Evolution of the neocortex: a perspective from developmental biology

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Abstract | The enlargement and species-specific elaboration of the cerebral neocortex during evolution holds the secret to the mental abilities of humans; however, the genetic origin and cellular mechanisms that generated the distinct evolutionary advancements are not well understood. This article describes how novelties that make us human may have been introduced during evolution, based on findings in the embryonic cerebral cortex in different mammalian species. The data on the differences in gene expression, new molecular pathways and novel cellular interactions that have led to these evolutionary advances may also provide insight into the pathogenesis and therapies for human-specific neuropsychiatric disorders.

The neocortex, as the name implies, is the newest addition to our brain and is considered to be the crowning achievement of evolution and the biological substrate of human mental prowess. Although its origin can be traced to reptiles[1,2] in the Carboniferous Period, it first appears as a uniform, six-layered sheet consisting of radially deployed neurons in small mammals that emerged during the transition from the Triassic period to the Jurassic period. Increases in size and complexity of the cerebral cortex have culminated in the modern human that separated from rodents between 90 and 100 million years ago and from the Old World monkeys, such as macaques, 25 million years before the present time[3,4] (FIG. 1). Given that the cerebral neocortex is the centre of extraordinary human cognitive abilities, it is surprising how little has been done to research its emergence. It seems that we are sometimes so seduced by similarities between species that we neglect the differences.

There are several possible explanations for this tendency. First, the mouse is the best and most economical experimental model system for studying the cerebral cortex[5]. Second, the concept, aptly advocated by Charles Darwin, that the biological world is unified, and that "there is no fundamental difference between man and the higher mammals in their mental faculties"[6] is still engrained in our mind and we hope that human traits can be deduced from this commonality. Third, research on the development of the human brain has been mainly descriptive. However, the recent application of the most advanced methods of molecular and cell biology to study the developing human brain have revealed quantitative and qualitative differences in the gene composition and expression between the neocortex of rodents and primates, and we have only begun to explore the developmental and evolutionary origin of these differences.

As the excavated skulls of our common predecessors are empty, perhaps the only way to understand cortical evolution at the cellular level is by comparison of the differential gene expression and developmental events that occur during the embryonic development of living mammals[7]. This approach, known as evo–devo[8] (BOX 1) reflects the realization that developmental neurobiology can explain how the genetic information contained within progenitor cells regulates the number, phenotype, migration and allocation of neurons in the developing brain, where they establish species-specific circuits. For obvious ethical and logistic reasons, experimental manipulation is not possible in humans and thus, comparative analysis of the developmental events in other mammalian species is used to reveal the origin of evolutionary novelties in the cerebral cortex.

In this Review, I provide selective examples of what evolutionary concepts can be deduced from evo–devo studies that have been carried out in embryonic brains of rodents (mostly *Mus musculus*), non-human primates (mostly *Macaca mulatta*) and humans (*Homo sapiens*). Although there is a large body of data on other mammalian species, I focus on only these three species because of space limitations. These three presently living species effectively illustrate different levels

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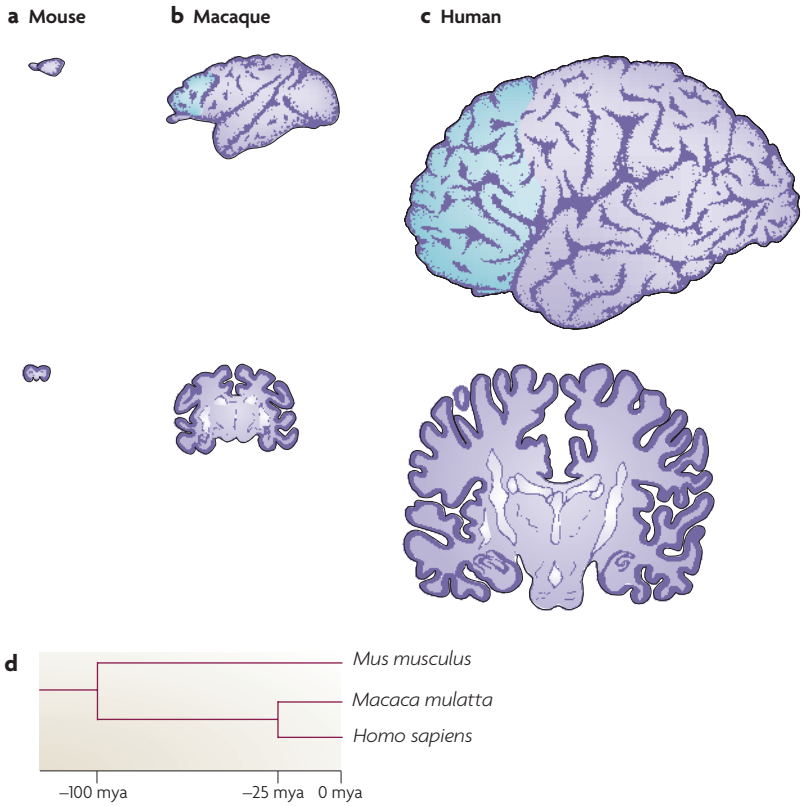

Figure 1 | **Broad comparison of the brain of a mouse, a macaque monkey and a human, and the phylogenetic divergence of these species.** In the top panels of **a**, **b** and **c** the cerebral hemispheres of a mouse, a macaque monkey and a human brain, respectively, are drawn to approximately the same scale to convey the overall difference that exits in the size and elaboration of the cerebral cortex in these three species. The prefrontal cortex, which has no counterpart in mouse, is shaded in blue in the macaque and the human. In the bottom panels of **a**, **b** and **c**, diagrams of cerebral sections are shown for these same species to illustrate that there has been a relative small increase in the thickness of the cortex (dark purple outline) compared with the large increase in surface area (1: 100: 1000 X in mouse, macaque monkey and human, respectively). In part **d**, the timescale of phylogenetic divergence of mouse (*Mus musculus*), macaque monkey (*Macaca mulatta*) and human (*Homo sapiens*) is also shown based on the available DNA sequencing data (reviewed in REFS 3,4). mya, million years ago.

of neocortical expansion and elaboration (FIG. 1a,b,c), and this is often taken, implicitly and erroneously, as an indication of the lineage continuity between them with the human species at the top; however, comparative anatomical and DNA sequencing data clearly indicate that these three species belong to different branches on the phylogenetic tree (FIG. 1d).

Peculiarities of the cerebral cortex

The evolution of the cerebral neocortex cannot be fully appreciated without understanding the way in which it is built. There are several features of cortical development that differ from the development of other organs of the body and even from the rest of the brain.

First, the cerebral cortex in all mammalian species, including humans, is a cellular sheet composed of projection (or pyramidal) and local circuit neurons (or interneurons) deployed in horizontal layers, intersected by vertical (or radial) columns that are stereotypically interconnected in the vertical dimension and share extrinsic connectivity[9–10]. However, this apparent cytoarchitectural uniformity exhibits different degrees of variability in each area depending on their function. This compartmentalization is particularly pronounced in the large and convoluted human neocortex, with more than 50 distinct cytoarchitectonic areas[11].

