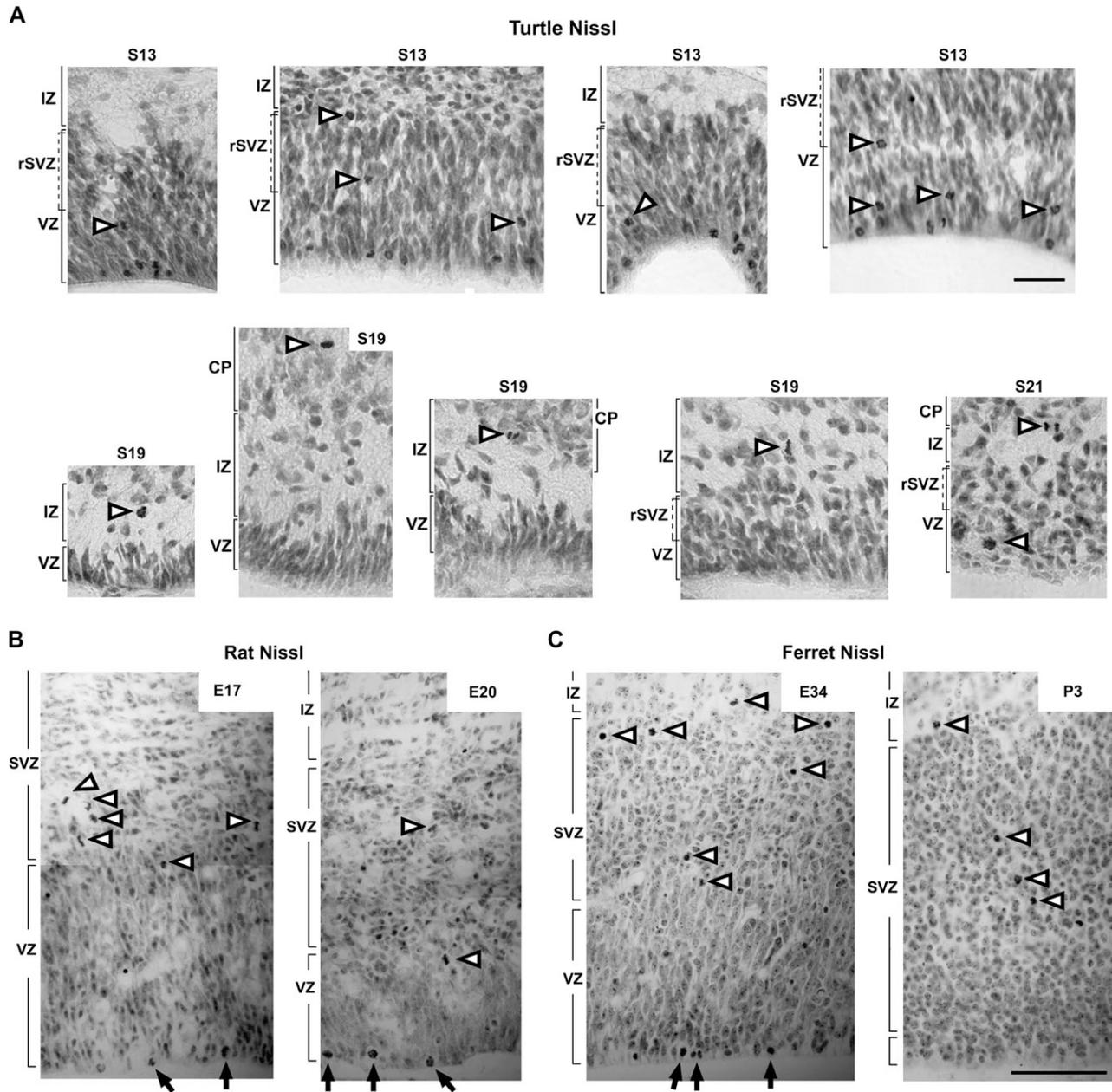


Figure 4. Proliferative zones in turtle, rat, and ferret. (A) High-power photomicrographs of the proliferative zone in Nissl-stained embryonic turtle dorsal cortex from stage 13 (S13) to S21, which encompasses the period of embryonic neurogenesis in turtle. Condensed chromatin of dividing cells can be seen both at the ventricular surface and also away from the ventricle (white arrowheads). (B, C) High-power photomicrographs of Nissl-stained tissue showing the proliferative zones of rat and ferret developing neocortex at comparative stages of cortical development. White arrowheads indicate abventricular divisions, and black arrows point to ventricular surface divisions. Stage of development (E, embryonic; P, postnatal) is indicated in upper right of panel. Scale bar in (A) = 40 μ m and applies to all panels; scale bar in (C) = 50 μ m and applies to (B) and (C).

contrast, $72.3 \pm 3.2\%$ of abventricular mitoses was located in the rat SVZ ($n = 358$ cells), and $73.8 \pm 5.7\%$ ($n = 570$ cells) was located in the ferret SVZ. The concentration of abventricular mitoses in rat and ferret SVZ highlights the abundance of these cells in developing mammal cortex.

To examine the contribution of the 2 progenitor populations per unit area of the cortex, we quantified the number of ventricular surface and abventricular mitoses in 70- μ m-wide bins that extended radially from the ventricle to the pia in coronal sections of motor/sensory cortex in each species. At

