OPINION

Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion

Arnold Kriegstein, Stephen Noctor and Verónica Martínez-Cerdeño

Abstract | The dramatic evolutionary expansion of the cerebral cortex of *Homo sapiens* underlies our unique higher cortical functions, and therefore bears on the ultimate issue of what makes us human. Recent insights into developmental events during early proliferative stages of cortical development indicate how neural stem and progenitor cells might interact to produce cortical expansion during development, and could shed light on evolutionary changes in cortical structure.

One of the most significant steps in the evolution of the cerebral cortex was the transformation of the lissencephalic cortex, which is characteristic of rodents, into the highly folded gyrencephalic cortex, which is characteristic of primates. Although rodents evolved independently from primates, when compared with other placentals, rodents are actually closer to primates than to many other mammals. Recent genome sequencing data and fossil evidence both place primates and rodents in the same mammalian superorder, Euarchontoglires. As such, there is a closer relationship between primates and rodents than that which exists between primates and dogs, cows, elephants or dolphins[1]. Interestingly, the recent identification and description of what might be a precursor to placental mammals, the mouse-sized *Eomaia scansoria*[2], suggests that it shared several morphological features with modern rodents. Accordingly, we do not suggest that rodents represent the true stem mammal. Rather we discuss rodents as living examples of mammals that possess a six-layered lissencephalic cerebral cortex that could provide insights into the transformation of smooth into gyrencephalic cortex.

Although the laminar organization of the cortex is relatively similar in all mammals, an enormous expansion in the cortical surface area underlies the transformation from smooth cortex to the highly folded primate cortex, and the associated alteration of cortical architecture that is the substrate for the 'higher' cortical functions that distinguish *Homo sapiens* from other mammalian species. The enormous functional consequences of this evolutionary step underscore the importance of understanding the genetic changes that underpin this transition and the mechanistic steps that accompanied evolutionary change.

Since the fossil record is largely mute with respect to the evolution of soft structures like the brain, current concepts concerning evolution of the human cerebral cortex are generally based on comparative studies of living species, using genetic and anatomical features. However, developmental studies have also frequently influenced thinking regarding the evolution of brain structure and function. In the last few years there has been a dramatic change in our understanding of the process of neurogenesis at early stages of brain development. These new concepts are likely to influence thinking about the origin of hallmark features of the primate brain and, in particular, the size and structure of the cerebral cortex. In this article we describe how recent studies of the early stages of brain development support a novel view of the way in which cortical evolution might have progressed.

Overview of cortical development

During the early phases of mammalian brain development, the anterior portion of the neural tube closes to form the vesicles that will give rise to the telencephalon, the most anterior of which form the fluid-filled lateral ventricles in the cerebral hemispheres. The developing cortex is initially composed of a layer of proliferative neuroepithelial cells lining the lateral ventricles — the ventricular zone (VZ). The VZ is a densely packed cell layer formed by morphologically homogeneous cells that are radially oriented and maintain contact with the ventricular lumen and the pial surface of the brain. The first neuronal layer to appear during cortical histogenesis, the preplate, forms above the VZ. A second proliferative layer called the subventricular zone (SVZ) subsequently forms between the VZ and the preplate. The SVZ is formed by loosely arranged cells that are not aligned in a radial fashion and contains mitotic cells, as does the VZ. As cortical neurogenesis proceeds, newly generated neurons migrate radially out of the proliferative zones and insinuate themselves into the preplate, splitting this layer into a superficial marginal zone and a deeper subplate layer, and in so doing form a new laminar structure between them: the cortical plate (FIG. 1). The cortical plate subsequently grows as newly generated neurons migrate past earlier-generated cells to settle in progressively more superficial layers, ultimately forming a six-layered cortex in which a neuron's laminar position is determined by its birth date. As neurogenesis proceeds, the VZ becomes smaller, and after neurogenesis is completed the VZ is replaced by a single layer of ependymal cells that line the lateral ventricles. The SVZ also disappears postnatally in most cortical regions except along the lateral wall of the lateral ventricles, where it persists and continues to provide olfactory neurons into adulthood[3].

Patterns of cortical neurogenesis

At early stages of embryonic development, most neuroepithelial progenitor cells in the VZ undergo symmetric division — two neuroepithelial cells are produced at each division, thereby expanding the population of founder cells that will ultimately produce the neocortex

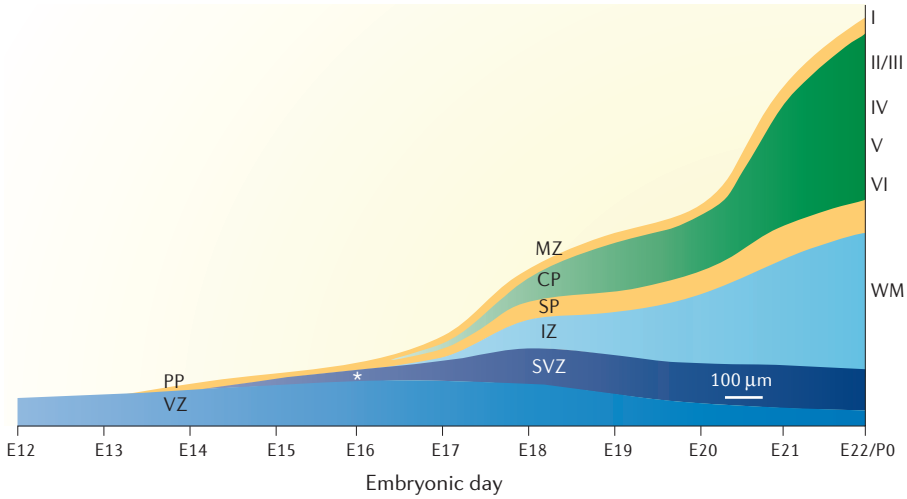

Figure 1 | **Histogenesis of the cerebral cortex.** This schematic drawing provides an approximate representation of the appearance and relative size of cortical structures between embryonic day (E)12 and E22 in the rat. At the onset of cortical histogenesis, the ventricular zone (VZ, blue), or neuroepithelium, is the only structure present in the cerebral cortex. Elements of the preplate (PP, yellow) appear above the VZ between E13 and E14. The subventricular zone (SVZ, dark blue) appears above the VZ, and beneath the PP after E14. After E16, cortical plate neurons migrate into the PP, splitting this structure into the superficial marginal zone (MZ) and deeper subplate (SP), and in doing so form the cortical plate (CP, green). Elements of the intermediate zone (IZ, light blue) invade the cerebral cortex at E16. The asterisk indicates the stage at which SVZ and IZ elements are intermingled in the same layer. The cortical layers I – VI and the white matter (WM) are depicted on the right margin of the scheme. P0, postnatal day 0. The cortical structures were drawn to scale based on unpublished observations (S.N., V.M.-C. and A.K.) and measurements taken from sagittal sections shown in REF. 94 © (1991) Raven.