Second, a remarkable feature of cortical development in all mammalian species is that none of its constituent neurons is generated within the cortex itself. Rather, they are generated in the proliferative transient embryonic zones (such as the ventricular zone (VZ) and subventricular zone (SVZ)), which are situated near the surface of the cerebral lateral ventricles, and acquire their proper areal and laminar positions through long-distance radial and tangential migration across the intermediate zone (reviewed in REFS 12–17). Neurons migrating radially to the increasingly distant cortex follow the shafts of a transient population of radial glial cells[18–20]. A detailed description of the complex migratory process and its underlying complex and multiple molecular mechanisms cannot be described in this short Review. FIG. 2 conveys the basic concepts and further details can be viewed in the Rakic laboratory animated video of a migrating cortical neuron and animated video of radial migration.

Third, not only do the postmitotic neurons migrate to the overlaying cortex, but also each generation bypasses the previous one, a phenomenon known as the 'inside-out gradient of neurogenesis' (REFS 21,22) (see the animated video of radial migration). The sole exception to the inside-out sequence of neurogenesis occurs in the layer I of primates, in which, contrary to rodents, neurogenesis continues throughout the entire period of corticogenesis[23]. The biological significance of the inside-out sequence of settling of neurons in the cerebral cortex is not clear[24], but if disturbed, by either genetic or environmental factors, neurons display abnormal cortical function[25]. Only recently have methodological advances given us an opportunity to study mechanisms involved in neuronal migration at the molecular and cellular level (reviewed in REFS 14,26–28). In most mammals, including humans, corticogenesis is completed before or around the time of birth[29].

Human ascent through cortical expansion

The first step in the evolutionary ascent of the human cerebral cortex is its enlargement, which occurs mainly by expansion of the surface area without a comparable increase in its thickness (FIG. 1). The modest, only about twofold, difference in cortical thickness between rodents and primates is in part due to the enlargement of neurons and neuropile in the extracellular space.

An explanation of the cellular mechanism that underlies the enormous surface expansion of the cortex without a comparable increase in its thickness has been offered by the radial unit hypothesis[12] (FIG. 2). According to this model, an increase in the number of neural stem cells by symmetrical divisions before the onset of neurogenesis would result in an exponential increase in the number of founder cells that give rise to radial cortical columns (FIG. 2a). At later stages, the number of neurons increases linearly within each column, mostly

Transient embryonic layers
Layers identified in the embryonic brain, such as the proliferative ventricular and subventricular zones (VZ/SVZ) or migratory intermediate zone that lack direct counterparts in the adult brain, as defined by the Boulder Committee.

Neuropile
The tissue situated between neuronal cell bodies, composed of a complex network of neuronal and glial processes including dendrites, dendritic spines, axonal terminals and synapses, used often to measure connectedness of a given structure.

by asymmetrical divisions of neural progenitors[30]. For example, less than 7 extra rounds of cell divisions in the VZ at the early embryonic stages can account for the 1,000-fold difference in total cortical surface area between mice and humans (FIGS 1,2a). The fact that the forebrain primordium in humans is much bigger than in mice and monkeys, even at embryonic stages before the onset of cortical neurogenesis[19,31,32], supports this model. Furthermore, this model predicts that neurons generated from the founder cells will follow adjacent shafts of radial glial cells and thus, form more columns, which results in an expanded laminar rather than nuclear shaped structure. However, the radial unit hypothesis does not imply homogeneity of radial units across the cortex within or between species. On the contrary, there is a large body of evidence to support that radial columns differ across the cerebral hemisphere and change considerably in size and composition during cortical evolution (BOX. 2).

Experimental tests of the radial unit hypothesis. The radial unit model has been tested by either diminishing the rate of programmed cell death (apoptosis) or by increasing the rate of neural stem cell proliferation in the VZ. Mice deficient in caspase 3 and 9, two important components of programmed cell death (apoptosis) — an essential process during development[33,34] — have a larger number of neural stem (founder) cells and radial glial cells. These cells, in turn, produce more than the normal number of radial columns and, hence, result in a larger cortical surface that forms incipient convolutions[35] (FIG. 3a).

Box 1 | **Evo–devo approach to reconstruct neocortical evolution**

The use of advanced methods to study embryonic brain development in living species provides insights into possible cellular and molecular mechanisms that have enabled the evolutionary expansion and elaboration of organs in common ancestors. The new subfield of developmental biology called evo–devo, was created to elucidate how new features may have been created by gene mutation and, if inherited, propagated during evolution by natural selection[7,8,139]. The contemporary evo–devo approach should be distinguished from Haeckle's law of ontogenetic recapitulation of phylogeny that arose in the nineteenth century (for historical reviews see REFS 1,2,7). The evo–devo approach embraces the concept of modularity or functional cellular units, such as segments, somites, limb buds and cortical columns. Changes in these modules, if inheritable, often result in lethality; but, if useful can enhance the survival and propagation of the species.

The present Review is, by necessity, based on selected data on cortical development in humans, non-human primates and rodents, which are relevant for understanding neocortical evolution. For example, experimental inhibition or overexpression of specific genes in the embryo allows us to test their effect on development of the cerebral cortex and to draw conclusions on their involvement in increasing evolutionary complexity[35,38,74]. In addition, it is possible to explore the timing of expression of new genes and their modification directly in the embryonic human cortex[128]. Based on the available data we can propose models of how gene expression within the neural stem cells in the proliferative embryonic zones control the number of columnar units, regulate neuronal migration and allocation into proper laminar and radial locations in the cortical plate, where they promote differentiation into species-specific phenotypes, establish new patterns of synaptic connections and determine particular neurotransmitters and receptors within the cytoarchitectonic areas subserving specific functions[140]. Thus, the evo–devo approach represents a scientific way to unravel the events that led to the evolution of the neocortex, which we will never be able to verify in real time.

Similarly, an even more dramatic increase in cortical surface occurs with an experimentally induced increase in neuronal production. It is counterintuitive that such a large increase in neocortical size is achieved by a substantial prolongation, rather than reduction in duration of the cell cycle[36–37]. It is the changes in pool size of the neural stem cells and the rate of the exit from the cell cycle that have a crucial role. For example, the β-catenin-mediated signalling pathway acts on the decision of the neuronal precursor cells to exit the cell cycle[38] and, hence, has an influence on the number of postmitotic neurons. Thus, transgenic mice that express a stabilized form of β-catenin transgene in neural precursors develop an increased number of precursor (founder) cells in the VZ, which in turn lead to a larger number of radial columns and consequently the formation of elaborate cerebral convolutions, contrasting the smooth (lissencephalic) hemispheric surface of their wild type counterparts[38,39] (FIG. 3b). Despite severe additional brain abnormalities in these transgenic mice, many of the overproduced neurons migrate radially, settle into apparently normal laminar and radial positions and generate a much larger surface of the neocortex of relative uniform thickness. By contrast, overexpression of cyclin-dependent kinase (CDK) inhibitor p27, which acts on neuronal progenitors at later developmental stages, decreases the production of neurons destined for the more superficial layers within the radial columns without increasing its surface[40].