the earliest stage of cortical development, we found that ventricular surface proliferation was roughly similar in the 3 species, averaging 5.75 ± 0.9 mitotic cells per 70 μ m of VZ surface in turtle, 6.2 ± 0.8 in rat, and 8.0 ± 0.6 in ferret. However, at later stages of development, the number of VZ surface mitoses decreased in turtle but remained high in rat and ferret through midstage neurogenesis, before dropping during the end stages (Fig. 8). These data suggest that cortical proliferation in mammals relies on a mechanism that does not rapidly deplete the VZ progenitor pool.

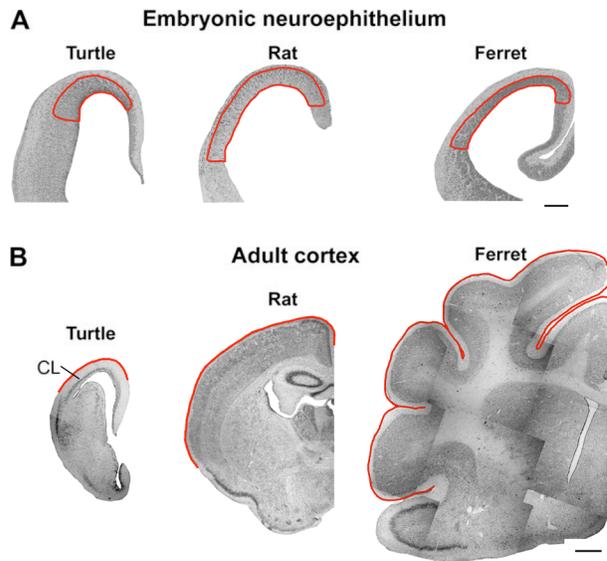


Figure 5. Comparison of embryonic and adult neocortex in turtle, rat, and ferret. (A) The size of proliferative VZ (outlined in red) is similar in each species at the start of cortical neurogenesis. (B) Coronal sections of adult cortex from turtle, rat, and ferret, at the same scale. Different patterns of neurogenesis at work in each species generate significant differences in cortical cytoarchitecture and in surface area of the cortex (indicated with red line). Adult turtle dorsal cortex has a single, dense layer of neurons (cellular layer, CL) positioned close to the lateral ventricle. Adult rat neocortex has expanded in the radial dimension and has 6 neuronal layers. Adult ferret neocortex also has 6 neuronal layers but, in addition, has expanded tangentially and has a greater surface area (red line) than either rat or turtle. Scale bar in (A) = 100 μ m, (B) = 1 mm.