(FIG. 2a). However, at the onset of neurogenesis, neuroepithelial cells take on some, though not all, of the characteristics of glial cells, including the expression of specific intermediate filaments and, soon after, the expression of cytoplasmic glycogen granules[4–7]. These features support the classification of radial glial cells as a form of specialized glia[4]. Radial glial cells are one of the most characteristic components of the developing cerebral cortex. They are proliferative[8,9–11], and serve as migratory guides for newly generated neurons[12]. Recently it has been found that, in addition to their role in guiding migrating neurons, radial glia generate cortical neurons[8,13–16], and that they seem to do so in two ways[17]. First, radial glia undergo asymmetric division, generating with each division one 'self-renewing' radial glial cell that inherits the radial fibre and one daughter cell that becomes a neuron[8,17] (FIG. 2b). Radial glia are therefore able to retain their radial fibres throughout the cell cycle, providing a continuous substrate for neuronal migration. The asymmetric division of neurogenic radial glia provides a mechanism for generating diverse subtypes of neurons that eventually occupy different layers within a radial segment or column of the cortex, as has been observed in studies following retrovirally labelled neuron clones in the primate cortex[18]. Radial glial cell divisions all take place in the VZ.

A second mechanism for neuron generation in the dorsal cortex involves the production of intermediate progenitor cells, rather than neurons, from radial glial cells[17]. The intermediate progenitor cells migrate to the embryonic SVZ where they produce neurons through symmetric division (FIG. 2c). This two-step pattern of neurogenesis expands the number of neurons of a particular subtype, which will ultimately occupy the same cortical layer. Given this concept, we propose that an evolutionary adaptation that resulted in prolonged intermediate progenitor cell production might have increased the number of neurons in each cortical layer and therefore contributed to the evolutionary expansion in cortical surface area. However, it is also possible that cortical surface area might have expanded owing to an increase in the number of neuroepithelial or radial glial cells prior to the commencement of neurogenesis. Such an increase in the founder cell population, either through prolonged proliferation or attenuated cell death, has been proposed by the radial unit hypothesis to underlie cortical expansion[19,20]. In the discussion below we consider how these concepts fit with developmental observations.

Models of cortical expansion

Evolutionary changes in cerebral size. The cerebral cortex of all primates demonstrates an expanded, gyrencephalic cortical mantel; however, there has also been a considerable increase in overall brain size during hominid evolution. It has recently been proposed that the microcephalin (*MCPH*) genes, which are mutated in human primary autosomal recessive microcephaly, are responsible for the dramatic evolutionary increase in cerebral cortical size[21–23]. Humans with primary microcephaly have a substantially smaller brain with a particularly shrunken cerebral cortex and a simplified gyral pattern[24]. This condition is most commonly caused by a mutation in the *ASPM* (abnormal spindle-like microcephaly-associated) gene[24], or in related genes including the *MCPH* genes[25]. Evolutionary studies of the *ASPM* and *MCPH* genes indicate that they have undergone positive selection during hominid evolution and have probably led to the evolutionary increase in cerebral size[21,25–29].

ASPM is an ortholog of the *Drosophila* abnormal spindle protein (*asp*) gene[30]. *Drosophila asp* mutants have severe defects in spindle microtubule organization[31] and fail to undergo cytokinesis[32]. Similarly, ASPM is a component of the mitotic spindle in mammals, and might regulate the proliferation of neural progenitors during brain development. *MCPH* genes are expressed in the proliferative zones of the forebrain, consistent with a role in neurogenesis during cerebral cortical development[24]. The amino (N) terminal domain of ASPM has also recently been shown to be a member of a family of domains associated with the basal bodies of cilia[33], a finding that supports the suggestion that the protein is localized to centrosomes during mitosis. The mechanism by which these proteins regulate neuronal number is unclear, but could be related to an influence of spindle anchoring on the mode of cell division[24].

The evolutionary contribution of the *MCPH* genes seems to be to increase the overall size of the cerebral cortex, presumably by influencing neuronal number; however, they are not likely to be determinants of the cortical surface area expansion associated with gyration because gyral folding is present in microcephaly. The microcephaly genes are expressed in both the VZ and SVZ[24] — the proliferative zones that contain radial glia and intermediate progenitors, respectively. These genes might therefore have a similar effect on both radial glia and intermediate progenitors, possibly regulating the number of neurons produced by both

cell types and thereby influencing total brain volume rather than selectively altering radial growth or cortical surface expansion.

Other mechanisms of producing macrocephaly have been described, including mutations in the tumour suppressor gene, *Pten*. Mice lacking *Pten* have enlarged, morphologically abnormal brains as the result of several actions, including increased cell proliferation, decreased cell death and enlarged cell size[34]. Although *Pten* mutation is associated with prolonged neural stem cell proliferation[35], the macrocephaly in the *Pten*-null mouse is the result of multiple factors and does not seem to reproduce the features of developmental or evolutionary brain expansion.

The role of the founder cell population. The basic cortical plan of the six-layered neocortex evolved during the transition from reptilian ancestors to primordial mammals, prior to the dramatic increase in cortical surface area[36]. Moreover, with the transition of the lissencephalic to gyrencephalic cortex, the basic six-layered cortical architecture was essentially conserved. Evolutionary cladograms indicate that both the lissencephalic and gyrencephalic cortices are represented in each of the major mammalian lineages; monotremes, marsupials, and placentals[37]. This suggests that the gyrencephalic cortex arose independently in multiple lineages, but there might have been similar underlying mechanisms in each instance. According to the radial unit hypothesis[38], the evolutionary expansion in cortical surface area resulted from an increase in the number of founder cells prior to neurogenesis. This would increase the number of radial units, and if the program for generating neurons remained unaltered, each radial unit would generate a column of six layers of neurons[19,38]. This would have the effect of increasing cortical surface area without increasing cortical thickness. But is this hypothesis consistent with the morphology of the embryonic cortex during stages of neurogenesis and cortical expansion?

An increase in the number of founder cells[39] or a decrease in cell death[40] could both increase the number of radial units and account for cortical expansion. But, although the size of the precursor pool is an important factor that will help determine the number of neurons eventually produced, increases in the number of radial units will increase both the area of the cortical surface and of the lateral ventricle[41]. So, ventricular surface area will be an index of the size of the population of ventricular cells available for subsequent neuron production (FIG. 2a). Indeed, this has been demonstrated through electroporation of the dominant-active form of β-catenin in the chick neural tube[42], and in mice engineered to overexpress β-catenin in neuronal precursor cells[20]. β-catenin, a component of the adherens junctions that couple radial glia at the ventricular border, is part of the WNT signalling pathway implicated in cell growth regulation[43]. Dominant-active β-catenin has been shown to reduce neuronal differentiation and increase the surface area of the ventricular zone in the chick neural tube[42]. Furthermore, when murine neuroepithelial cells were engineered to express a constitutively active form of β-catenin, increased numbers of cortical progenitor cells were generated, and the mice developed a dramatically increased cortical surface area that displayed folds suggestive of gyrencephaly[20]. However, consistent with an increase in the founder cell population, the size of the VZ increased proportionately with the expansion of cortical