Although, it is unlikely that mutation in caspase 9, β-catenins, or CDK genes were involved in neocortical evolution, as mice that are deficient for these particular genes are not viable, these experiments can serve as an example of the evo–devo approach to draw theories on how a single gene mutation, that may have occurred in our common, long-extinct ancestors, could have increased the number of proliferative founder cells in the VZ, which triggered a cascade of events that led to an increase in the number of radial units and cortical surface expansion, including formation of convolutions without an increase in cortical thickness.

Development of convolutions. It has been recognized as common sense that the increase in the initially smooth cortical surface must have led to its folding (FIG. 1). However, understanding how these convolutions formed involves rather complicated logistical problems: one of them being that the pattern of convolutions is highly reproducible within each species with relatively small individual variations. To account for this stereotypic pattern, a role of the long cortico-cortical axonal tracts in this process has been proposed[41–43]. Indeed, the formation of the convolutions in the human cerebrum is associated with the formation of the voluminous intermediate zone that eventually transforms into subcortical white matter, crisscrossed by the large number of distinct fascicles of cortico–cortical connections. It has been speculated that the tension created by these fascicles is responsible for the stereotyped shape and orientation of the convolutions in gyrencephalic brains[43].

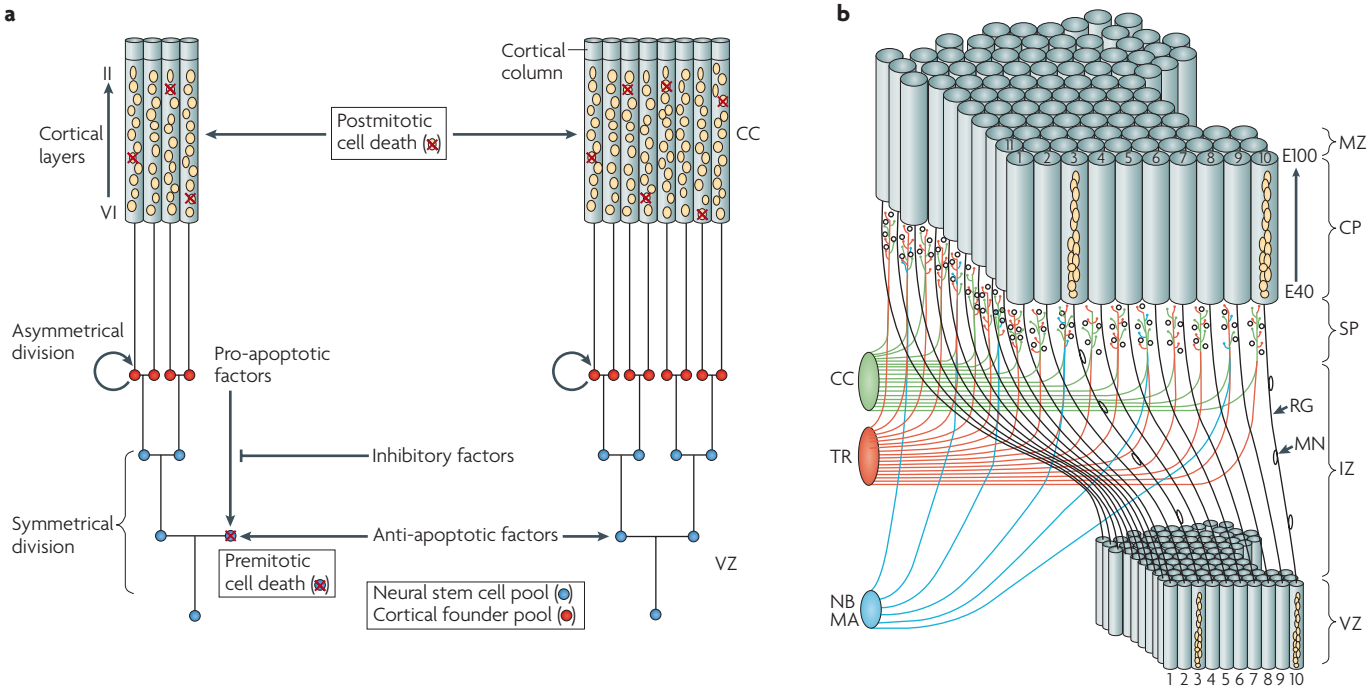

Figure 2 | **Radial unit lineage model of cortical neurogenesis. a** | Based on the radial unit hypothesis[12,30], the model illustrates how changes in the mode and the rates of cell proliferation and/or programmed cell death within the neural stem cell pool (blue circles) in the ventricular zone (VZ) that divide symmetrically at early embryonic stages causes an exponential increase in the number of radial columns, which, in turn, results in surface expansion of the cerebral cortex without changes in its thickness. By contrast, similar changes in proliferation kinetics occurring in the founder cells (red circles), which divide asymmetrically, cause a linear increase in the number of neurons within radial columns without a change in the cortical surface area. **b** | The model of radial neuronal migration that underlies columnar organization based on REFS 12,30. The cohorts of neurons generated in the VZ traverse the intermediate zone (IZ) and subplate zone (SP) containing 'waiting' afferents from several sources (cortico-cortical connections (CC), thalamic radiation (TR), nucleus basalis (NB), monoamine subcortical centers (MA)) and finally pass through the earlier generated deep layers before settling in at the interface between the cortical plate (CP) and marginal zone (MZ). The timing of neurogenesis (E40–E100) refers to the embryonic age in the macaque monkey[12,22,32]. The positional information of the neurons in the VZ and corresponding protomap within the SP and CP is preserved during cortical expansion by transient radial glial scaffolding. Further details can be viewed in the Rakic laboratory animated video of radial migration. RG, radial glia cell; MN, migrating neuron. Part **a** is modified with permission from REF. 147 © (2005) Macmillan Publishers Ltd. All rights reserved. Part **b** is reproduced, with permission, from REF. 140 © (2007) Elsevier.

Evolutionary adaptation of radial glial cells. The transient population of radial glial cells in the developing cerebral cortex serves both as neural stem cells and as a guide for the migration of their offspring and adjacent neuronal progenitors[44–46]. However, formation of convolutions requires long, curvilinear migratory pathways and durable radial glial shafts that serve as guides for neuronal migration to the distant cerebral cortex at later fetal stages. Thus, in the primate forebrain, many radial glial cells transiently stop dividing while their fibres span the entire cerebral wall, retaining their endfeet at the ventricular and pial surfaces[47]. A single radial glial fibre in primates may serve as a guide for cohorts of up to 30 simultaneously migrating neurons, including some of the interneurons that originate from the expanded SVZ[48,49] and from the larger pool of progenitors with short radial processes in the VZ[46,50,51]. The greater stability and longevity of the radial glial scaffolding may be an evolutionary adaptation that enables proper allocation of neurons to the expanded and convoluted cerebral cortex[32,49]. Indeed, the radial glial in both human and non-human primates differentiate precociously and express mature glial markers such as the glial fibrillary acidic protein (GFAP) at the onset of corticogenesis, whereas in rodents they are expressed only after birth[48,50–55]. Therefore, even basic cell types such as the radial glial, have diversified during evolution to accommodate the need to develop cerebral convolutions.

