Developmental mechanisms underlying the evolution of human cortical circuits

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Abstract| The brain of modern humans has evolved remarkable computational abilities that enable higher cognitive functions. These capacities are tightly linked to an increase in the size and connectivity of the cerebral cortex, which is thought to have resulted from evolutionary changes in the mechanisms of cortical development. Convergent progress in evolutionary genomics, developmental biology and neuroscience has recently enabled the identification of genomic changes that act as human-specific modifiers of cortical development. These modifiers influence most aspects of corticogenesis, from the timing and complexity of cortical neurogenesis to synaptogenesis and the assembly of cortical circuits. Mutations of human-specific genetic modifiers of corticogenesis have started to be linked to neurodevelopmental disorders, providing evidence for their physiological relevance and suggesting potential relationships between the evolution of the human brain and its sensitivity to specific diseases.

[H1] Introduction

Approximately 6-8 million years ago, the common ancestor of great apes (chimpanzees and bonobos) and species of the *homo* lineage lived in Africa. Following the divergence of hominins, one branch, through multiple radiation (in which new species emerge) and extinction events, gave rise to *Homo sapiens* approximately 300,000 years ago [1]. As a result of this evolution, the brain of modern humans displays remarkable cognitive abilities that enable functions such as elaborate working memory, self-awareness, advanced forms of communication, complex tool making and cultural development [2,3]. The mechanisms underlying the evolutionary emergence of human cognitive abilities constitute a long-standing topic of interest in neuroscience that has recently been transformed by the coalescence of major advances in comparative genomics **[G]**, developmental neurobiology and the development of new experimental models to study human neural development and function (**Box 1**).

Here, we present an overview of these recent advances, which have linked developmental mechanisms with the evolution of human neural circuits. We focus on the cerebral cortex, arguably the most complex and among the most divergent of human brain structures, compared with other species. We first describe some of the most notable qualitative and quantitative differences between the human cortex and that of other animals at the cellular level. We then review the cellular mechanisms that underlie specific features of human corticogenesis and their molecular links with upstream human-specific genomic changes. Finally, we illustrate how the identification of human-specific modifiers of cortical development and function could lead to the discovery of previously unknown aspects of human brain structure, function and disease.

[H1] Human-specific brain features

The staggering structural and functional complexity of brain organization can be studied at multiple scales [4]. At a macroscopic scale, neural circuits are organized in interconnected networks of neurons by short-range and long-range axonal projections. At a microscopic scale, neurons connect to each other via precise synaptic connections. One main goal of neuroscience is to understand how variation at these different levels underlies the brain's functional properties and ultimately shapes

behavior. Studying this challenging problem is essential if we are to decipher the mechanisms that mediated the emergence of the cognitive capacities of modern humans[5].

[H2] Cellular composition

[H3] More cortical neurons

The human brain contains approximately 85 billion neurons, each forming thousands of individual synapses with other neurons[3,6,7]. The numbers of neuronal and non-neuronal cells in specific brain structures in 41 mammalian species have been estimated, leading the authors of those studies to the conclusion that the brain of modern humans can be considered a "scaled-up primate brain" [6,7]. However, two brain structures have expanded significantly more than others (in terms of neuron number) among primates and particularly in humans: the cerebral cortex (**Fig. 1a**) and the cerebellum [6,7]. With 16 billion neurons, the human cerebral cortex contains a larger number of neurons than that of our closest relative the chimpanzee (6 billion neurons) or the more distantly related rhesus macaque (1.7 billion neurons) [6]. As a comparison, the neocortex of two mammalian species often used in neuroscience, the mouse and rat, contain approximately 14 and 31 million neurons, respectively [6,7].

[H3] Altered cortical neuron composition and diversification

Beyond absolute cell number, a key substrate of the complexity of the human cortex is its cell composition and diversity (**Fig. 1b**). The neocortex is composed of six layers containing both long-distance-projecting excitatory pyramidal neurons and locally-projecting inhibitory interneurons. These broad neuronal classes can be further divided into several dozen subclasses and subtypes, each of which displays specific molecular, cellular and hodological features[8,9]. Some subtypes are partially specific to particular cortical layers: for instance, deep layer (DL) neurons (those found in layers 5 and 6) mostly send long-range projections to subcortical targets, while upper layer (UL) neurons (found in layers 2 and 3) project mostly locally and to other cortical areas. Finally, layer 4 neurons receive most of the monosynaptic connections from the thalamus that relay sensory information from the periphery (**Fig. 1b**) [10]. While these basic principles are largely conserved in all mammals, important cellular features that display divergence in humans have been uncovered. For example, the thalamo-recipient cortical layer 4 is expanded in human and non-human primates, compared with non-primate species, and displays a more complex cytoarchitecture[11].

One key human feature is a significant expansion of the fraction of cortical neurons that are UL pyramidal neurons: these neurons constitute more than 40% of the neurons in the human cerebral cortex, while their proportion is around 25% in the mouse (with intermediate values for non-human primates and hominids)[11-13] (**Fig. 1b**). Recent multimodal analyses combining single cell transcriptomic, electrophysiological and morphological profiling have revealed more heterogeneity and diversity among UL neurons in the human cortex, where at least 5 UL subtypes have been identified, compared to 3 in the mouse (**Fig. 1b**). Interestingly, the 2 additional UL subtypes found in the human display patterns of gene expression that are reminiscent of DL neurons[14,15]. The resulting

increase in the proportion of long-range projecting neurons in external upper layers of the cortex[16] could be an important substrate of the increased cortico-cortical connectivity found in the human brain[14,16] (**Fig. 1b**).

Beyond UL neurons, single cell transcriptomic comparisons of adult human cortical neurons with their mouse and primate counterparts have revealed considerable gene expression divergence in homologous cell subtypes in each species, as well as changes in their relative proportion[15,17,18]. For example, Betz neurons, a subtype of DL corticospinal neurons that connect to the spinal cord to mediate fine motor control, are enriched in primates [17]. Von Economo (VEN) neurons represent a cell type that are characterized primarily by bitufted dendritic morphology and found in several higher mammals including humans, where they are located mostly in anterior cingulate cortex (ACC) and frontal areas. Recent evidence identified some of the transcriptional and electrophysiological signatures of VEN cells, which suggest that this cell type is transcriptionally homologous to extratelencephalic (ET) excitatory neurons that project to subcortical targets [19].