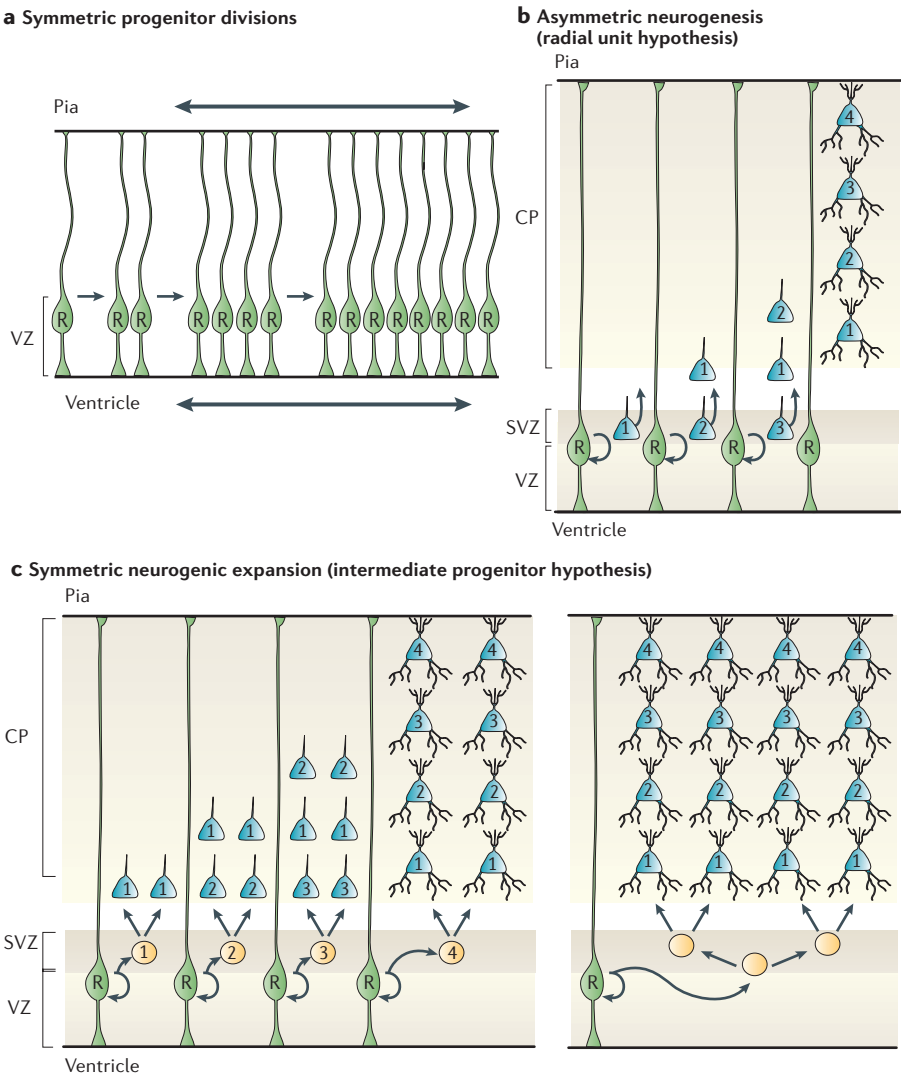

Figure 2 | **Patterns of cell division in the embryonic cortex.** Schematic drawings illustrating division patterns observed in the embryonic cortex during development. **a** | Symmetric progenitor divisions in the ventricular zone (VZ) increase the founder cell (radial glia (R), green) population. **b** | Asymmetric neurogenic divisions in the VZ will yield self-renewal of radial glial cells and produce neurons (blue) destined for different layers in the cortical plate (CP), thereby resulting in a radial array or column of neurons. **c** | The left panel shows symmetric neurogenic divisions of 'intermediate progenitor cells' (yellow) in the subventricular zone (SVZ) resulting in the amplification of cells of the same type that have the same birth dates (illustrated by numbers in the cells) and occupy the same cortical layer. The right panel shows that additional symmetric progenitor divisions in the SVZ before terminal neurogenic divisions would further amplify the number of cortical neurons generated for a given cortical layer, without requiring the presence of more radial glial cells in the VZ. Numbered cells 1–4 represent the neuronal progeny produced by four sequential radial glial cell divisions.

area, and the lateral ventricle became dramatically larger and also developed folds. These findings show that gyrencephaly can result from an increase in cell number and an expansion of cortical surface area. However, during comparable stages of cortical development in gyrencephalic animals, neuronal cell number increases without a significant change in ventricular size. Therefore, β-catenin overexpression does not model the disproportionate increase of cortical surface area accompanied by a relatively modest increase in ventricular size that characterizes cortical development in gyrencephalic animals. The answer might lie, in part, in an evolutionary shift towards a non-epithelial neuronal precursor, the intermediate progenitor cell, that could increase neuron number in each cortical layer without increasing ventricular size (FIG. 2c).

The role of SVZ intermediate progenitor cells. The embryonic SVZ, once thought to be primarily a site of gliogenesis, is increasingly appreciated to be a significant neurogenic region. Several lines of evidence indicate that neurons, particularly cortical projection neurons, are born in the embryonic mammalian SVZ. One line of evidence comes from molecular studies of the developmental patterns of transcription factor expression in rodents. Transcription factors such as the sublineage genes *Svet1*, *Cux1*/*Cux2* and *Tbr1*/*Tbr2* are expressed by embryonic SVZ precursor cells that generate upper cortical layer neurons[44–46]. This developmental pattern suggests that transcription factor expression begins in SVZ precursor cells and persists in the neurons generated by these cells. More direct evidence comes from retroviral labelling and time-lapse imaging studies using embryonic rodent slice cultures. These studies demonstrate that radial glia in the VZ generate intermediate precursor cells that move to the SVZ, where they divide symmetrically to produce pairs of neurons that subsequently migrate to the cortical plate[17]. Intermediate progenitors can also divide symmetrically to produce pairs of progenitors that could subsequently each generate a pair of neurons[17]. These findings are supported by earlier work showing that the SVZ of the ganglionic eminence is a neurogenic compartment[47]. The generation of cortical neurons through intermediate progenitor cells would significantly increase the number of neurons generated by a single radial glial stem cell. As each intermediate progenitor produces two neurons with the same birth date, and because laminar position is correlated with birth date, the number of neurons destined for a particular cortical layer would be increased[48]. The radial unit hypothesis and the intermediate progenitor hypothesis are compared in FIG. 2.