Multiplication and elaboration of cortical maps

During evolution, functionally distinct cortical areas have expanded at individual rates and new areas have been added, forming an elaborate mosaic[56]. For example, Broca and Wernicke language-related areas or the prefrontal granular cortex have not been, either anatomically or functionally, identified in rodents[4,10,57] (FIG. 1). Numerous ideas about the developmental mechanisms that could explain how this elaborate cortical parcellation occurred in evolution have been discussed. Early debates about whether structural differences between areas are induced in the initially homogeneous population of cortical neurons by the patterned activity of the inputs arriving from the periphery through the thalamus (the tabula rasa hypothesis) or, whether the cortical

Cortical parcellation
Regionalization of the cerebral neocortex into areas with distinct structural and functional attributes.

Patterning center
Group of cells in the embryonic brain that secrete molecules (morphogens) that initiate differential expression of transcription factors that specify formation of the cortical areas.

progenitors themselves are targets of evolution (the protomap hypothesis) have been recently reviewed[28,58,59] and are not repeated here. In brief, the consensus that has emerged is that the species-specific diversity of the neocortical areas originates from differential gene expression in the neural stem cells in the embryonic VZ and this information is transferred radially through migrating postmitotic neurons to the overlying cortical plate. Thus, each area attracts appropriate inputs, rather than being specified by them. Activity-dependent mechanisms have a significant role at subsequent stages that refine the already formed synaptic connections[12,60–62].

Duplication through morphogenetic factors. The evolution of the cerebral cortex involves both an increase in the number of new cytoarchitectonic areas, as well as duplication of sensory representations[63]. It is generally accepted that the regionalization of the neocortex is initiated by patterning centres that secrete signalling molecules, such as the fibroblast growth factors (FGFs), wingless (WNTs) and bone morphogenetic protein (BMP), that regulate the position and size of cortical areas (reviewed in REFS 28,64,65). For example, the mouse forebrain has an anterior patterning centre at the commissural plate that secretes FGF8 and FGF17. Perturbations of the patterning centre and expression of FGF8 can profoundly change the somatosensory map of the mouse cerebral cortex: overexpression of FGF8 in the embryonic forebrain results in formation of an additional barrel field, with a mirror image topographical representation of mouse whiskers[64]. The cellular mechanism of this remarkable duplication is unknown, but it is instructive that FGF8 expression is coordinated by distinct sets of transcription factors, namely zinc-finger and sonic hedgehog transcription factors that are involved in neuronal production and phenotypic and areal specification[65–69]. However, this FGF study shows dramatically that a change in expression of a single factor can result in a duplication of an entire functional area.

Decisions in the proliferative centers. Numerous recent studies have demonstrated that the subdivisions and area enlargements of the cortical areas in embryonic mice could be traced back to the proliferative VZ and SVZ (reviewed in REFS 28,58,65). The generation of transgenic mice in which the number of neurons and radial units is increased serves as an example of how a larger-than-normal number of founder cells can create a cortex with an increased surface area and number of neurons that eventually form new connections. For example, the expression of FGF8 in the anterior parts of the telencephalon suppresses the posteriorly expressed chick ovalbumin that normally functions upstream of COUP transcription factor 1 (COUP-TF1) and homeobox (EMX2) in the telencephalic neuroepithelial cells[65,70–73], and this suppression results in a decrease in the anterior but an increase in the posterior expression of these molecules. The development of frontal and sensory subdivisions of the cortex is dependent on the graded action of FGF8 (REF. 73) and FGF17 (REFS 74,75) and provides an elegant example of how one area, such as the frontal cortex, can expand differentially and independently of the growth rate of other areas[75,76] (FIG. 4c). Furthermore, the size of an area can be regulated by the change in FGF17 expression at early embryonic stages independent of the synaptic input from the periphery through the thalamus[74] (FIG. 4).

The fact that it is now possible to enlarge and/or duplicate distinct cytoarchitectonic areas in the neocortex by genetic manipulation opens an unprecedented opportunity to explore how these maps develop in each individual, as well as how they may have been introduced during neocortical evolution. As a next step, it is important to search for additional genes and morphoregulatory molecules that may be involved in cortical specification; and, to develop rodent and possibly non-human primate models, of cortical dysgenesis that mimic specific genetic or acquired cortical disorders in human[77]. In the spirit of evo–devo reasoning, these studies illustrate how a single mutation could have a sudden and profound effect during evolution on the pattern of cortical parcellation and presents some insights into how it could have occurred at the genetic and cellular level[78].

More neurons, more connections. Obviously, an increase in the number of neurons, radial columns and cytoarchitectonic areas is not sufficient to explain the multitude of functional advances that evolved to generate the human cerebral cortex and which must also involve the elaboration of neuronal connections[2,62,79]. There is a large amount of comparative anatomical data showing that, in spite of considerable inter-individual variability, the basic pattern of neuronal connection is species-specific and highly reproducible. It is generally agreed that functional usefulness of evolutionarily new cortical connections must be validated through the process of

Box 2 | **Evolutionary elaboration of radial columns**

During evolution, the neocortical surface expands by the addition of radial columns[23,30] (FIG. 2), but the composition of the columns also undergoes changes. The notion of the homogeneity of the columns[141] has been abandoned in favour of their heterogeneity, both in different functional areas of an individual, as well as across species[142,143]. In all mammals, functional columns consist of an intermix of projection (excitatory, glutaminergic) neurons and inhibitory (GABAergic) interneurons that are stereotypically interconnected in the vertical dimension[144] but receive diverse extrinsic input[9,10,140]. To achieve proper cellular composition, several neuronal clones move between their parental radial glial cells within the subventricular zone (SVZ) and the intermediate zone (IZ) of the embryonic cerebral wall[145]. The disturbance of this intermixing, essential for proper cortical operations, may underlie disorders of selected cognitive functions[143]. However, although this lateral shift of migrating neurons in the SVZ–IZ was recognized 35 years ago based on reconstruction from serial electron microscopic sections[145], the molecular mechanisms that regulate this intermix of neuronal clones has been elusive.

A recent study using a combination of the most advanced molecular technology showed that, during radial neuronal migration, the lateral intermixing of neurons that belong to different clones within a given functional column depends on the expression levels of A-type Eph receptor tyrosine kinase and their ligands, ephrin-As[146]. Eph/*Ephrin*-dependent intermixing in the SVZ and IZ may be only one of several mechanisms for allocating proper composition of neuronal phenotypes that have been operating during mammalian evolution. However, it can serve as an example of how developmental studies in mice may provide an insight into mechanisms of evolutionary elaboration of columnar composition that may have happened in our extinct ancestors.