Orthogonal to its laminar organization, the cerebral cortex is parcellated into numerous cortical areas populated by neurons displaying specific patterns of gene expression and connectivity[20,21]. While areas subserving first-order motor control and sensory processing are well-conserved in mammals, the number of cortical areas has considerably expanded in the primate lineage, rising from approximately 20 in the mouse to more than 150 areas in the human cortex [22-24]. In particular, there has been a diversification of association areas **[G]** [22-24]. The cortical areas involved in language processing and production are present in both humans and non-human primates, but their long-range input and output connectivity are divergent, which might explain human-specific linguistic capacities[25]. Most importantly, the size of the human prefrontal cortex (PFC), involved in complex social behaviors and executive planning[26], is larger than that of non-human primates[27,28] (although whether this reflects an absolute increase in size or an increase relative to the size of other areas is still debated[29]). The increase in the size of the PFC also reflects an increase in the size and/or number of its subdivisions (**Fig. 1a**). The developmental mechanisms underlying the diversification of association and PFC areas remain largely unknown. However, proposed mechanisms include changes in morphogen **[G]** signaling[30] (see below) and the 'untethering' of cortical neuron patterning from the constraining influence of local signaling centers, due to the significant expansion of the cortical surface [31].

Despite the significant progress achieved through multimodal single cell analyses, the field is only starting to evaluate the degree of cortical neuronal diversity in different species. More work is needed to explore further the relationship between areal diversity, connectome **[G]** properties and specific developmental programs underlying neuronal diversification. Nevertheless, current data indicate that the human cortex is characterized by an expansion and diversification of UL and PFC neurons. Both of these sets of neurons make extensive cortico–cortical connections, which are correspondingly increased in the primate and human cortex (**Fig. 1b,c**). Intriguingly, UL and PFC neurons are also characterized by their delayed generation and/or development: UL neurons are the last to be

generated during neurogenesis **[G]** and PFC areas are the last to reach mature patterns of structural and functional connectivity[32]. This is consistent with an important influence of extended developmental timing on the evolution of the human cortex (see below).

[H3] Altered interneuron composition.

Approximately 20% of rodent cortical neurons are locally-projecting interneurons; however, this proportion is greater in human cortex (approximately 25-30%)[17,33]. Cortical interneurons can be subdivided into at least 25 subtypes based on their morphology, connectivity, electrophysiological properties and gene expression profiles[9]. These subtypes appear to be largely conserved in mouse and human, at least based on transcriptomic profiling [34,35]. However, there are significant species differences in the repertoire of genes expressed in each interneuron subtype and also in their relative abundance and laminar position [34,35]. For instance, Rosehip interneurons, characterized by their specialized axon arborization that targets the dendrites of pyramidal neurons, have been found in the human cortex but not in mouse cortex[36]. Future studies should assess whether the qualitative and/or quantitative distribution of interneuron subtypes differs between human and non-human primates and/or represents a feature conserved in other mammals. It will also be important to determine how differences in interneuron composition affect circuit properties in a species-specific manner.

[H3] Altered glial cell type composition.

The cortex also contains non-neuronal cell types including astrocytes, oligodendrocytes and microglial cells. However, only a few studies have explored whether these cell types differ in their transcriptional profile, morphology or function in the brain of various types of mammals[34,37]. Comparison between several non-human primates and humans revealed that three main types of cortical astrocytes (interlaminar, protoplasmic and fibrous) display distinct morphological features [38,39] and vary in abundance depending on their laminar position and gene expression profile [34,37]. Recent single cell RNA sequencing **[G]** (scRNAseq) also revealed a significant degree of subtype diversity among microglial cells, especially in humans [40,41]. Many differences in glial cell gene expression profiles have been reported between human and chimpanzee[18] and several studies have also suggested that glial cells constitute a larger proportion of the total number of cells found in the human brain than in other mammalian species [40-42]. A recent study used a combination of cell fate mapping **[G]**, morphological analysis and gene expression profiling to illustrate the diversity of the astrocyte lineage and cell composition in the human cortex [43]. Future investigations will undoubtedly document whether these differences are observed in non-human primates and if they play a role in human-specific traits of brain development and/or adult brain function.

[H2] Cellular properties

[H3] Increased neuronal size, complexity and connectivity.

All mammalian cortical pyramidal neurons have some generic morphological features, including a single axon that projects towards the white matter, an apical dendrite that is oriented towards the

cortical surface and basal dendrites that branch extensively (with all dendrites being decorated by dendritic spines **[G]**) [44]. However, human pyramidal neurons display some additional species-specific properties. Their dendrites are longer and more branched than their mouse, macaque and chimpanzee counterparts[45-48]. Moreover they bear a higher density of spines than macaque, marmoset and mouse cortical pyramidal neurons[47,49] and more spines than cortical pyramidal neurons in any other primates including great apes such as chimpanzee and bonobo [45,50] (**Fig. 1c**). The average size of the spines (including the length of spine neck) is also larger in human pyramidal neurons, suggesting distinctive functional properties[47,49].

As a result of their larger dendritic length and increased spine density, human pyramidal neurons receive more synaptic inputs than those of non-human primates or other mammalian species (approximately ~30,000 synapses for human pyramidal neurons versus ~9,000-15,000 in mouse and rat)[3,47,51]. A recent study estimated that human pyramidal neurons receive approximately twice as many synapses per neuron as those of any other primate[50]. Interestingly, electron microscopy studies revealed that the ratio between the number of excitatory and inhibitory synapses received by pyramidal neurons in the human and rat cortex is constant (approximately 90:10). This indicates that there has been a similar increase in both types of synapses in humans, leading to a remarkable conservation of the excitation/inhibition (E/I) balance [3,12].

The emergence of new approaches to study connectomics and to create single neuron reconstructions from human post-mortem brain samples will enable us to determine whether there are variations in the number of excitatory and inhibitory synaptic connections received by individual neuronal subtypes in different cortical layers and areas. This information could then further inform computational approaches to model the impact of such variation in synaptic connectivity on circuit function.

[H3] Altered functional and circuit properties

Several recent studies, taking advantage of live biopsies of healthy human cortical tissue, have compared the functional properties of human and rodent cortical pyramidal neurons. Multiple features that differ between humans and other mammalian species and lead to differences in neuronal excitability or input-output relationships, have been identified [42,46,48,52-56]. These results suggest that the biophysical properties of the dendrites of human layer 5 and layer 2/3 pyramidal neurons are specialized compared to other mammals.