We have observed the tangential movement of intermediate progenitor cells in the SVZ (S.N., unpublished observations), and subpopulations of cortical neurons have been shown to migrate tangentially in the embryonic cortex[49]. This lateral migration might contribute to the tangential dispersion of cell clones previously observed in retroviral labelling experiments in the rodent cortex[50–52]. A potential complication of this model is that despite the increased number of neurons leaving the proliferative zones, the model does not provide an increased number of radial glia to support the migration of these extra neurons. However, this would not be a problem if multiple neurons, including those destined for the same cortical layer, could migrate along a single radial glial fibre. This is likely to occur, and has been demonstrated in time-lapse images of retrovirally labelled cells in slice cultures that show two daughter cells produced by the division of a single intermediate progenitor cell migrating along the same radial fibre (S.N., V.M.-C. and A.K., unpublished observations).

The production of neurons by way of intermediate progenitor cells might well precede the development of the SVZ. Even before the SVZ is formed, proliferating neuron-producing cells, presumed to be intermediate progenitor cells, can be observed dividing away from the ventricular surface, in contrast to radial glial cells which divide at the ventricular surface. This is illustrated by studies of mice expressing green fluorescent protein (GFP) from the *Tis21* locus[53]. *Tis21* is an anti-proliferative gene that is selectively expressed in neuron-generating cells, beginning in the cell cycle just before neurogenic division[54]. By examining GFP expression in these mice it was shown that, at the onset of cortical neurogenesis, neuronal precursors appear in the basal neuroepithelium, the region of the future SVZ, even though it is not yet a distinct architectonic zone. Moreover, although the fates of daughter cells were not determined in this study, the daughter cells of VZ progenitors migrated to different locations at different rates, consistent with asymmetrical cell fate, whereas the daughters of intermediate progenitors migrated together, suggesting a possible symmetrical fate[53]. The observations summarized above suggest that a pattern of neurogenesis based on expanded numbers of intermediate progenitor cells would produce an increase in cortical area without increasing the size of the VZ, consistent with the developmental changes in cortical architecture observed in the gyrencephalic cortex. We therefore predict that an evolutionary shift towards increased numbers of intermediate progenitor cells could have contributed to the increase in cortical surface area that accompanied mammalian evolution (FIG. 2c).

Intermediate progenitor model

The model outlined above leads to several predictions. First, the size of the embryonic SVZ would be significantly larger in species that demonstrate greater degrees of gyrification, as this is the zone that would contain the large increase in intermediate progenitor cells. Comparing the size of the SVZ in cross-sections prepared from rat embryos with those from ferrets during comparable stages of neurogenesis shows that the ferret SVZ is twofold larger[55]. Moreover, among mammalian species, the SVZ is larger in primates[56] and especially large in *Homo sapiens*[57], a species in which great cortical expansion has taken place. In addition,

Glossary

Asymmetric division
A cell division that produces two cells with different fate potential.

Cladogram
A tree-like diagram depicting evolutionary relationships between different species. In these diagrams, branches that share the same node are closely related.

Cortical plate
The cellular layer of the developing cerebral cortex that will become layers II–VI of the adult cortex.

Gyrencephalic cortex
Adult six-layered neocortex that develops a folded surface associated with gyri and sulci.

Lissencephalic cortex
Adult six-layered neocortex without folds created by gyri and sulci, which therefore has a smooth surface.

Preplate
The first cortical layer to develop, containing the earliest-generated cortical neurons. This layer is split by migrating cortical plate neurons that settle here, dividing it into a superficial layer that becomes the marginal zone and a deep layer that becomes the subplate.

Stem mammal
Putative vertebrate species from which all mammals evolved.

Symmetric division
A cell division that produces two cells with identical fate potential.

Telencephalon
The anterior portion of the forebrain, which includes the cerebral hemispheres, basal ganglia and the olfactory bulbs.

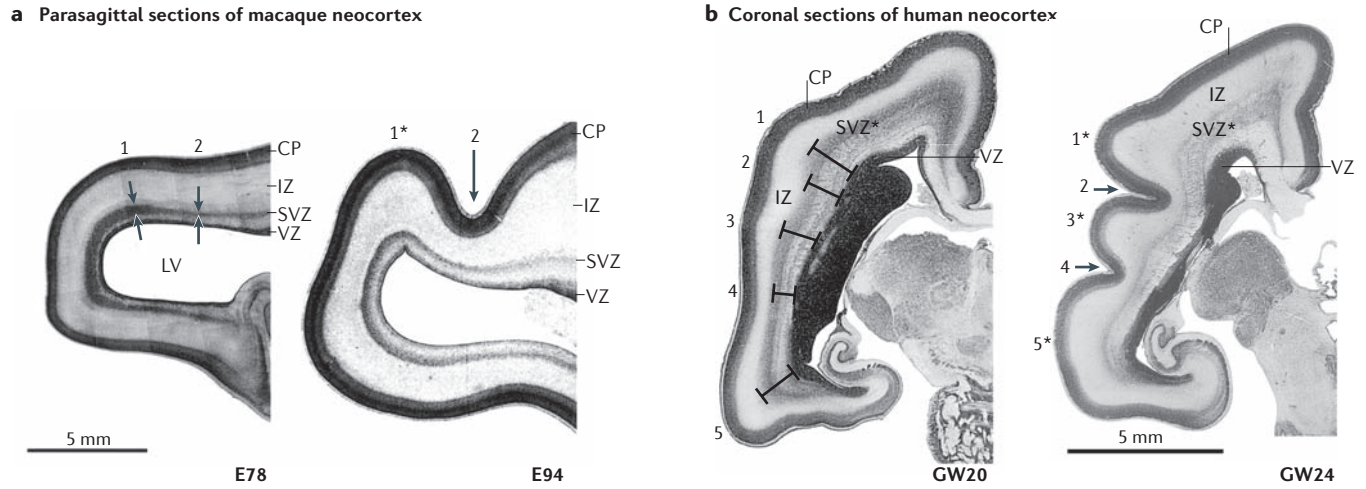

Figure 3 | **SVZ size predicts sites of gyral and sulcal formation.** The subventricular zone (SVZ) is thicker in areas underlying gyrus formation and thinner in areas underlying sulcus formation. **a** | Parasagittal sections of the macaque occipital lobe. In embryonic day (E) 78 macaque cortex, a thickened SVZ (indicated by black arrows under 1) presages the gyral formation that can be seen just over 2 weeks later at E94 (1*). By contrast, a much thinner SVZ (indicated by black arrows under 2) is located under a region of sulcal formation (2, arrow). **b** | Similar features are observed in coronal sections of the developing human cortex. At gestational week (GW) 20, areas of thickened SVZ (indicated by brackets under 1, 3 and 5) presage gyral formation that can be seen in the same region of the cortex four weeks later at GW24. By contrast, areas of thinner SVZ (indicated by brackets under 2 and 4) are located under regions of sulcal formation that are observed four weeks later at GW24. CP, cortical plate; IZ, intermediate zone; LV, lateral ventricle; SVZ*, encompasses stratified transitional fields 1–6; VZ, ventricular zone. Panel **a** modified, with permission, from REF. 56 © (2002) Oxford Univ. Press. Panel **b** modified, with permission, from REF. 63 © (2005) Taylor & Francis.

the period of neurogenesis is prolonged in animals with a gyrencephalic cortex, being threefold longer in ferrets than in rodents[58], and eightfold longer in primates[59]. This means that, in spite of a longer cell cycle time, each progenitor can divide more times in animals with a gyrencephalic cortex. For example, in the macaque there are approximately 28 cell cycles during the neurogenic period[59], substantially increasing the potential neuronal output of the SVZ in primates compared with rodents, which have 11 neurogenic cell cycles[60]. In most mammalian species, the SVZ — and therefore the number of symmetrically generated neurons — is larger at developmental stages when upper cortical layers are being formed than when deeper layers are produced. The model predicts that the surface area of the superficial layers would therefore be greater than the area of the deeper layers. In fact, the ratio of cortical neurons in upper layers compared to lower layers is much greater in humans than it is in rodents[61]. Therefore, superficial layers are expanded compared to deeper layers, a phenomenon that might also contribute to the development of gyri[62].