In reptilian turtle cortex, the number of abventricular mitoses remained consistently low throughout development. In mammals, the number of abventricular mitoses started low but rose steadily throughout neurogenesis and represented the majority of cortical mitoses by mid to late stages of cortical development (Fig. 8).

We noted differences in proliferation in rats and ferrets. The number of ventricular and abventricular mitoses reached similar peak levels in rat and ferret, but proliferation remained higher for an extended period in ferret. Thus, cortical proliferation is apparently prolonged in the ferret and does not deplete the progenitor pool as rapidly as it occurs in rat (Fig. 8).

To examine the contribution of abventricular cells to the generation of specific cortical layers in mammals, we quantified the number of abventricular divisions per radial unit of cortex during formation of each cortical layer. The greatest number of abventricular mitoses was present during the formation of the upper cortical layers in both ferret and rat. In E15 rat, we found 1.36 ± 0.46 abventricular mitoses per 70- μ m-wide section of cortex during layer V/VI formation. At E17, during layer IV formation, the number had increased to 4.18 ± 0.82 . At E20, during layer II/III formation, the number had further increased to 4.5 ± 0.59 . In ferret, we found approximately twice the number of abventricular mitoses per unit area of the cortex when compared with rat. At E28–E31, during layer V/VI formation, we found 3.21 ± 0.46 mitoses. At E34, during layer IV formation, we observed 5.83 ± 0.66 mitoses. At P3, when layers II–III are forming, this number had increased to 6.8 ± 0.69 (Fig. 9).