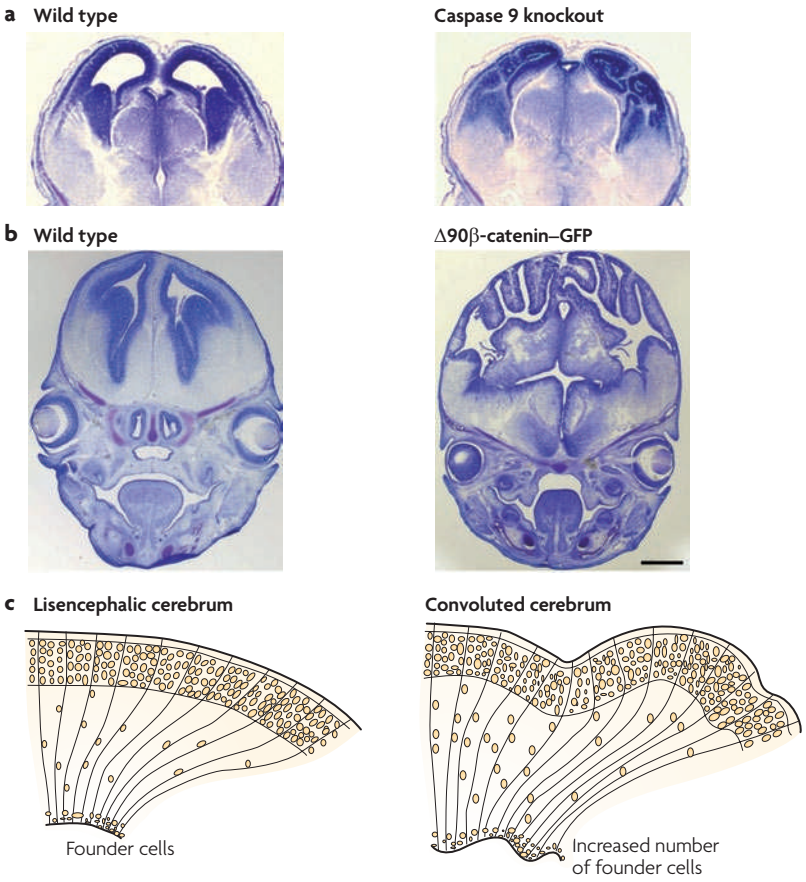

Figure 3 | **Enlargement of cortical surface by decrease in programmed cell death or increase in proliferation. a** | Brain sections of a wild type mouse and that of a littermate in which both copies of caspase 9 were deleted are shown. Knockout of caspase 9 leads to expansion of the progenitor pool, which in turn results in increased number of radial columns and consequently a convoluted cortical surface. **c** | Mid-coronal section through the forebrain (stained with cresyl violet) of an embryonic day 15.5 wild type mouse and of a transgenic mouse that expresses a Δ90β-catenin–GFP (green fluorescent protein) fusion protein in neural precursors. Expression of the fusion protein results in an increase of the precursor population and of the number of cortical columns. **c** | Diagrams showing how an increased number of progenitor cells can be distributed through radial glial scaffolding to form a sheet, rather than a lump of postmitotic neurons. Parts **a** and **c** are reproduced, with permission, from REF. 35 © (1999) Oxford University Press. Part **b** is reproduced, with permission, from REF. 38 © (2002) American Association for the Advancement of Science.

natural selection and, if proven to be advantageous, may contribute to the survival and propagation of the species. However, as neurons are generated before they form axonal connections, an increase in neuronal number, diversification of their types and attainment of their positions will precede the establishment of new, useful connections in cortical evolution.

As reviewed in the next section, the new classes of neurons allow the formation of new patterns of connections. In this complex process, innate neuronal activity is essential for the validation and refinement of synaptic connections and leads to their selective stabilization and/or elimination through competitive interactions[80–86]. This is a large research field that cannot be considered in sufficient detail in this Review. However, it is important to mention that there should be a clear distinction between the formation of stereotypic, species-specific connections that are established by surface mediated attractions and innate, activity-dependent refinements, from the experience-dependent synaptic plasticity that shapes connections in cooperation with the outside environment in each individual. These later-occurring, experience-dependent changes are not inheritable and, thus, cannot contribute to evolutionary advances, which require gene mutation in germinal cells.

New cell types and migratory pathways

Apart from an increase in the number of neurons and their allocation into distinct cytoarchitectonic areas, the evolution of the cerebral cortex has also been accompanied by the introduction of new cell types that migrate through old or new routes to appropriate, often distant locations. During 100 million years of separation from a common ancestor, the mouse and human cerebral cortex have acquired a number of different features, only some of which are briefly described in the following subsections.

The subpial granular layer. The subpial granular layer is a prominent and voluminous transient cellular layer situated below the pial surface of the fetal human cerebrum that was first described by O. Ranke more than a century ago. However, there are only a handful of studies on its developmental history and function in human and non-human primates[19,23,87,88] and it has not yet been observed in rodents[17]. It appears around the eleventh gestational week in the human marginal zone, peaks in size during mid-gestation and disappears by the time of birth[17,31,89]. Analysis of proliferative activity in the developing cerebrum of cats and primates indicates that the subpial granular layer produces neurons that may descend to the underlying cortical plate[23,87]. Based on the uneven size and high incorporation of tritiated thymidine in the subpial granular layer, which is located above the developing cortical plate, it may contribute to the wealth of interneurons in certain cortical areas, such as the primary visual cortex, which has an extraordinary large layer IV compared with rodents[23]. Thus, better characterization of the subpopulations of interneurons that originate in the subpial granular layer may elucidate their function as well as their possible involvement in human cortical disorders that do not occur or cannot be mimicked in mice.

Migration from the ganglionic eminence to the thalamus. A human-specific migratory pathway formed during mid-gestation by neurons streaming from the ganglionic eminence of the ventral telencephalon to the dorsal thalamus of the diencephalon, known as the corpus gangliothalamicum (CGT), was discovered four decades ago[90]. This voluminous, easily identifiable stream of migrating bipolar neurons in humans could not be found in any other species examined to date, including rodents, carnivores and New and Old World primates[31,91]. More recently, cell labelling in organotypic slice cultures of human embryonic brain tissue enabled tracing the migration of these cells directly from the ganglionic eminence to the dorsal thalamic association

Homotypic–neurophilic guidance
Mode of neuronal migration along the surface of other neurons that depends on membrane-bound adhesion molecules present on both migrating and guiding neurons as opposed to heterotypic gliophilic migration that is guided by the shafts of radial glial cells.

nuclei[92]. These migratory cells, which rely on homotypic–neurophilic guidance and express distal-less homeobox 1 (DLX1) and DLX2 proteins, which guide their tangential migration, eventually form GABAergic neurons in the dorsal thalamus, in particular, the pulvinar and mediodorsal nuclei, which are anatomically related to the association neocortex involved in higher cognitive functions, including symbolic reasoning or language[92]. Thus, it seems that the human thalamus, to accommodate the increased axonal input from the expanding association neocortex, recruits an additional complement of neurons from the nearby, still mitotically active ganglionic eminence in the ventral telencephalon after the diencephalic proliferative VZ becomes exhausted[92].