An increase in the number of cells in upper layers compared with deeper layers will produce a wedge-shaped cortical section and help to contribute to the increased cortical surface area in areas of gyral formation, but in areas of sulcal formation the cortical surface area is reduced compared with the deeper layers. Therefore, a second prediction of the model is that the SVZ would be non-uniform in size, with greater numbers of intermediate progenitors — and therefore a larger SVZ — in areas underlying gyral formation, and fewer progenitors — and therefore a smaller SVZ — in areas underlying sulcal formation. This situation might help to explain regional variations in the sizes of the proliferative zones during the early stages of neurogenesis in species that have a gyrencephalic cortex. For example, in the embryonic monkey cortex, the SVZ is relatively large in areas where gyri subsequently develop, and relatively thin in areas underlying future sulci[56]. Inspection of histological sections of the developing monkey and human cortex support this prediction[56,63]. Areas of thickened SVZ are visible in locations where gyri subsequently form, and areas of relatively thin SVZ presage locations where sulci lie (FIG. 3). This observation is consistent with the hypothesis that the primate SVZ is the source of upper layer cortical neurons, and supports the concept that regional patterns of neurogenesis caused by intermediate progenitor cells in the SVZ help determine patterns of gyrification.

A third prediction is that the SVZ of the medial ganglionic eminence (MGE) would be proportionately larger in gyrencephalic animals in order to provide the increased number of interneurons required for cortical expansion. In rodents, the ganglionic eminence is the predominant source of cortical inhibitory interneurons[64–71], which migrate tangentially into the cortex during development[65,67,72]. It is presumed that they also undergo a two-step pattern of neurogenesis, with intermediate progenitors arising from radial glia lining the ventricle, followed by expansion through symmetric divisions of intermediate progenitors in the MGE SVZ. This concept is consistent with retroviral labelling studies suggesting that clones observed in the MGE are larger than those in the cortex[73,74]. It would be expected that the SVZ of the MGE in gyrencephalic animals would be proportionately larger than that of lissencephalic animals in order to supply a larger number of interneurons. One observation mitigating this prediction, however, is the report that in the human brain, unlike in the rodent brain, inhibitory interneurons might also arise from the cortical SVZ[76]. Nonetheless, the primate MGE is very large in comparison with the rodent brain, with most of the increase consisting of an expanded SVZ[63]. The presence of a large SVZ is consistent with an amplification of cortical interneurons by intermediate progenitor cells in the MGE.

Implications for neuronal diversity

The asymmetric division pattern of radial glial cells could allow genes governing cell identity to be differentially inherited by intermediate progenitor cells generated in

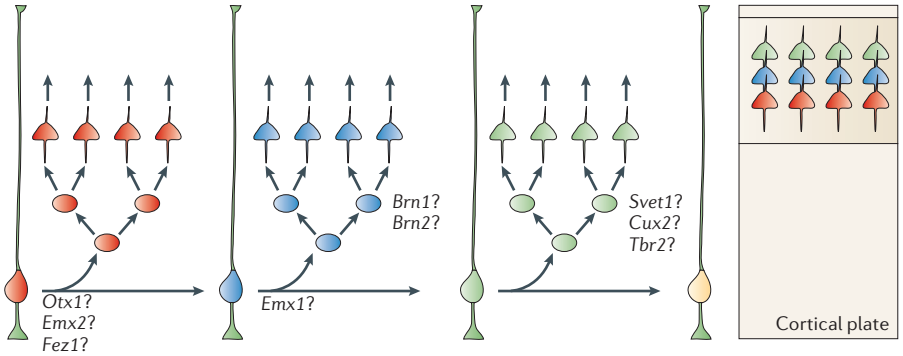

Figure 4 | **Model of how temporal patterns of gene expression may regulate layer formation.** Schematic of a proposed mechanism for generating multiple radial arrays of neurons, each consisting of a diverse number of cell types. This outcome is the result of temporally ordered changes in gene expression coupled to a pattern of asymmetric and symmetric progenitor cell divisions. Radial glia are proposed to undergo temporally ordered changes in cell identity gene expression (red, blue, green, yellow). Examples of temporally regulated genes are shown on the figure. Each subsequent asymmetric division would generate intermediate progenitors that each express different specific identity gene(s). Symmetric divisions of the intermediate progenitors, in turn, could permit stable inheritance of identity genes by their progeny, and in their final symmetric neurogenic divisions each family of intermediate progenitors would produce neurons of the same identity. This model would produce radial arrays of neurons in a relatively short time period.

different cell cycles. The inheritance of a specific cell identity gene or a combination of genes could then instruct intermediate progenitor cells produced in a given cell cycle to produce large numbers of neurons of an identical subtype appropriate for that particular stage of development, but different from those produced by intermediate progenitors that were generated in previous or subsequent cell cycles. For example, in the developing CNS of *Drosophila*, neuronal precursor cells sequentially express a series of distinct transcription factors. Each change in the expression of these cell-identity genes defines a temporal window in which neuroblasts can generate a sublineage of a particular identity. At each subsequent division, the neural progeny maintain expression of the transcription factor that was active at the time that they were generated[77]. For example, the genes *hunchback* (*hb*), *Krüppel* (*Kr*), *pdm1/pdm2* (*pdm*) and *castor* (*cas*) are expressed in dividing neuroblasts in this order, in early-, mid- and late-born neurons respectively[78,79,80,81]. These genes are therefore candidates for regulating temporal identity in neuroblast lineages. The absence of a particular temporally expressed gene results in a failure to generate the particular cell fate determined by that temporal window[78,79,82]. When a factor is expressed outside its normal sequence, the fates of later sublineages are redirected. For example, when neuroblasts are forced to ectopically express *hb* outside of the normal temporal window of *hb* expression, they generate neurons with the characteristics of neurons that are normally produced earlier[79,82]. The mechanism for generating neuronal diversity in the developing *Drosophila* brain is therefore a result of temporally ordered changes in gene expression coupled to a pattern of asymmetric neuroblast divisions.