Both UL and DL human pyramidal neurons display a much increased apical dendrite length, which might — depending on the degree of passive attenuation and the extent of active conductance mechanisms within these dendrites— contribute to increased electrical compartmentalization (defined as the ability of distal synaptic inputs to influence dendritic and somatic spiking) and thus affect synaptic integration[57] (**Fig. 1c**). Indeed, local depolarization of the distal dendrites of human layer 5 pyramidal neurons was shown to provide limited excitation to the soma, compared to rat pyramidal neurons[53]. This increased compartmentalization was accompanied by reduced inducibility

of dendritic spikes **[G]**, which could result from decreased ion channel densities. This is supported by biophysical modeling: if it is assumed that there is no change in the expression levels of the ion channels that propagate synaptic potentials or in the levels of voltage-gated ion channels, then their density per unit membrane surface will decrease as dendritic arbor size increases[53]. However, even for human DL pyramidal neurons, there seems to be considerable variability in the electrophysiological properties of different cell types. For example, a recent study showed that a subset of molecularly identified layer 5 pyramidal neurons in the human temporal cortex exhibit increased inducibility of dendritic spiking, compared to other layer 5 neurons [58]. Studies that aim to identify the electrophysiological properties that characterize human pyramidal neurons will therefore need not only to integrate their morphological variations but also to examine these properties in molecularly-defined subtypes, which remains challenging.

Another distinctive feature of some classes of human pyramidal neurons is a reduction in their intrinsic excitability compared with several other species, including primates [52,53], which could critically affect information processing and neural circuit plasticity. Another study showed that I_h channels (mediating hyperpolarization-activated cation currents) are expressed at higher levels in human UL pyramidal neurons than in mouse UL pyramidal neurons, conferring the human pyramidal neurons with distinct dendritic processing **[G]** properties [55]. Moreover, it has been shown that human UL pyramidal neurons display calcium-mediated dendritic spikes that are triggered only by selective classes of stimuli that drive both bottom-up (driven by sensory afferents) and top-down (driven by mixed selectivity feedback cortico-cortical projections) inputs [54]. More work is needed to determine whether these dendritic spikes are mediated by the molecular effectors that drive the dendritic calcium spikes observed in DL cortical and CA1 hippocampal pyramidal neurons in other mammalian species [57]. It will be also important to test, using both experimental and computational approaches, whether the putative increased functional compartmentalization of human UL pyramidal neurons alters their dendritic integration properties and the functional properties of cortical circuits [59-61]: this has been suggested by biophysical modeling, but remains unclear on the basis of rodent studies.

Finally, compared with rodents, human cortical neurons display distinctive synaptic features: some synapses between human pyramidal neurons and interneurons are remarkably strong and plastic [62-65] and synapses between human pyramidal neurons display properties enabling them to relay information during periods of particularly high frequency synchrony (which could change the modalities of information that can be transfered through cortico-cortical connections) [66]. These comparative studies should be carefully calibrated with recordings from non-human primates, but do suggest that human pyramidal neurons may have evolved unique input–output integration properties that could underlie enhanced information processing.

Where could the increased connectivity of human UL and DL pyramidal neurons originate from? Evidence suggests that it is likely to have arisen, at least in part, from the increased number of pyramidal neurons, as well as from the increase in total surface area and number of cortical areas

characterizing the human neocortex. Cortical circuits exhibit several canonical organizational principles (**Fig. 1b**)[67,68]. UL neurons transform and relay the sensory information that they receive locally to other UL pyramidal neurons (via recurrent excitation), to DL pyramidal neurons that provide feedforward projections to subcortical brain regions, and to UL pyramidal neurons in more distant associative cortical areas (via long range feedforward projections). In turn, the UL pyramidal neurons receive feedback inputs from local layer 5 pyramidal neurons and from long-range feedback cortical-cortical inputs (**Fig. 1c**). Thus, one emerging model suggests that the dendrites of human UL pyramidal neurons integrate and perform complex dendritic computations on the inputs they receive. By contrast, these computations are thought to be restricted to DL pyramidal neurons in rodents [54]. The increase in cortical-cortical connectivity in human cortical circuits, especially between areas such as the PFC and parietal cortex, could thus be a critical substrate for the evolution of higher cognitive functions in humans[69]. Importantly, the increased contribution of cortical-cortical connectivity might be linked to evolutionary changes in the patterns of cortical neurogenesis.

[H1] Evolution of neurogenesis mechanisms

What are the developmental mechanisms underlying human-specific features of cortical organization? Corticogenesis involves a highly complex developmental choreography, from the early steps of neurogenesis to the final stages of cortical circuit formation and refinement. While most of the underlying mechanisms are conserved in all mammals, some striking features have diverged significantly in non-human primates and humans.

[H2] Changes in neurogenesis

Neurogenesis determines how many and which subtypes of neurons are generated. Studies using the mouse as a model, together with human neuro-embryology, genetics and pluripotent stem cell (PSC)-based modelling (**Box 1**), have identified several features of cortical neurogenesis that underwent specific evolution in primates and humans (**Fig. 2**). As these were reviewed recently and extensively[70-73], they will only be summarized here.

Cortical neurogenesis starts with the expansion of neuroepithelial cells (NEC), which divide symmetrically without further differentiation. This step is thought to have a crucial influence on brain size by determining the initial size of the cortical neural progenitor pool [2]. NEC later convert into radial glial cells (RGC), which then start generating cortical neurons. Cortical neurogenesis from RGC typically occurs through asymmetric divisions that enable them to restore the progenitor pool while expanding neuronal production and leads to the formation of a ventricular zone, from which the cortical neurons migrate to form the cortical plate (**Fig. 2a-b**).

These key steps of neurogenesis are highly conserved among mammals, with the exception of one crucial point: timing. NEC amplification lasts for about one day in the mouse but up to two weeks in primates[72]. Similarly, cortical neurogenesis takes one week in the mouse, 2 months in the macaque and almost 4 months in the human (**Fig. 2a-d**)[70]. By allowing an increased number of NEC and RGC divisions to occur, this prolonged human neurogenesis is likely to constitute a key substrate of

cortical expansion. Moreover, as UL neurons are the last to be generated, the prolonged neurogenesis may favour their expansion. Further experimental testing of this idea is however needed and will require us to determine the mechanisms underlying the timing differences in human neurogenesis. Importantly, the timing of cortical neurogenesis in humans is largely conserved in human cortical cells in vitro[74-79], even when they are co-cultured with macaque cells or transplanted into the mouse brain[75,78], indicating that the underlying mechanisms are largely intrinsic to human cortical progenitors (see discussion below). The timing of other events important for neurogenesis is also extended in the human compared with the mouse, in a cell-intrinsic fashion. These include a longer cell cycle length, which could influence neurogenic fate specification[80,81] and a longer critical period during which cell fate remains plastic immediately after RGC division [82].