Two origins of neocortical interneurons — a working hypothesis. It was Ramón y Cajal[93] who suggested that extraordinary human mental abilities are closely related to the increase in the number and diversity of cortical interneurons, which he classified using the Golgi silver impregnation method. However, since then, many other subclasses of primate-specific interneurons have been characterized[94,95]. In spite of this, our knowledge of the origin and development of the interneurons has been derived almost exclusively from rodents as these are more amenable to more advanced methods. Thus, tracing cell lineages using retroviral vectors in mice first showed that interneurons and projection neurons originate from different progenitors[96]. Next, ingenious experiments in mice demonstrated that the majority, if not all, of the cortical interneurons in this species originate from the ganglionic eminence in the ventral pallium and then migrate tangentially to the dorsal telencephalon[14,97–100] (FIG. 5).

The suggestion that, in addition to those derived from the ganglionic eminence, a large number of interneurons in humans originate from the voluminous SVZ was initially based on classical histological observations[31]. However, more recently, several studies that have succeeded to explore the origin of interneurons in primates with more advanced methods, have uniformly suggested that cortical GABAergic neurons originate from the ganglionic eminence and the VZ and SVZ[101–106] (FIG. 5b). This stands in contrast to the exclusive ganglionic eminence origin of interneurons in rodents. Importantly, fate-mapping experiments in human mid-fetal slice cultures suggest that the percentage of cortical GABAergic neurons that arise from the dorsal pallium VZ and SVZ might be higher than those arising from the ventral progenitors[101]. Furthermore, it has been shown that proliferative cells (as identified by bromodeoxyuridine incorporation) in slices of the dorsal human VZ and SVZ during mid-gestation express the ventral transcription factors (DLX1, DLX2 and NKX2.1)[105]. Finally, bipolar morphology and orientation of DLX-expressing cells also indicate their local origin and radial migration[89]. These studies collectively support the idea that in primates, in addition to interneurons born in the ganglionic eminence of the ventral telencephalon, there is a population that originates in the VZ and SVZ of the dorsal telencephalon (FIG. 5b). Interneurons derived from the ganglionic eminence and the VZ and SVZ are GABAergic, but their morphology and molecular characteristics indicate that their function might be different and that the latter subclass may be involved in human-specific higher cortical functions.

Support of the dual origin of interneurons in humans comes from analysis of a congenital disorder named holoprosencephaly (HPE) in which the ventral forebrain structures, including the medial ganglionic eminence, in some cases fail to develop. Examination of the distribution of various classes of cortical GABAergic interneurons showed that the neocortex and hippocampus of HPE specimens with severe ventral forebrain hypoplasia lack specific subtypes of cortical GABAergic neurons that in mice are known to derive from the ganglionic eminence[106]. By contrast, other subtypes of interneurons seem to be little or not affected by ventral midline defects, which suggests that they probably originate in the preserved dorsal pallium VZ and SVZ. The detailed analysis of the expression of various cell-specific markers in the fetal and infant brains of HPE specimens with mild and severe ventral forebrain defects revealed that subclasses of GABAergic cells, distinguished by co-staining of nitric oxide synthase 1 (NOS1), neuropeptide Y (NPY), somatostatin (SST) and parvalbumin

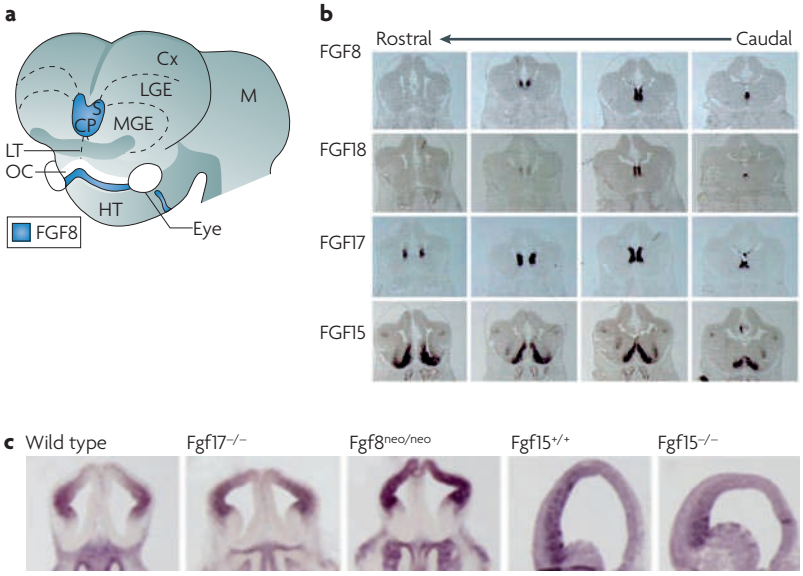

Figure 4 | **Control of arealization of the frontal cortex by FGF expression.** **a** | Diagram of the forebrain depicting the rostral patterning centre (which is also known as the commissural plate (CP)) of fibroblast growth factor 8 (FGF8). **b** | Coronal sections of the telencephalon of an embryonic day 12.5 mouse showing the expression of FGF isoforms in the rostral patterning centre. **c** | Coronal sections of the telencephalon of an embryonic day 12.5 mouse showing the regulation of COUP-TF1 (COUP transcription factor 1) expression by different FGF isoforms. COUP-TF1 expression is increased in FGF17$^{-/-}$ null mutant mice and FGF8 mutant mice (hypomorphic neo/neo mutant), which indicates that these factors repress COUP-TF1 expression in the wild type background. By contrast, COUP-TF1 expression is decreased in FGF15$^{-/-}$ null mutants, which suggests that FGF15 promotes COUP-TF1 expression. Cx, cortex; HT, hypothalamus; LGE, lateral ganglionic eminence; LT, lamina terminalis; M, midbrain; MGE, medial ganglionic eminence; OC, optic chiasm; S, septum. Part **a** is modified, with permission, from REF. 148 © (1973) American Association for the Advancement of Science. The panels in part **b** are courtesy of J. L. Rubenstein and reproduced, with permission, from REF. 75 © (2008) Wiley-Liss. The panels in part **c** are courtesy of J. L. Rubenstein and reproduced, with permission, from REF. 76 © (2008) Biomed central.