Temporally ordered changes in gene expression patterns have also been observed in neuronal precursors during stages of cortical layer formation in the mammalian cortex, suggesting that the molecular mechanism for generating neuronal diversity through asymmetric division might be conserved in invertebrates and mammals[83]. A series of neuronal sublineage genes have been described that are expressed by subsets of neurons in specific cortical layers, as well as by neuronal precursor cells during the specific developmental periods when those neurons are generated. For example, *Otx1* and *Fezl* are expressed by neuronal precursor cells in the VZ and SVZ early in cortical development and, later, by the neurons that they generate in deep cortical layers[84,85]. Similarly, later in cortical development, the sublineage genes *Svet1* (REF. 44), *Cuz2* (REFS 45,86), and *Tbr2*/*Tbr1* genes are expressed in precursor cells in the SVZ that generate upper layer cortical neurons.

These observations suggest a possible two-step model of neurogenesis where the first step — asymmetric radial glial cell division — generates neuronal diversity, and the second step — symmetric intermediate cell division — produces large numbers of neurons of the same subtype. In this model, a temporal sequence of gene expression changes in asymmetrically dividing radial glial cells would underlie the changing gene expression patterns in intermediate progenitors in the SVZ. The intermediate progenitor cells produced within a specific temporal window would, in turn, generate large numbers of similar cell types through symmetric division (FIG. 4). The scheme proposed here is, of course, overly simplified. For example, individual neuronal cell type identity might be established by combinatorial patterns of gene expression and, in addition, fate determination of cells generated later might also depend on the suppression of early fate signals. This seems to be the case for *Foxg1*, which has been shown to suppress the fate of early-born neurons (Cajal–Retzius cells) in order to permit the generation of later born neurons[87,88]. Nonetheless, the two-step pattern described for cortical neurogenesis supports a model in which radial glia could undergo temporally ordered changes in the expression of cell identity genes and, through asymmetric division, generate intermediate progenitors, each with a different cell fate. Symmetric divisions of the intermediate progenitors could, in turn, permit a stable inheritance of identity genes by both of their progeny, thereby expanding the numbers of neurons that share the same identity.

Conclusion

Recent changes in our understanding of the mechanisms of cortical development have provided fresh insights into possible steps involved in the evolution of the cortex. One example could be the role of intermediate progenitor cells as the source of the large numbers of neurons that could contribute to a lateral expansion of the cortex. The role of radial glial cells as a source of cortical neurons through asymmetric division, as well as their role as guides for radial migration, might underlie the columnar distribution of neurons across layers. Additionally, the production of neurons by intermediate progenitor cells, through symmetric cell division, might provide a mechanism for the amplification of cell numbers in each layer. Most intermediate progenitor cells reside in a specific proliferative region, the SVZ, and a first-pass examination of the role of the SVZ in development and in evolution seems to support this hypothesis. This basic scheme is clearly over-simplified, but might serve as a useful departure point for building a more complete model of the cellular dynamics that underlie cortical development and evolution.

Future studies might unravel the heterogeneity that undoubtedly exists among

cortical radial glial and intermediate progenitor cells. Clonal analysis suggests that neural stem and progenitor cell subsets exist[75,89–93], but we have yet to discover how they are spatially and temporally distributed in the developing cortex. As new transcription factor genes are discovered and their developmental expression patterns are explored, the molecular underpinnings of neural stem and progenitor cell diversity will become clearer. When these insights are coupled with an understanding of the signalling cascades that precisely regulate the expression patterns of the key genes, we will be better able to test concepts of cortical evolution at the genetic and molecular level. More importantly, a better understanding of the cellular and molecular events that underlie human cortical expansion could shed light on the origins of a wide range of neurodevelopmental disorders, ranging from cortical malformations such as lissencephaly to more subtle disorders, possibly including schizophrenia and autism. Such insights might ultimately help to identify new therapeutic strategies to treat or circumvent some of these diseases.

Arnold Kriegstein, Stephen Noctor and Verónica Martínez-Cerdeño are at the Institute for Stem Cell and Tissue Biology, University of California, San Francisco, 513 Parnassus Ave, HSW, 1201, San Francisco, California, USA.
Correspondence to A.K.
e-mail: kriegsteina@stemcell.ucsf.edu