Another feature of primate and human cortical neurogenesis is the expansion of specific populations of progenitors located outside the ventricular zone, known collectively as basal progenitors. Some basal progenitors, called intermediate progenitor cells (IPC), are found in large numbers in the mouse embryonic cortex, but are increased in number in higher mammals (including primates), where they are thought to have contributed to cortical expansion [83]. Moreover, another population of basal progenitors, called outer radial glia cells (oRGC, also known as basal RGC), are barely present in the mouse but strikingly expanded in the human cortex (**Fig. 2**)[84-88]. oRGC have distinctive features that are critical to their contribution to human cortex expansion: they display remarkable self-renewing capacities (in vitro clonal experiments have revealed that single oRGC can generate hundreds of neurons[89]) and their expansion occurs at late stages of corticogenesis, coinciding with UL neuron generation. In the human cortex oRGC are thus thought to constitute the main progenitor source of origin for UL neurons[90] (**Fig. 2**).

While their relative contribution to cortical expansion remains to be determined, it can be hypothesized that the prolonged timeline of NEC amplification and neurogenesis, together with the late amplification of oRGC, synergize to increase cortical surface area (thus allowing more diversification of cortical areas to occur) and the generation of UL neurons (thus providing an ideal substrate for the expansion of cortico-cortical connectivity, **Fig. 2**). Indeed, in a recent study in which UL neuron generation was enhanced pharmacolgically in the mouse visual cortex[91], mice displaying a 20% increase in UL neuronal number showed enhanced functional correlations among UL neuron assemblies, more functionally clustered neuronal ensembles and increased perceptual discrimination.

[H2] Changes in neuronal differentiation

Following neurogenesis, neurons undergo several major cellular and molecular transitions, leading to the growth and patterning of axons and dendrites. This is followed by synapse formation and pruning, leading to the formation of functional neural circuits that are further refined by various plasticity mechanisms. While these steps are widely conserved, their developmental timeline is strikingly different across species, taking weeks in the mouse, months in the macaque and years in the human (**Fig. 2c, d**). The most prolonged steps of neuronal development in humans include

dendritic outgrowth, dendritic spine and synapse formation (taking months to several years) and synaptic pruning (taking up to two decades in the PFC)[92-99]. The resulting human brain neoteny (retention of juvenile features in a mature organism) is one of the most specific and functionally relevant features of human brain development and is likely to underlie the prolonged periods of motor, sensory and cognitive development that characterize the human species. Morever it could lead to longer critical periods (stages of development during which experience-dependent plasticity, thought to be crucial for the acquisition of higher cognitive features, takes place)[2,100]. Disrupting this timeline of cortical development could lead to brain pathology: for instance, accelerated brain development has been associated with autism spectrum disorders (ASD)[101,102]. Notably, one study has reported prolonged cortical neuronal development in the chimpanzee, suggesting that neotenic features of cortical development might be graded among great apes[103].

An intriguing aspect of human brain neoteny is that it appears to be cell-type dependent, with UL and PFC neurons being the most neotenic[95,104]. The resulting differences in the rate of maturation between cortical layers and areas could have a significant impact on cortical circuit development[105,106]. Moreover the differentiation of some cortical interneurons is even more prolonged: human interneurons continue to migrate and integrate during the first postnatal months in the PFC[107], and other forebrain regions like the amygdala may contain immature neurons for years postnatally[108]. Finally, non-neuronal cells, most strikingly oligodendrocytes, also display protracted development in humans[109]. leading to heterochrony **[G]** of myelination that could also be important for the timing of human brain circuit assembly[110].

What are the mechanisms underlying human brain neoteny? Important hints have come from xenotransplantation **[G]** experiments. Mouse pyramidal neurons transplanted in the neonatal mouse brain develop along their physiological timeline[76], whereas human cortical pyramidal neurons transplanted in the mouse cortex take up to 6-11 months to mature [75,111] (**Fig. 2e-g**). Interestingly, one study showed that, while transplanted human neurons at 9 months display electrophysiological features that are similar to those of adult neurons, they still display less mature patterns of morphogenesis, consistent with the fact that human cortical neurons take up to several years to reach full maturity [92-99]. An even more prolonged development is observed for transplanted human cortical interneurons[112,113]. Remarkably, despite this protracted timeline of development, human transplanted pyramidal neurons integrate functionally into the host cortical circuits and display physiological responses to sensory stimuli (such as visual tuning): thus, they are not stalled in the unusual host environment, but rather develop physiologically at the pace characterizing the human brain in vivo[111]. Human xenotransplanted neurons develop over months even when transplanted as single neurons into mouse cortical tissue to allow optimal cellular and synaptic integration[111] (**Fig. 2e, f**) or when faster maturation is induced by molecular reprogramming[114], and chimpanzee PSC-derived transplanted cortical neurons develop at a faster pace than human neurons[115] (**Fig. 2g**). Similarly expanded timelines of human cortical neuronal maturation are also observed using in vitro systems compared with non-human primate neurons[78,114]. Overall these data suggest that the

mechanisms underlying neoteny of cortical neuron maturation are largely cell-intrinsic (see further discussion below).

Another striking feature of primate and human cortical neuron development is the expansion of transient populations of neurons that are only present and functional during development. These include Cajal-Retzius neurons found at the surface of the cortex and subplate neurons located below the cortical plate, both of which play a crucial roles in the assembly of mature cortical circuits[116].

[H1] Genetic substrates of cortical evolution

Three main types of genetic changes are thought to lead to alterations in gene expression and/or protein function relevant to evolution (**Fig. 3a**): base-pair substitutions in non-coding enhancer **[G]** and promoter **[G]** regions that can alter gene regulatory networks **[G]**; non-synonymous substitutions **[G]** in coding regions of genes leading to amino-acid changes that result in modified protein function (**Fig. 3b**); and the emergence of new genes, mostly through large segmental duplications (**Fig. 3c**) [117,118]. All three mechanisms have been involved in the emergence of human-specific traits of brain development and circuit function.