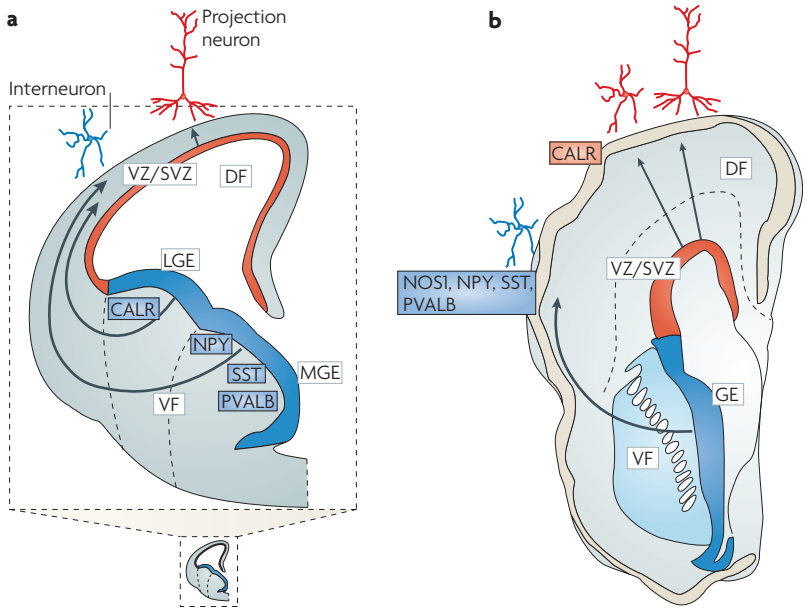

Figure 5 | **Rodent and human fetal forebrains at the peak of corticogenesis.** Diagrams of cross sections of half of a rodent (**a**) and a human (**b**) fetal forebrain are shown to scale (a zoom in of the rodent section is also provided for legibility). In rodents, the main source of interneurons is the ganglionic eminence (GE) — which is comprised of the lateral ganglionic eminence (LGE) and the medial ganglionic eminence (MGE) — of the ventral telencephalon. These neurons migrate tangentially to the neocortex in the dorsal telencephalon. By contrast, interneurons in the human forebrain originate both in the GE as well as locally in the ventricular and subventricular zones (VZ/SVZ) of the dorsal telencephalon subjacent to the neocortex. Interneurons originating from the cortical VZ/SVZ and GE express a different set of markers, namely nitric oxide synthase 1 (NOS1), neuropeptide Y (NPY), somatostatin (SST) and parvalbumin (PVALB) when originating from the GE, and calretinin (CALR) when originating from the VZ/SVZ. DF, dorsal forebrain; VF, ventral forebrain. Data to generate part **a** was derived from REF. 149; data to generate part **b** was derived from REFS 101,106.

(PVALB) are consistently absent in both fetal and infant stages. By contrast, the number of calretinin-positive GABAergic cells seem to be little or not affected in the HPE cases. Furthermore, the absence of cells expressing NOS1, NPY, SST and PVALB correlated with the dramatic depletion of the thyroid transcription factor 1 (NKZX-1/TITF1)-positive medial ganglionic eminence progenitors, which are the main source of these subtypes of interneurons in mice. Although not conclusive, the results strongly suggest that cells expressing NOS1, NPY, SST and PVALB originate in the ganglionic eminence and migrate tangentially into the cortex, whereas the calretinin-positive cells could have arisen from the dorsal neuroepithelium of the lateral ventricular wall and migrated to the cortex radially together with glutamatergic projection neurons. Admittedly, this study cannot eliminate the possibility that the calretinin-positive cells could have arisen from less affected ganglionic eminence progenitors. However, it is noteworthy that the neurons expressing NOS1, NPY and SST are most numerous in the derivatives of the marginal zone and subplate zone in monkeys[107], whereas the calretinin-expressing interneurons populate cortical layers II–VI, which include the double bouquet cells that are absent from the rodent cortex[108,109,110] (reviewed in REF. 95).

Association areas
Areas of the neocortex that are particularly large in the human cortex (for example, prefrontal granular cortex or language-related Broca and Wernicke areas) are considered as analysers for integration of information from various sensory and motor areas.

The finding of evolutionarily novel origins that produce different subtypes of interneurons in human and non-human primates does not negate the significance of the tangential migration of cortical GABAergic cells from the ganglionic eminence that were discovered in the mouse. In fact, studies in human[101,102,106] and the cynomolgus monkey[103] indicate that the ganglionic eminence produces a significant portion of interneurons that migrate to the neocortex through the tangential route. A better understanding of the underlying mechanisms that generate the greater diversity of inhibitory interneurons in human and non-human primates could uncover a possible role of these cells in human psychiatric disorders[95,111].

Predecessor neurons. The most recently discovered type of neurons in human, the predecessor cell, has so far not been observed in other species[32]. These large, bipolar cells are the earliest generated neurons in the human forebrain that emerge under the pial surface of the ventrolateral cerebral wall at the end of the first gestational month, before onset of neurogenesis in the VZ and SVZ of the dorsal telencephalon. They express TU20 and T-box brain gene 1 (TBR1), have long horizontal processes and form an extensive network over the forebrain primordium[32]. My research team has proposed that predecessor cells may be a transient population, involved in determining the number of functional radial units in the cerebral cortex. However, it is important to establish whether these cells exist in non-human primates to experimentally determine their lineage, pattern of gene expression, function and fate.

The selected examples of human-specific developmental features and cellular events described earlier are by no means exclusive. Such evolutionary novelties are probably the result of gene mutations and/or the introduction of new genes and/or the addition of the developmental mechanisms and cell–cell interactions that act during the progenitor's exit from the mitotic cycle. They generate a different outcome depending on the evolutionary context. Recent studies indicate that even subtypes of the pyramidal cells destined for layer V, which are the main efferent system that connects the cortex with subcortical structures, are determined at the time of their last division in the VZ[112–114]. These experimental studies in mice indicate that evolutionary novelties, including introduction of new neuronal types, may start with a mutation that regulates the fate of neuronal stem cells in the proliferative zones.

Small is big: genetic differences

One frequently repeated statement, which has almost become a cliché, is the emphasis on how small the genetic differences are between humans and the rest of the animal kingdom. Indeed, only about 1% of the genes are human specific and yet, their effect on timing, sequence and level of gene expression has obviously great functional significance. For example, the human brain is characterized by expansion in size and complexity of the association areas of the neocortex, most notably the prefrontal cortex, but also the perisylvian areas that

are related to speech and language processing[115]. During human development these areas have a several-fold larger subplate zone that consists of multipolar neurons and incoming 'waiting' thalamic axons[116] and mature at a slower pace than in other areas[117]. Furthermore, some, if not most, of the specific functional prefrontal cortex subdivisions do not exist in non-primate species and their development, therefore, cannot be modelled easily in mice[4,10]. Yet, it is precisely these areas that are probably most significant for the unique human mental abilities, as well as neuropsychiatric disorders such as dyslexia, autism or schizophrenia. Although microarray analysis and other techniques have been available for more than a decade and have been applied in mice[118,119], so far there have been only few genome-wide expression studies of the human brain. The most comprehensive studies in terms of brain regions were done on aged brains[120,121], or on comparisons between diseased and healthy brains[122,123–124].