doi:10.1038/nrn2008
Published online 11 October 2006

1. Lindblad-Toh, K. Genome sequencing: three's company. *Nature* **428**, 475–476 (2004).
2. Ji, Q. *et al.* The earliest known eutherian mammal. *Nature* **416**, 816–822 (2002).
3. Lois, C. & Alvarez-Buylla, A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc. Natl Acad. Sci. USA* **90**, 2074–2077 (1993).
4. Gadisseux, J. F. & Evrard, P. Glial–neuronal relationship in the developing central nervous system. A histochemical–electron microscope study of radial glial cell particulate glycogen in normal and *reeler* mice and the human fetus. *Dev. Neurosci.* **7**, 12–32 (1985).
5. Bruckner, G. & Biesold, D. Histochemistry of glycogen deposition in perinatal rat brain: importance of radial glial cells. *J. Neurocytol.* **10**, 749–757 (1981).
6. Choi, B. H. & Lapham, L. W. Radial glia in the human fetal cerebrum: a combined Golgi, immunofluorescent and electron microscopic study. *Brain Res.* **148**, 295–311 (1978).
7. Kriegstein, A. R. & Gotz, M. Radial glia diversity: a matter of cell fate. *Glia* **43**, 37–43 (2003).
8. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. & Kriegstein, A. R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714–720 (2001).
9. Misson, J. P., Edwards, M. A., Yamamoto, M. & Caviness, V. S. Jr. Mitotic cycling of radial glial cells of the fetal murine cerebral wall: a combined autoradiographic and immunohistochemical study. *Brain Res.* **466**, 183–190 (1988).
10. Levitt, P., Cooper, M. L. & Rakic, P. Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: an ultrastructural immunoperoxidase analysis. *J. Neurosci.* **1**, 27–39 (1981).
11. Schmechel, D. E. & Rakic, P. Arrested proliferation of radial glial cells during midgestation in rhesus monkey. *Nature* **277**, 303–305 (1979).
12. Rakic, P. Neuronal migration and contact guidance in the primate telencephalon. *Postgrad. Med. J.* **1**, 25–40 (1978).
13. Miyata, T., Kawaguchi, A., Okano, H. & Ogawa, M. Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron* **31**, 727–741 (2001).
14. Malatesta, P., Hartfuss, E. & Gotz, M. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**, 5253–5263 (2000).
15. Anthony, T. E. *et al.* Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* **41**, 881–890 (2004).
16. Tamamaki, N. *et al.* Radial glia is a progenitor of neocortical neurons in the developing cerebral cortex. *Neurosci. Res.* **41**, 51–60 (2001).
17. Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. & Kriegstein, A. R. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neurosci.* **7**, 136–144 (2004).
18. Kornack, D. R. & Rakic, P. Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. *Neuron* **15**, 311–321 (1995).
19. Rakic, P. Radial unit hypothesis of neocortical expansion. *Novartis Found. Symp.* **228**, 30–42; discussion 42–52 (2000).
20. Chenn, A. & Walsh, C. A. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* **297**, 365–369 (2002).
21. Ponting, C. & Jackson, A. P. Evolution of primary microcephaly genes and the enlargement of primate brains. *Curr. Opin. Genet. Dev.* **15**, 241–248 (2005).
22. Gilbert, S. L., Dobyns, W. B. & Lahn, B. T. Genetic links between brain development and brain evolution. *Nature Rev. Genet.* **6**, 581–590 (2005).
23. Woods, C. G., Bond, J. & Enard, W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. *Am. J. Hum. Genet.* **76**, 717–728 (2005).
24. Bond, J. *et al. ASPM* is a major determinant of cerebral cortical size. *Nature Genet.* **32**, 316–320 (2002).
25. Evans, P. D. *et al.* Adaptive evolution of ASPM, a major determinant of cerebral cortical size in humans. *Hum. Mol. Genet.* **13**, 489–494 (2004).
26. Evans, P. D. *et al.* Microcephalin, a gene regulating brain size, continues to evolve adaptively in humans. *Science* **309**, 1717–1720 (2005).
27. Kouprina, N. *et al.* Accelerated evolution of the *ASPM* gene controlling brain size begins prior to human brain expansion. *PLoS Biol.* **2**, E126 (2004).
28. Zhang, J. Evolution of the human *ASPM* gene, a major determinant of brain size. *Genetics* **165**, 2063–2070 (2003).
29. Mekel-Bobrov, N. *et al.* Ongoing adaptive evolution of *ASPM*, a brain size determinant in *Homo sapiens*. *Science* **309**, 1720–1722 (2005).
30. Ripoll, P., Pimpinelli, S., Valdivia, M. M. & Avila, J. A cell division mutant of *Drosophila* with a functionally abnormal spindle. *Cell* **41**, 907–912 (1985).
31. do Carmo Avides, M. & Glover, D. M. Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers. *Science* **283**, 1733–1735 (1999).
32. Wakefield, J. G., Bonaccorsi, S. & Gatti, M. The *Drosophila* protein asp is involved in microtubule organization during spindle formation and cytokinesis. *J. Cell. Biol.* **153**, 637–648 (2001).
33. Ponting, C. P. A novel domain suggests a ciliary function for *ASPM*, a brain size determining gene. *Bioinformatics* **22**, 1031–1035 (2006).
34. Groszer, M. *et al.* Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene *in vivo*. *Science* **294**, 2186–2189 (2001).
35. Groszer, M. *et al.* PTEN negatively regulates neural stem cell self-renewal by modulating G0–G1 cell cycle entry. *Proc. Natl Acad. Sci. USA* **103**, 111–116 (2006).
36. Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C. *The Central Nervous System of Vertebrates* Vol. 3, 2219 (Springer, Berlin, 1998).
37. Striedter, G. F. *Principles of Brain Evolution* 436 (Sinauer Associates, Sunderland, 2005).
38. Rakic, P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci.* **18**, 383–388 (1995).
39. Rakic, P. Developmental and evolutionary adaptations of cortical radial glia. *Cereb. Cortex* **13**, 541–549 (2003).
40. Roth, K. A. *et al.* Epistatic and independent functions of caspase-3 and Bcl-X_L in developmental programmed cell death. *Proc. Natl Acad. Sci. USA* **97**, 466–471 (2000).
41. Smart, I. H. & McSherry, G. M. Growth patterns in the lateral wall of the mouse telencephalon. II. Histological changes during and subsequent to the period of isocortical neuron production. *J. Anat.* **134**, 415–442 (1982).
42. Megason, S. G. & McMahon, A. P. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* **129**, 2087–2098 (2002).
43. Parr, B. A., Shea, M. J., Vassileva, G. & McMahon, A. P. Mouse *Wnt* genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development* **119**, 247–261 (1993).
44. Tarabykin, V., Stoykova, A., Usman, N. & Gruss, P. Cortical upper layer neurons derive from the subventricular zone as indicated by *Svet1* gene expression. *Development* **128**, 1983–1993 (2001).
45. Zimmer, C., Tiveron, M. C., Bodmer, R. & Cremer, H. Dynamics of *Cux2* expression suggests that an early pool of SVZ precursors is fated to become upper cortical layer neurons. *Cereb. Cortex* **14**, 1408–1420 (2004).
46. Englund, C. *et al. Pax6*, *Tbr2*, and *Tbr1* are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J. Neurosci.* **25**, 247–251 (2005).
47. Anderson, S. A. *et al.* Mutations of the homeobox genes *Dlx-1* and *Dlx-2* disrupt the striatal subventricular zone and differentiation of late born striatal neurons. *Neuron* **19**, 27–37 (1997).
48. Krubitzer, L. & Kahn, D. M. Nature versus nurture revisited: an old idea with a new twist. *Prog. Neurobiol.* **70**, 33–52 (2003).
49. Britanova, O. *et al.* A novel mode of tangential migration of cortical projection neurons. *Dev. Biol.* 30 Jun 2006 (doi:10.1016/j.ydbio.2006.06.040).
50. Reid, C. B., Liang, I. & Walsh, C. Systematic widespread clonal organization in cerebral cortex. *Neuron* **15**, 299–310 (1995).
51. Walsh, C. & Cepko, C. L. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. *Science* **255**, 434–440 (1992).
52. Walsh, C. & Cepko, C. L. Clonally related cortical cells show several migration patterns. *Science* **241**, 1342–1345 (1988).
53. Haubensak, W., Attardo, A., Denk, W. & Huttner, W. B. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc. Natl Acad. Sci. USA* **101**, 3196–3201 (2004).
54. Iacopetti, P. *et al.* Expression of the antiproliferative gene *TIS21* at the onset of neurogenesis identifies single neuroepithelial cells that switch from proliferative to neuron-generating division. *Proc. Natl Acad. Sci. USA* **96**, 4639–4644 (1999).
55. Martinez-Cerdeno, V., Noctor, S. C. & Kriegstein, A. R. The role of the intermediate progenitor cells in the evolutionary expansion on the cerebral cortex. *Cereb. Cortex* **16**, 152–161 (2006).
56. Smart, I. H., Dehay, C., Giroud, P., Berland, M. & Kennedy, H. 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 (2002).
57. Zecevic, N., Chen, Y. & Filipovic, R. Contributions of cortical subventricular zone to the development of the human cerebral cortex. *J. Comp. Neurol.* **491**, 109–122 (2005).
58. Noctor, S. C., Scholnicoff, N. J. & Juliano, S. L. Histogenesis of ferret somatosensory cortex. *J. Comp. Neurol.* **387**, 179–193 (1997).
59. Kornack, D. R. & Rakic, P. Changes in cell-cycle kinetics during the development and evolution of primate neocortex. *Proc. Natl Acad. Sci. USA* **95**, 1242–1246 (1998).
60. Takahashi, T., Nowakowski, R. S. and Caviness, V. Jr. The cell cycle of the pseudostratified ventricular epithelium of the embryonic murine cerebral wall. *J. Neurosci.* **15**, 6046–6057 (1995).
61. DeFelipe, J., Alonso-Nanclares, L. & Arellano, J. I. Microstructure of the neocortex: comparative aspects. *J. Neurocytol.* **31**, 299–316 (2002).
62. Richman, D. P., Stewart, R. M., Hutchinson, J. W. & Caviness, V. S. Jr. Mechanical model of brain convolutional development. *Science* **189**, 18–21 (1975).
63. Bayer, S. A. & Altman, J. *The Human Brain During the Second Trimester* (Taylor & Francis, Boca Raton, 2005).
64. Nery, S., Fishell, G. & Corbin, J. G. The caudal ganglionic eminence is a source of distinct cortical and