[H2] Gene regulatory changes

Functionally relevant evolutionary changes in the genome are often linked to gene regulatory mechanisms[119]. Identifying human or primate genomic evolutionary changes, and linking them to gene regulation during human brain development, has proved a huge challenge because of the high degree of conservation of gene regulatory programs during mammalian neural development[120]. However, many human-specific cis-regulatory elements **[G]** (CREs, or *Cis* mechanisms), and upstream transcriptional regulators (*Trans* mechanisms), including long-range enhancers, have been identified that are uniquely regulated during human corticogenesis, leading potentially to human-specific control of gene expression (**Fig. 3, Table 1**).

***[H3] Cis* regulatory mechanisms**

Comparative genomics identified human accelerated regions (HARs): DNA sequences that are very strongly conserved in mammals or vertebrates, but display sequence changes in the human genome only[121,122]. Mouse transgenics[123] suggests that most HARs correspond to regulatory elements, although some (such as HAR1) are transcribed to produce non-coding long RNAs expressed in developing neurons in the fetal and adult cortex[121]. HARs are enriched in the vicinity of genes involved in neural and/or cortical development, suggesting their importance for gene regulation during corticogenesis[18,124-129]. Supporting this, the enhancer activity of more than 3000 HARs was recently tested in vitro by massively parallel reporter assays (MPRA) **[G]** [130,131,132]. More than half of the tested HARs displayed enhancer activity in neural progenitor cells and this activity was, in most cases, either increased or decreased in human HARs, when compared to their ancestor sequences. When tested in mouse and human cells, most HARs displayed similar levels of activity, further pointing to *Cis*-variation as the main driver of HAR evolutionary changes[130,131]. Most HARs were mapped to genomic loci that are transcriptionally active in cortical progenitors or postmitotic neurons

during fetal development, further pointing to their prominent regulatory role during all stages of corticogenesis.

The HAR5 element was the first of these elements to be functionally explored in the context of cortical neurogenesis (**Fig. 3a, Table 1**). HAR5 lies upstream of the *FZD8* gene, which encodes a receptor for WNT proteins (the mediators of a pathway that controls the balance between self-renewal and differentiation in cortical progenitors[133,134]). Using a mouse transgenic model in which the *Fzd8* coding sequence was put under the control of HAR5 variants, human HAR5 was shown to enhance the expression of *Fzd8* and the proliferation of RGC (resulting in increased cortical size), while chimpanzee HAR5 did not have these effects[135]. Several other genes, including *PPP1R17*, which encodes a cell cycle regulator, are similarly controlled by HAR elements that have a selective influence on their expression in human cortical progenitors[130].

Beyond neurogenesis, the involvement of HARs in cortical neuron development and maturation is supported by human genetic studies[124]. Sequence analysis of HARs revealed that these elements are often highly invariant in the human population, suggesting the functional importance of their ultra-conserved structure. Moreover several rare HAR variants are found in individuals affected by neurodevelopmental disorders[124]. Some of these pathogenic HAR variants were studied in the mouse, revealing their impact on the expression patterns of genes required for cortical neuron development. Consistent with the potential role of HARs in human cortical circuit evolution, one class of HAR-linked genes is also preferentially expressed in association cortical areas in the adult brain[128].

Overall, these data point to HARs as potentially important molecular effectors of human cortical evolution. Future studies should consider inserting the human version of these regions into the endogenous ancestral locus in the mouse or other non-human, in order to circumvent the pitfalls of the viral and transgenic assays (including MPRAs) that have been used so far to study these elements. Most importantly, new approaches should be developed to determine the repertoire of *Trans* factors that bind to HAR elements and how it is affected by human-specific substitutions.

Comparative epigenetic profiling **[G]** of human, macaque and mouse at early stages of corticogenesis has identified another class of interesting CREs, called human gained enhancers (HGEs), that have gained activity in humans[136]. MPRAs revealed differential transactivation activity of a third of the tested HGEs in human versus chimpanzee neural stem cells[131]. Like HARs, HGEs are enriched in the vicinity of genes related to corticogenesis, including genes preferentially expressed in oRGC[137]. HGEs are also enriched in the vicinity of genes encoding proteins involved in the Notch pathway[138], which promotes clonal expansion and prolonged neurogenesis of human RGC and oRGC[85,139,140]. These genes include *HEY2*, for which nearby HGE sequence variants were associated with changes in cortical surface area[141]. Similar comparative annotation of CREs in the adult brain also revealed a large number of elements that are more active in human than non-human primates, including a few that are not present in chimpanzee[142]. Some HGEs even display *Homo*

sapiens-specific changes in sequence that are not found in the genome of Neanderthals[143], suggesting a contribution to the most recent evolutionary changes in human corticogenesis.

The three-dimensional nuclear organization of DNA and chromatin structures associated with complex gene regulation — such as topology associated domains (TADs) **[G]** and chromatin loops **[G]** [144] — has also been shown to have human-specific features during corticogenesis[138,145]. For example, hundreds of (TADs) and chromatin loops are present in the human compared with macaque and mouse[145]. The boundaries of TADs and anchors of the loops were found to often be enriched for HARs and other enhancers that are specifically active in human cortical cells. Among these, one enhancer was found that interacts with the promoter of *EPHA7*, which encodes an axon guidance factor involved in corticogenesis[146]. Acute disruption of this enhancer in human neurons *in vitro* led to decreased *EPHA7* expression and increased dendritic outgrowth[145].

Finally, in addition to the point mutation mechanisms depicted above, the evolutionary loss or gain of entire CREs has also been found in the human genome, compared with non-human hominids, and in some cases could be linked to changes in the regulation of associated genes during cortical development [147,148].

There is thus a rich repertoire of CREs that regulate gene expression during corticogenesis by influencing transcription factor binding and/or chromatin structure, pointing to a need to study their biological impact on human corticogenesis and their relation to human evolution. An innovative approach was recently described in which PSC derived from different species were fused[149,150]. Fused chimpanzee-human induced pluripotent stem (iPS) cells were differentiated into cortical organoids**[G]**, followed by transcriptome profiling, leading to the identification of genes uniquely upregulated in human astrocytes[149]. The use of composite cell lines of different species, associated with a direct comparison with contributor cells of origin, will constitute a promising tool to study the relative contribution of *trans* versus *cis*-mechanisms of control of gene expression during corticogenesis.