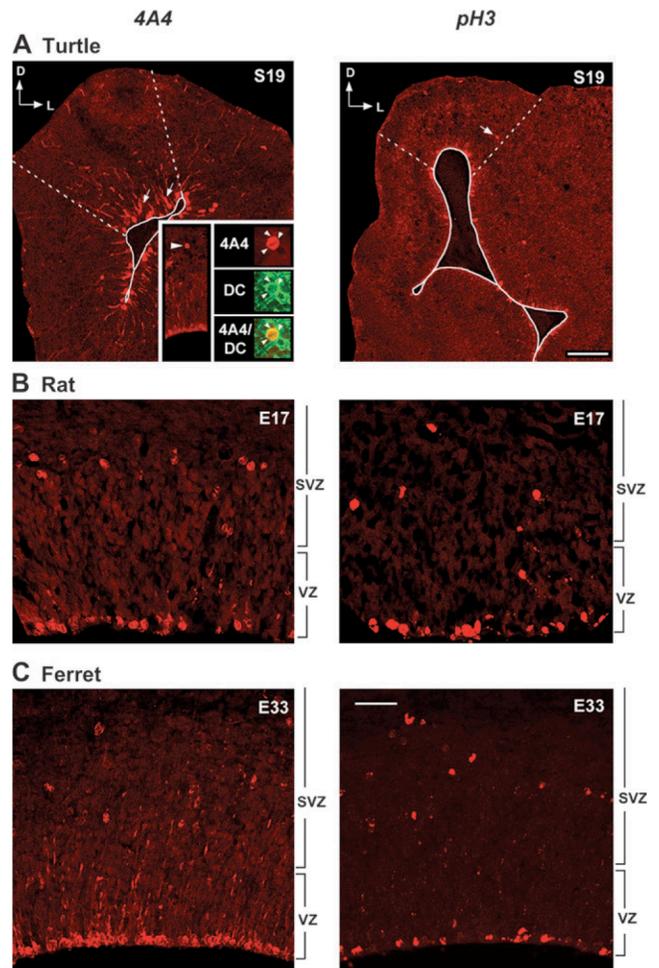


Figure 6. Abventricular mitoses in the embryonic turtle share characteristics with embryonic mammalian SVZ cells. (A) Ventricular surface and abventricular mitoses (white arrows) in the stage 19 (S19) turtle express phosphorylated vimentin (4A4, left panel). Inset: (A) 4A4+ abventricular cell in the embryonic turtle (white arrowhead, red) costained with the neuronal-specific marker doublecortin (green), suggesting that these cells are neuronal. Turtle abventricular mitoses also stain with the mitotic cell marker phosphohistone H3 (pH3, right panels). Solid white line indicates ventricular surface. Dotted white lines mark the boundaries of dorsal cortex. (B, C) In rats and ferrets, mitotic radial glial cells at the ventricular surface express 4A4 (left panels) and pH3 (right panels). IP cells that divide away from the ventricle also express these markers. Stage of development is indicated in upper right of each panel. Scale bar in (A) = 75 μ m, in (C) = 35 μ m and applies to (B).

Conclusions

Our understanding of the cell types comprising the embryonic cortical VZ and SVZ has grown considerably in recent years. In addition to guiding neuronal migration (Rakic 1971), radial glial cells in the VZ have been shown to proliferate (Misson and others 1988), to generate neurons (Malatesta and others 2000; Miyata and others 2001; Noctor and others 2001; Tamamaki and others 2001), to divide asymmetrically (Miyata and others 2001; Noctor and others 2001, 2004; Haubensak and others 2004), and to produce self-renewed radial glial cells as well as IP cells that populate the SVZ (Noctor and others 2004).

The SVZ is seeded by the progeny of VZ progenitor cells through asymmetric division (Smart 1973; Bayer and Altman 1991; Takahashi and others 1995; Haubensak and others 2004; Miyata and others 2004; Noctor and others 2004), but SVZ cells appear to divide symmetrically to amplify the SVZ progenitor

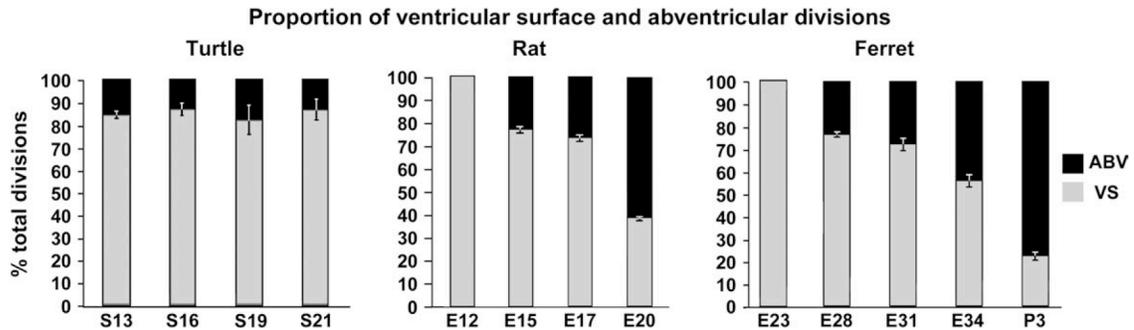


Figure 7. The proportion of ventricular surface divisions (VS, gray) and adventricular divisions (ABV, black) changes during cortical development in the rat and ferret but not in the turtle. In the turtle (left panel), the majority of progenitor cells divide at the ventricular surface throughout cortical development. However, in rat (middle panel) and in ferret (right panel), the proportion of VS versus ABV changes during development. The vast majority of divisions occur at the surface during early stages of cortical development, but the proportion of adventricular divisions rises throughout development. At end stages of rat and ferret neurogenesis, the majority of divisions occur away from the ventricle. Error bars indicate standard error of the mean. x axis indicates the stage of development for each species.