subcortical cell populations. *Nature Neurosci.* **5**, 1279–1287 (2002).
65. de Carlos, J. A., Lopez-Mascaraque, L. & Valverde, F. Dynamics of cell migration from the lateral ganglionic eminence in the rat. *J. Neurosci.* **16**, 6146–6156 (1996).
66. Lavdas, A. A., Grigoriou, M., Pachnis, V. & Parnavelas, J. G. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* **19**, 7881–7888 (1999).
67. Anderson, S. A., Marin, O., Horn, C., Jennings, K. & Rubenstein, J. L. Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development* **128**, 353–363 (2001).
68. Anderson, S. A., Eisenstat, D. D., Shi, L. & Rubenstein, J. Interneuron migration from basal forebrain to neocortex: dependence on *dlx* genes. *Science* **278**, 474–476 (1997).
69. Wichterle, H., Garcia-Verdugo, J. M., Herrera, D. G. & Alvarez-Buylla, A. Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nature Neurosci.* **2**, 461–466 (1999).
70. Wichterle, H., Turnbull, D. H., Nery, S., Fishell, G. & Alvarez-Buylla, A. *In utero* fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development* **128**, 3759–3771 (2001).
71. Sussel, L., Marin, O., Kimura, S. & Rubenstein, J. L. Loss of *Nkx2.1* homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359–3370 (1999).
72. Tamamaki, N., Fujimori, K. E. & Takauji, R. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J. Neurosci.* **17**, 8313–8323 (1997).
73. Halliday, A. L. & Cepko, C. L. Generation and migration of cells in the developing striatum. *Neuron* **9**, 15–26 (1992).
74. Walsh, C. & Cepko, C. L. Clonal dispersion in proliferative layers of developing cerebral cortex. *Nature* **362**, 632–635 (1993).
75. Reid, C. B. & Walsh, C. A. Evidence of common progenitors and patterns of dispersion in rat striatum and cerebral cortex. *J. Neurosci.* **22**, 4002–4014 (2002).
76. Letinic, K., Zoncu, R. & Rakic, P. Origin of GABAergic neurons in the human neocortex. *Nature* **417**, 645–649 (2002).
77. Brody, T. & Odenwald, W. F. Programmed transformations in neuroblast gene expression during *Drosophila* CNS lineage development. *Dev. Biol.* **226**, 34–44 (2000).
78. Kambadur, R. *et al.* Regulation of POU genes by *castor* and *hunchback* establishes layered compartments in the *Drosophila* CNS. *Genes. Dev.* **12**, 246–260 (1998).
79. Isshiki, T., Pearson, B., Holbrook, S. & Doe, C. Q. *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* **106**, 511–521 (2001).
80. Cui, X. & Doe, C. Q. *ming* is expressed in neuroblast sublineages and regulates gene expression in the *Drosophila* central nervous system. *Development* **116**, 943–952 (1992).
81. Mellerick, D. M., Kassis, J. A., Zhang, S. D. & Odenwald, W. F. *castor* encodes a novel zinc finger protein required for the development of a subset of CNS neurons in *Drosophila*. *Neuron* **9**, 789–803 (1992).
82. Novotny, T., Eiselt, R. & Urban, J. Hunchback is required for the specification of the early sublineage of neuroblast 7–3 in the *Drosophila* central nervous system. *Development* **129**, 1027–1036 (2002).
83. Zhong, W. Diversifying neural cells through order of birth and asymmetry of division. *Neuron* **37**, 11–14 (2003).
84. Frantz, G. D., Weimann, J. M., Levin, M. E. & McConnell, S. K. Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. *J. Neurosci.* **14**, 5725–5740 (1994).
85. Molyneaux, B. J., Arlotta, P., Hirata, T., Hibi, M. & Macklis, J. D. *Fezl* is required for the birth and specification of corticospinal motor neurons. *Neuron* **47**, 817–831 (2005).
86. Nieto, M. *et al.* Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II–IV of the cerebral cortex. *J. Comp. Neurol.* **479**, 168–180 (2004).
87. Hanashima, C., Li, S. C., Shen, L., Lai, E. & Fishell, G. *Foxg1* suppresses early cortical cell fate. *Science* **303**, 56–59 (2004).
88. Shen, Q. *et al.* The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nature Neurosci.* **9**, 743–751 (2006).
89. Lavdas, A. A., Mione, M. C. & Parnavelas, J. G. Neuronal clones in the cerebral cortex show morphological and neurotransmitter heterogeneity during development. *Cereb. Cortex* **6**, 490–497 (1996).
90. Williams, B. P., Read, J. & Price, J. The generation of neurons and oligodendrocytes from a common precursor cell. *Neuron* **7**, 685–693 (1991).
91. Luskin, M. B., Parnavelas, J. G. & Barfield, J. A. Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: an ultrastructural analysis of clonally related cells. *J. Neurosci.* **13**, 1730–1750 (1993).
92. Walsh, C. & Cepko, C. L. Cell lineage and cell migration in the developing cerebral cortex. *Experientia* **46**, 940–947 (1990).
93. Temple, S. Division and differentiation of isolated CNS blast cells in microculture. *Nature* **340**, 471–473 (1989).
94. Bayer, S. A. & Altman, J. *Neocortical Development* (Raven, New York, 1991).

Acknowledgements
The authors thank A. Alvarez-Bullya and J. L. R. Rubenstein for helpful comments on the manuscript. This work has been supported by grants from the National Institutes of Health to A.R.K., and from the the Ministerio de Educación, Cultura y Deporte, Spain, to V.M.C.

Competing interests statement
The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
asp | *ASPM* | *Cux1* | *Cux2* | *cas* | *hb* | *Kr* | *pdm* | *Pten* | *Svet1* | *Tbr1* | *Tbr2* | *Tis21*
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Primary autosomal recessive microcephaly

FURTHER INFORMATION

Kriegstein's laboratory: http://www.stemcell.medschool.ucsf.edu/Research/labs/Kriegstein/
Access to this links box is available online.