[H3] Trans mechanisms and transcriptomics

In parallel with these studies, comparative transcriptomics of the developing cortex in mouse, human and other primates, as well as corresponding PSC-derived cortical organoid models, has examined human-specific patterns of gene expression [79,151-153]. While these studies revealed overall highly conserved patterns of gene expression, they suggest an 'hourglass' pattern of species-specific differences in gene expression, with greater divergence at embryonic to mid-fetal stages, and to a lesser extent during adolescence / young adulthood, and less divergence during late fetal stages[151-153].

These analyses also revealed a consistent protracted pattern of 'immature' gene expression in the human, consistent with the neotenic nature of neurogenesis and neuronal differentiation[77,79]. While the mechanisms upstream of cortical transcriptional heterochrony in human remain unclear, some

leads have started to emerge. Transcriptome profiling of human and non-human hominid cortical organoids revealed a delayed expression of the ZEB2 transcription factor in the human organoids, which was linked functionally to a delayed transition from NEC to RGC (and thereby potentially to increased size of the initial progenitor pool)[74]. Comparative transcriptomics in the human versus non human primate cortex also revealed quantitative differences in genes involved in signaling pathways functionally relevant to neurogenesis. These include the PDGF pathway, which was upregulated in the primate cortex and promotes RGC proliferation in human (but not in mouse) fetal cortex[154], and the mTOR pathway, which was selectively upregulated in human oRGC and controls their morphology and migration properties[155,156]. A final interesting case is the KRAB-zinc finger protein family, which are the largest group of transcription factors in mammalian genomes and are considerably amplified in primate and human genomes[157]. While these transcription factors are mostly involved in transposable element repression, they also control the expression of endogenous genes, particularly during neural development[158,159]. For instance, the KRAB-zinc finger protein, ZNF558, is expressed in human and not chimpanzee neural progenitors and its loss of function in neural organoids leads to a dysregulation of gene expression that is compatible with the more precocious neurogenesis observed in non-human primates [158].

Finally, beyond transcription factor-related mechanisms, several miRNAs {Arcila, 2014 #373;Nowakowski, 2018 #374}, and primate and/or hominid-specific long non-coding RNAs **[G]** [160-162] are expressed specifically during primate or human corticogenesis, some of which have been linked to the regulation of cortical progenitor proliferation and self-renewal.

[H3] Divergent gene regulatory mechanisms in human cortical neurons and glia

It has been more challenging to explore conserved and divergent transcriptomic patterns during later stages of human cortical neuron and circuit development than in the early stages of corticogenesis. Neurons are typically harder to profile than progenitors using single cell transcriptomics and the postnatal stages of human development (which are the most relevant to neuronal differentiation and circuit formation) are among the least accessible for molecular or cellular analyses. Nevertheless, recent studies have started successfully to link divergent transcriptional regulation to cortical neuron connectivity (**Fig. 3, Table1**). A striking example is provided by cerebellin-2 (*CBLN2*), a gene that regulates synaptogenesis in many brain areas, which was found to be more broadly expressed in the primate PFC than in the mouse PFC[163]. This upregulation was linked to the loss of a regulatory binding site for the transcription factor SOX5, a key repressor during corticogenesis, and this was found to directly impact the development of neuronal connectivity in the mouse PFC[163]. Moreover, retinoic acid signaling was found to regulate the expression of *CBLN2*, and was shown to be increased and expanded in the primate PFC at early stages and drive to PFC expansion in mouse[30] and human cortical organoids[164]. Together with scRNAseq efforts that have started to uncover the developmental dynamics of gene expression across the human fetal cortex[165], these data provide important hints about the species-specific transcriptional mechanisms that might participate in the development of connectivity in particular cortical areas.

Another interesting example linked to the patterning of connectivity is provided by the species-specific regulation of the plexin A1 guidance receptor gene, *PLXNA1* [166] (**Fig. 3, Table 1**). In primates, but not rodents, motor area-derived corticospinal pyramidal neurons make abundant synapses with motor neurons, which might underlie increased dexterity. Juvenile mouse corticospinal neurons also develop these connections but they are pruned at adult stages, in large part through the action of semaphorin repellent guidance cues [166]. The sempahorin receptor plexin A1 was found to be strongly expressed in corticospinal neurons in the mouse, but not human motor cortex, and the lack of expression of *PLXNA1* in the human cortex was linked to primate-specific changes in upstream enhancer regions that confer binding to the FEZF2 transcriptional repressor[166].

Following circuit formation, neurons undergo synaptic plasticity in response to changes in activity. The specific gene regulatory programmes involved in this plasticity[167] were shown to display significant differences between human and non-human neurons[168-170]. For example, *OSTN* encodes a well-conserved secreted protein that is expressed in muscle and bone in the mouse and human, but only expressed in the brain in humans and macaques [168]. *OSTN* expression is induced in an activity-dependent manner in layer 4 of macaque primary visual cortex [168]. Loss of function of *OSTN* in human neurons in vitro increased dendritic outgrowth, suggesting that it acts as a negative modulator of activity-dependent dendritic branching (although more functional exploration is needed to determine its biological functions in vivo). The activity-dependent expression of *OSTN* in human neurons could be linked to the presence, in primates but not in other mammals, of binding sites for MEF2 transcription factors[168]. Interestingly, MEF2A (and downstream genes) was previously identified as displaying a 'neotenic' pattern of expression in the human cortex [171]. Together with the identification of HARs upstream of MEF2C that are mutated in specific neurodevelopmental disorders[124], this indicates that the MEF2 family and its downstream regulatory network are an attractive target for the evolution of cortical neuronal development programs.

Human-specific gene regulation has also been identified in interneurons and glial cells. For example, a recent study performed in human PSC derived cortical interneurons identified human- or primate-specific regulatory sequences responsive to neuronal activity[172] that are enriched for genes associated with neurodevelopmental or psychiatric disorders including the ASD-linked genes *SHANK3* and *FMR1*. Another interesting example involves secretagogin (SCGN), a calcium-binding protein that is strongly expressed in a subset of interneurons in primates but not in mice[173]. Forced expression of SCGN in mouse interneurons led to increased dendritic complexity, suggesting that SCGN functions in the development of a subtype of primate cortical interneurons. Finally, a recent study documented hominin-specific changes in regulatory elements affecting gene expression in oligodendrocytes that are dysregulated in the brain of individuals with ASD [174]. However, more work is required to test their cellular functions and determine how they might impact circuit development and/or function.