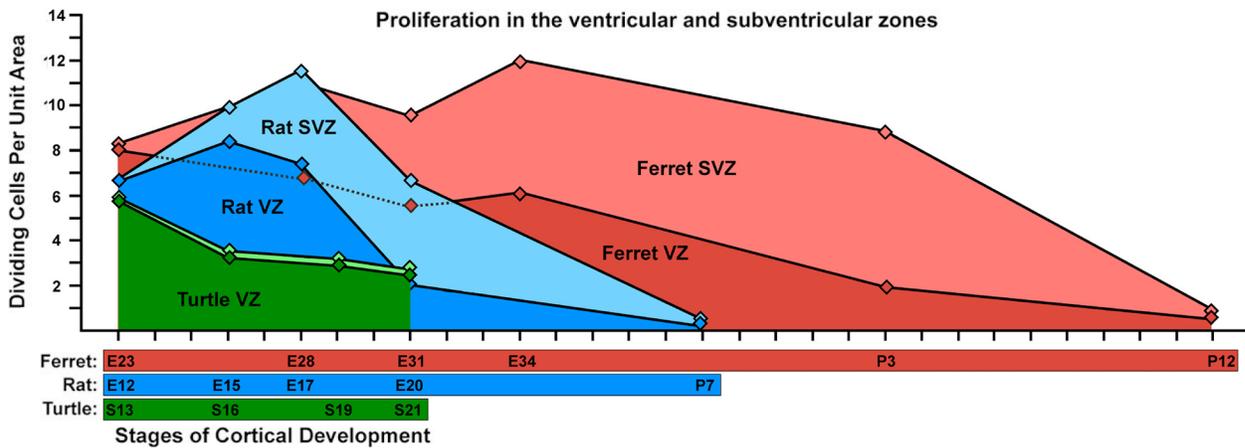


Figure 8. Comparison of ventricular mitoses (VZ) and adventricular mitoses (SVZ) in the cortex of developing turtle, rat, and ferret. The number of mitotic cells in 70- μ m-wide sections of Nissl-stained cortex is shown for turtle, rat, and ferret. The sum of divisions in the VZ and SVZ equals the total proliferation within each 70- μ m-wide section of cortex. The number of VZ divisions is similar in rat and turtle but slightly higher in ferret at the beginning of neurogenesis. VZ divisions drop in turtle but remain high in rat and ferret as neurogenesis proceeds. The number of adventricular divisions in turtle is low throughout cortical development but rises dramatically in rat and ferret during formation of the cortical layers. The period of proliferation extends much longer in ferret than in rat or turtle. x axis indicates the embryonic stage of development for turtle and embryonic or postnatal day for rat and ferret.

pool or to produce neurons (Haubensak and others 2004; Noctor and others 2004). The postnatal and adult rodent SVZ was shown to be a site of neurogenesis through tritiated thymidine pulse labeling more than 40 years ago, but because no labeled neurons were found in the overlying cortex, the newborn neurons were presumed to degenerate within the SVZ (Smart 1961). Alvarez-Buylla and others, however, have demonstrated that the adult SVZ produces functional neurons destined for the olfactory bulb in many species including rodents (Lois and Alvarez-Buylla 1993; Lois and others 1996) and humans (Sanai and others 2004). The embryonic SVZ has also been linked with cortical neurogenesis (Smart 1961, 1973; Smart and others 2002) but was generally thought to be the prime site for gliogenesis (Smart 1973; Privat 1975; Takahashi and others 1995). Recent technical advances have provided evidence that the embryonic SVZ is an important site of cortical neurogenesis (Tarabykin and others 2001; Letinic and others 2002; Haubensak and others 2004; Miyata and others 2004; Nieto and others 2004; Noctor and others 2004; Englund and others 2005). Interestingly, the SVZ is not present during development of the 3-layered turtle cortex (Blanton and Kriegstein 1991b) but is

especially prominent during development of the 6-layered mammalian neocortex (Smart 1961, 1973; Smart and others 2002), suggesting that the SVZ plays an important role in cortical histogenesis of mammals.