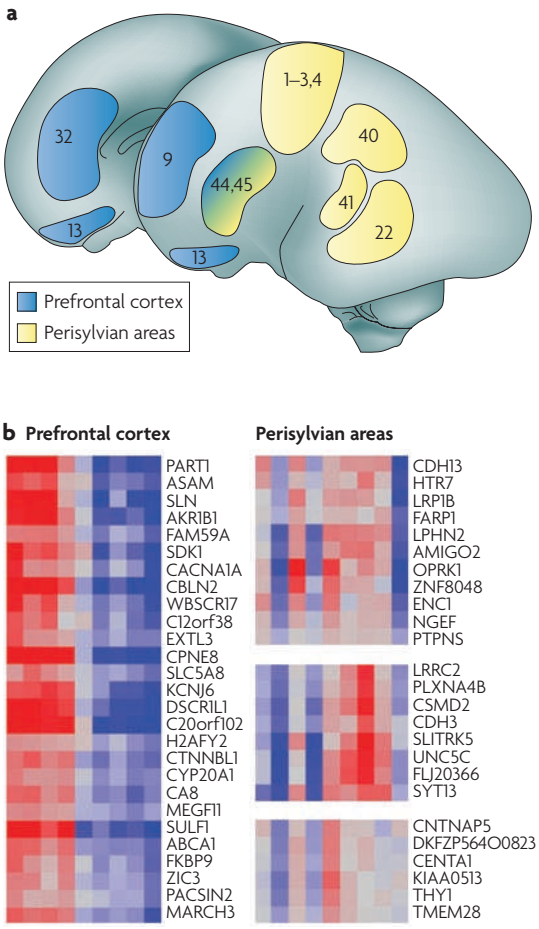

Figure 6 | **Gene expression patterns of neocortical areas of the human fetal cerebral hemispheres.** **a** | The neocortical areas investigated included four distinct subdivisions of the prefrontal cortex (blue), namely orbital, medial, dorsolateral and ventrolateral (Brodmann's areas: 9,13,32,44,45), and perisylvian areas (1–3,4,40,41,42) (yellow). In addition to the ventrolateral prefrontal cortex, which encompasses the prospective Broca's area (44/45), two other speech and language-related areas were assayed: parietal and temporal association cortices, which encompass the prospective Wernicke's–Geschwind's areas (22/39). **b** | Examples of different clusters of correlated gene expression patterns in the perisylvian and cortical areas. Selected highly correlated clusters of genes with the most restricted expression patterns are listed at the right side of columns. Red is higher level of expression, blue is lower expression. Part **b** is modified with permission from REF. 125 © (2009) Cell Press.

Region specific differential gene expression. A recent study examined 13 brain regions from both cerebral hemispheres of the fetal human brain[125]. Using the most advanced generation of microarrays, over one million known and predicted exons in nine distinct neocortical areas were profiled, including subdivisions of the prefrontal cortex, as well as the temporal and parietal association areas of the perisylvian cortex (FIG. 6a). The data revealed a huge number of transcriptional differences, including genes that were both differentially expressed and alternatively spliced. For example, although the greatest distinction within the neocortex is between the prefrontal and non-frontal areas, there are also expression patterns common to the perisylvian areas (prospective Broca's, Geschwind's and Wernicke's area), which, although distributed across three lobes, are functionally linked by their involvement in speech and language[125]. The functions of these genes commonly enriched in perisylvian areas might therefore hold some clues to the evolution of structure and connectivity that allowed for the development of language in our species. In addition, the prospective Broca's area (and its right hemisphere homologue) is, on the basis of gene expression patterns, more similar to the neighbouring motor cortex than to the prefrontal cortex[125]. Interestingly, no statistically significant left–right transcriptional asymmetry at the population level was identified, suggesting that such asymmetries most likely occur earlier during fetal gestation as previously suggested[126]; but, also noting that more sensitive technologies, as well as greater sample sizes, may yet uncover such asymmetry at the late mid-fetal stage.

The application of weighted gene co-expression network analysis to microarray-derived expression data has previously provided unique insights into the genetic mechanisms of human brain evolution and disease[127]. Applying this analysis to data from the developing human brain led to the identification of networks of genes that are linked both to brain regions, including the prefrontal cortex, and to biological processes such as alternative splicing[125]. Genes with the highest degree of interaction within such networks, known as 'hubs', may represent critical transcription or other regulatory factors, and are prime candidates for functional characterization.

Several recent studies indicate that tissue- and cell type-specific enhancers have crucial roles in neuronal specification and development[128]. For example, the transcription factor SOX5 (SRY (sex determining region Y) -box 5) regulates connections of the neurons in the subplate zone and cortical layer 6. Thus, subsets of such transcription factors and their enhancers, may have

Network of genes
A collection of genes that are co-regulated or interact with each other.

Enhancers
A short region of DNA to which proteins including transcription factors can bind to enhance transcription levels of genes in gene clusters.

led to the emergence of new gene expression patterns and neocortical areal phenotypes through recent evolutionary modification[129]. These spatially comprehensive expression data in the fetal neocortex have opened the door to the systematic investigation of the relationship between recently evolved enhancers and transcriptional specificity. Genes that are differentially expressed in the developing human neocortex were associated with putative recently evolved enhancers, providing a short list of candidate genes and regulatory elements for functional characterization. The number of such associations was statistically disproportionate relative to the genome-wide frequency of such putative enhancers, providing further evidence that the evolution of regulatory factors may have had an important role in the evolution of the human cortex[125].

The recent data in humans[125] have uncovered an order of magnitude of greater transcriptional differences between neocortical areas than has been obtained in comparable studies in rodents[130,131]. For example, the gene *CNTNAP2* (contactin associated protein-like 2), previously studied for its role in autism and specific language impairment[132–135] is selectively and highly enriched in the orbital prefrontal cortex, an area involved in regulation of social behaviour in humans and has no comparable analogue in rodents[124]. The mouse homologue *Cntnap2* has not been found to be expressed in any areal pattern or gradient in the mouse brain at any stage of development[136] (M.B. Johnson and N. Sestan, personal communication). This is an example of how unique structures and gene expression patterns that give rise to abilities, such as language, are also involved in disorders, such as autism, for which there is no accepted mouse model[137]. Future analyses contrasting fetal human brain gene networks with comparable data from mice (as has been done for human and chimpanzee adult brains[138] will provide new insights into the complex transcriptional and molecular underpinnings of cortical evolution and development of human specific neuronal and synaptic organization.

Conclusion

The evo–devo approach might eventually elucidate the advent of the human in the universe, achieved through expansion and elaboration of the cerebral cortex. Based on the recent findings from the descriptive and experimental embryonic studies of various mammalian species, we can develop realistic models of how gene mutations that influenced the fate of cell progenitors during evolution affect neuron number, regulate their migration into proper regions, promote formation of new phenotypes that establish new areas with new connections which express particular sets of neurotransmitters and receptors. Neglecting or minimizing evolutionary differences between the species may be among the reasons for the failures of many clinical trials that were based exclusively on the highly promising findings in rodents. However, the evo–devo approach for the study of corticoneurogenesis is not only important to the compelling problems of congenital disorders of higher cortical functions in humans, but also provides a hint about how we may have evolved to be masters of our destiny.

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DATABASES
UniProtKB: http://www.uniprot.org
calretinin | caspase 9 | COUP-TF1 | FGF8 | NPY | NOS1 | SST | PVALB

FURTHER INFORMATION
Pasko Rakic's homepage: http://rakiclab.med.yale.edu/
Video of a migrating cortical neuron: http://rakiclab.med.yale.edu/MigratingCorticalNeuron.html
Video of radial migration: http://rakiclab.med.yale.edu/RadialMigration.html

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