[H2] Protein coding changes

Genome sequencing of many mammalian species has uncovered human-specific amino acid substitutions in approximately 100 genomic loci with a size of approximately 100kB, representing thousands of protein coding genes [175,176]. Using stringent criteria, non-synonymous substitutions that show strong positive selection in human exons, compared to 29 other mammals (including chimpanzees), represent approximately 15,000 sites in around 4,400 proteins[177]. Surprisingly, however, the functional impact of these human-specific substitutions on human brain evolution has been explored for only very few of them.

Notable exceptions are the human-specific amino acid substitutions affecting the transcription factor FOXP2. *FOXP2* is mutated in individuals with a monogenic speech disorder[178] and human *FOXP2* contains two amino acid substitutions that are not found in chimpanzee[179]. A mouse transgenic model that displays these two substitutions in mouse *FoxP2* exhibited changes in dopamine concentrations in multiple brains regions (including the striatum and frontal cortex), decreased exploratory behaviors and altered ultrasonic vocalizations and increased neurite outgrowth and synaptic plasticity of striatal projection neurons[179] (**Table 1**). These results linked human-specific changes in the FOXP2 amino-acid sequence with alterations in cortico-basal ganglia circuits that might have played a role in the evolution of speech production[179]. Future experiments should explore whether human-specific coding substitutions in other genes are relevant to the emergence of human-specific traits.

Of particular interest for future exploration are about 100 proteins that display amino acid substitutions that are specific to humans when compared with closer hominins Neanderthals and Denisovans[180]. Some of these proteins (namely, CASC5, KIF18A and SPAG5) are expressed in neural progenitors, while others (SLITRK1 and LRTM2) are involved in synaptogenesis, making them potentially relevant for evolutionary changes in several aspects of human corticogenesis.

[H2] New genes

An important source of evolutionary innovation can be provided by new genes, which typically emerge as a result of large segmental genomic duplications **[G]** or retrotransposition **[G]** [118] (**Fig. 3, Table 1**). Some human-specific genes resulting from retrotransposition are expressed during corticogenesis[181], but recent evidence indicates that species-specific large segmental gene duplications represent an important source of genomic innovation during human evolution. The human genome contains approximately 30 gene families that show human-specific duplication patterns[182,183]. For most, both the ancestral copy of these genes and their human-specific paralogs are expressed throughout human fetal corticogenesis[140]. Since these genes are often located in hotsptots for genomic recombination, human-specific large segmental duplications can display variable degree of conservation in copy number in the human population, which can in turn be used to estimate selection pressure [184].

Several human-specific duplicated genes have been studied functionally during cortical neurogenesis, revealing their contributions to human cortical progenitor expansion and prolonged neurogenesis. The *NOTCH2NL* genes constitute a family of human-specific partial gene duplicates

of *NOTCH2*, which encodes a key receptor in the Notch signalling pathway. Three *NOTCH2NL* genes are present only in the human genome[139,140] and are located in a genomic region that is associated with changes in brain size[185] (see below). Experiments combining gain and loss of function of these genes in the mouse embryonic cortex and in human PSC models of corticogenesis have revealed that the *NOTCH2NL* genes are human-specific activators of the Notch pathway, driving RGC expansion, prolonged neurogenesis and increased cortical neuronal production[139,140,186]. Clinical human genetics further supports the importance of *NOTCH2NL* genes in human biology and disease (**Box 2**).

A surprisingly large number of human-specific gene duplications seem to have contributed to the evolutionary expansion of the cortical anlage (**Box 2**), including *ARHGAP11B*, *TBC1D3*, *TMEM14B* and *CROCCP2*, which all promote basal progenitor (including oRGC) expansion[187-192]. Among these CROCCP2 was found to act as a human-specific modifier of mTOR signaling[192], which is selectively upregulated in human oRGC[79].

Several human-specific gene duplicates are also selectively expressed in developing or mature postmitotic neurons, suggesting their involvement in cortical circuit assembly and function. For example, SLIT-ROBO Rho GTPase activating protein 2 (*SRGAP2*[193]) is expressed in postmitotic cortical pyramidal neurons, as well as other neuronal subtypes, throughout development and in the adult brain[194]. The ancestral gene, called *SRGAP2A,* encodes a multifunctional protein that is highly conserved among all mammals[195]. *SRGAP2A* has undergone a series of large-segmental duplications leading to two partial duplications (*SRGAP2B* and *SRGAP2C*) that contain only the first 9 exons of *SRGAP2A*[184,196]. *SRGAP2C* emerged approximately 2.4 million years ago (at the birth of the *Homo* lineage) and exhibits a highly conserved copy number among human populations, suggesting strong positive selection[184]. *SRGAP2C* encodes a truncated protein that can bind to and inhibit all known functions of SRGAP2A[196-198] (**Fig. 4a**).

Functional studies in vivo demonstrated that SRGAP2A is a postsynaptic protein that promotes the maturation of excitatory and inhibitory synapses in cortical pyramidal neurons and, at the same time, limits the total number of synapses formed by cortical layer 5 and layer 2/3 pyramidal neurons[196-198]. Induction of *SRGAP2C* expression in mouse layer 2/3 cortical pyramidal neurons phenocopies a partial loss of function of *SRGAP2A* and leads to a significant delay in synaptic maturation and an increase in the density of synapses (**Fig. 4a**), mimicking two important features of human pyramidal neurons[3]. Recent results demonstrate that transgenic expression of *SRGAP2C* in mouse layer 2/3 pyramidal neurons leads to a specific increase in the number of cortical-cortical connections between local excitatory pyramidal neurons and increased long-range cortical-cortical connections between cortical areas [199](**Fig. 4b-c**). In vivo imaging further revealed that layer 2/3 pyramidal neurons respond more reliably to sensory stimulations in the *SRGAP2C*-expressing mice (**Fig. 4d**) and that they also learn more efficiently a whisker-based sensory discrimination task, when compared to wild-type mice[199] (**Fig. 4d**). These results suggest that the emergence of *SRGAP2C* has contributed to

the evolution of some of the unique structural and functional features of cortical circuits in the human brain.