Previous studies have explored mechanisms that may contribute to the folding patterns of mammalian cortex, often invoking mechanical models (Richman and others 1975; Todd 1982; Van Essen 1997; Toro and Burnod 2005), or the expansion of intersulcal cortical tissue (Smart and McSherry 1986; Borrell V, Zhao C, Gage FH, Callaway EM, unpublished data). We investigated this problem with a comparative developmental approach. We considered the contribution of embryonic SVZ progenitor cells to cortical expansion by comparing the cytoarchitecture of the proliferative zones in the 3-layered reptilian cortex, 6-layered lissencephalic neocortex, and 6-layered gyrencephalic neocortex.

Adventricular Mitoses

The embryonic turtle dorsal cortex has a few scattered mitotic cells situated away from the ventricle but does not possess an

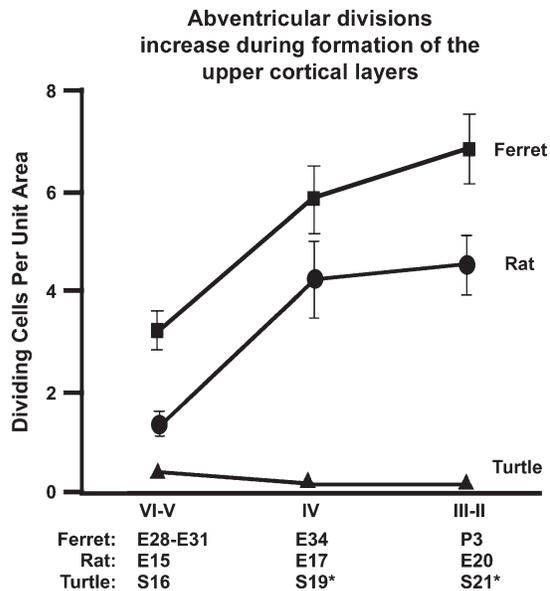


Figure 9. The number of abventricular mitoses during neurogenesis for each cortical layer. The number of divisions rises throughout cortical neurogenesis in mammals but not in the turtle. The number of abventricular mitoses in coronal sections of dorsal cortex rises to peak levels during formation of the upper cortical layers in rat and ferret but remains very low throughout neurogenesis in turtle. The number of abventricular mitoses in the ferret gyrencephalic cortex is nearly double that of the rat lissencephalic cortex during the neurogenesis of each layer. Error bars indicate standard error of the mean. *Turtles do not have the equivalent of upper cortical layers.

SVZ as does the embryonic mammal cortex (Blanton and Kriegstein 1991b). Nevertheless, abventricular mitoses in the developing turtle cortex share some basic characteristics with their mammalian counterparts, including expression of markers such as 4A4. The fate of daughter cells generated by these abventricular cells in developing turtle remains to be determined. The abventricular mitoses might represent glial cell divisions, similar to gliogenic progenitor cells that predominate in the early postnatal mammalian cortex (Levison and Goldman 1993). However, the expression of doublecortin suggests that embryonic turtle abventricular cells may be in a neuronal lineage and generate neurons, as do embryonic SVZ cells in rodents (Haubensak and others 2004; Miyata and others 2004; Noctor and others 2004). Perhaps the most significant difference between abventricular cells in turtle and mammals is quantitative. The greatly reduced number of turtle abventricular cells may result from differences in VZ proliferation we observed between turtles and mammals. The size of the mammal VZ expands during early stages of cortical neurogenesis, whereas that of the turtle VZ does not (Fig. 8). The increased size of the mammal VZ most likely results from a burst of symmetric proliferation that increases the VZ progenitor pool (Cai and others 2002). Our data suggest that turtles lack this phase of development. Consequently, the smaller turtle VZ may be sufficient to generate a small number of cortical neurons and the scattered abventricular cells we observed but may not be sufficient to form a proper SVZ. The absence of an SVZ in turtle indicates that reptiles and mammals utilize different patterns of proliferation, and this has a profound effect on cortical cytoarchitecture. The length of cortical neurogenesis is similar in turtles and rodents, but the resulting structures are dramatically different. A three-layered cortex of neurons is produced

over 1 week in turtle, whereas a complex 6-layered structure is generated in the same time span in rodent.

A Two-Step Pattern of Neurogenesis in Cortical Evolution

Our data show that the number of abventricular mitoses reaches a peak during formation of the upper cortical layers in mammals. We also find that the number of abventricular mitoses and the size of the SVZ, where most of the IP cells reside, are greater in the gyrencephalic ferret cortex than in the lissencephalic rodent cortex. Furthermore, previous work has shown that the cortical SVZ is significantly larger in highly gyrencephalic primates than in other mammals, such as rodents or ferrets (Smart and others 2002). Thus, the number of abventricular mitoses and the size of the SVZ appear to correlate with the evolutionary expansion of cortical surface area across species. However, additional changes in proliferation may also contribute to cortical expansion. For example, a longer period of symmetric proliferation before neurogenesis, a longer neurogenic period, or changes in the length of the cell cycle would each alter the total number of cortical neurons generated. Previous studies have examined in detail the proliferative output of the VZ and SVZ (Takahashi and others 1993, 1995) and the proportion of symmetric and asymmetric divisions during cortical development (Kornack and Rakic 1995; Mione and others 1997; Cai and others 2002). These studies indicate that symmetric proliferative divisions occur at early stages of development, that asymmetric divisions are more prevalent during midstages, and that symmetric terminal divisions are more frequent during late stages. But recent evidence indicates that asymmetric and symmetric divisions work in concert, rather than independently, to produce neurons. During late stages of cortical development, many radial glial cells undergo self-renewing asymmetric divisions in the VZ that also produce an IP cell, which subsequently divides symmetrically in the SVZ to generate neurons (Noctor and others 2004). This two-step pattern of neurogenesis represents a mechanism that amplifies neuronal number without depleting the primary pool of progenitor cells. This allows for an extended period of neurogenesis and amplifies the number of neurons within a given layer and perhaps may underlie cortical expansion in species with prolonged neurogenic intervals, such as primates. Our data show that VZ and SVZ proliferation is sustained for a longer period of time in ferret, which is consistent with the two-step pattern of neuron production.

During evolution, an altered or prolonged pattern of intercellular signaling may have prolonged the proliferation of neuroepithelial cells in some species. For example, prolonged expression of signaling factors such as β -catenin, which maintain VZ cells in a proliferative state (Chenn and Walsh 2002), might explain the larger VZ in mammals compared with reptiles. In addition, transcription factors may regulate developmental programs that differentially affect progenitor cell populations. For example, the transcription factor *Otx1* is expressed in the VZ during generation of the mammalian deep cortical layers V and VI (Frantz and others 1994) but is not expressed in the SVZ. On the other hand, transcription factors such as *Svet1* (Tarabykin and others 2001) and *Tbr2* (Englund and others 2005), which are restricted to proliferative SVZ cells, may reflect the appearance of genetic programs that resulted in the evolution of the upper cortical layers in mammals.

We propose that the evolution of a two-step pattern of neurogenesis supported a sustained period of neuron production and served to amplify cell numbers during cortical development. Our data suggest that this pattern of neurogenesis is responsible for the presence of the SVZ in the developing cortex and was a critical step in the evolution of multilayered and gyrencephalic neocortex. Future work aimed at revealing the signaling factors that regulate patterns of cellular proliferation during embryonic development will be crucial for understanding the radial and tangential expansion of the cerebral cortex.

Notes

Jeanelle Agudelo and William Walantus provided excellent technical assistance. We thank Francisco Clascá for comments on the manuscript and Alfonso Fairén, Sharon Juliano, and Francisco Clascá for the generous gift of ferret tissue. We thank Brian Clinton for maintaining the turtle colony. This work was supported by a grant from the Ministerio de Educación, Cultura y Deporte, Spain, to VMC and from the National Institutes of Health to ARK. *Conflict of Interest* None declared.

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