[H2] Beyond genetic mechanisms

The genetic mechanisms outlined above are likely to underlie many of the changes in developmental programmes that have driven human cortical evolution. However, emerging data suggest that more global changes in cellular processes could also link development to the evolution of the human brain. Human cortical neurons were shown to display much slower mitochondrial development and lower levels of oxidative metabolism than mouse neurons, and enhancing mitochondria function was demonstrated to speed up neuronal morphogenesis and synaptogenesis[200]. Similarly, recent studies have shown that rates of protein turnover are lower in human cells than in mouse cells [201,202], and that lower rates of translation can directly influence the timing of cortical neurogenesis [203,204]**.** Collectively, these data suggest that global cellular mechanisms could play an important role in the scaling and patterning of human brain developmental events, in synergy with gene regulation and signaling.

[H1] Conclusions and perspectives

In the past two decades, a flurry of studies have revealed human-specific genomic changes — ranging from HARs to human-specific gene duplications — that constitute candidate species-specific modifiers of human brain development. However only a small fraction of these have been explored functionally and often the function of the ancestral genes and/or regulatory sequences remain poorly understood. Moreover there remains a profound gap in our understanding of how the identified evolutionary changes in developmental programme are linked to the resulting divergence in the properties of human neural circuits.

To move forward, the field needs to develop new experimental tools (**Box 1**) allowing more high-thoughput and parallelized ways to probe the functions of these modifiers during brain development in animal and human models. This is especially true for human-specific modifiers that act relatively late (in postnatal development or even in adulthood) on key steps such as synapse development, which might impact circuit architecture and function: two aspects of human brain development that remain especially challenging to modify and study. The availability of PSC from humans, primates and other mammals, in combination with advances in genomic editing and emerging tools such as next generation organoids and xenotransplantation of human neurons, will provide new and exciting opportunities to move towards this goal. However, these should not overshadow the crucial need for animal models that will be essential to understand the mechanisms of corticogenesis in a genuine and robust in vivo context. These include invertebrates and mice, but also, importantly, higher mammals (such as ferrets) and primates (such as marmosets and macaque), in order to include a wide spectrum of phylogeny, body size and brain specializations (**Box 1**). In parallel, new approaches are needed to link the molecular features of cortical cells with circuit assembly and circuit properties, including high resolution comparative (multi-omic to connectomic) studies in animal models and the human brain.

Finally, it is striking to note that many species-specific gene variants are also the sites of pathogenic or polymorphic variants in the human population. This emphasizes the need to further integrate human genetics with evolutionary genomics to fully comprehend how human-specific genomic changes have made our brain functions uniquely expansive and at the same time susceptible to neurodevelopmental and neurodegenerative disorders.

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Competing interests

The authors declare no competing interests.

Table 1| Examples of human-specific genetic modifiers affecting gene expression and/or protein function.

Gene	Modification type	Species-specificity	Molecular effects	Phenotypic outcome	Reference
Gene regulatory changes					
FZD8	HAR5 (enhancer)	Human	Human HAR5 increased expression of *Fzd8* in mouse RGC	Increased RGC proliferation and increased cortical size	[135]
EPHA7	HSL	Human	Increased expression of EPHA7 in human subplate cortical neurons	Dendritic remodelling	[145]
OSTN	Gain of binding sites for the	Primate	Activity-dependent expression of	Activity-dependent dendritic remodelling	[168]

	transcription factor MEF2		OSTN in primate brain		
CBLN2	Loss of binding sites for transcription factor SOX5	Hominid	Increased expression of *CBLN2* in PFC neurons	Increased synapse formation and connectivity in the PFC	*163*
PLXNA1	Loss of binding sites for the transcription factor FEZF2	Primate	Decreased expression of *PLXNA1* in cortical neurons	Decreased axonal pruning of direct cortico-spinal projections	*166*
Protein coding changes					
FOXP2	Changes in the coding sequence	Human	Two non-synonymous amino-acid substitutions present in human FOXP2	Increased dendritic growth in human medium spiny striatal neurons.	*179*
New human-specific genes					
NOTCH2NLA/B/C	Human-specific gene duplication	Human	Increased Notch signalling in human cortical progenitors	Prolonged neurogenesis and increased cortical neuron number. CNVs in these genes associated with micro/macrocephaly	*139,140*
ARHGAP11B	Human-specific gene duplication	Human	Increased calcium signalling in mitochondria	Increased amplification of basal progenitors and increased cortical neuron number	*187,205*
CROCCP2	Human-specific gene duplication	Hominid	Decreased ciliary dynamics and increased mTOR signalling in	Increased amplification of basal progenitors and ncreased cortical neuron number	*192*

			cortical progenitors		
SRGAP2C	Human-specific gene duplication	Human	Inhibition of all functions of protein encoded by ancestral gene (postsynaptic SRGAP2A)	Neotenic synaptic maturation Increased cortico-cortical connectivity Changes in sensory coding Improved behavioral performance	*196,198,199*

CNVs, copy number variants; HAR5, human accelerated region 5; HSL, human-specific loop; PFC, prefrontal cortex; RGC, radial glial cells; See also Figure 3.

Fig. 1| Cortical circuit evolution. a, Schematic illustration of the expansion of cortical area size that has taken place in the human brain, when compared to the mouse (brains depicted approximately to scale). The expanded size of the human neocortex is accompanied by an increase in the number of cortical areas driven by the emergence of new cortical areas (such as Wernicke's area and Broca's areas). In addition there has been an increase in the size of certain cortical areas, including a pronounced expansion of the prefrontal cortex (PFC) compared to its putative homologous regions (PFC-like regions) in the mouse. **b**, In mammals, cortical neurons are organized in 6 layers that are generated in an inside-first outside-last manner: early born neurons generate deep layer (DL, layers 5/6) pyramidal neurons (PNs) (blue), then thalamo-recipient layer 4 neurons (green) and finally upper layer (UL, layers 2/3) PNs (shades of red). DL neurons project mostly to sub-cortical targets such as the dorsal thalamus (from layer 6) and the striatum, spinal cord and other sub-cortical targets (from layer 5). UL layer PNs project mostly locally to layer 5 and to other cortical areas (via feedforward cortico-cortical projections). Layer 2/3 UL neurons receive inputs from long-range feedback projections from other cortical areas in layer 1. The human cortex is characterized by an increased number of feedforward and feedback cortico-cortical connections (indicated by thicker lines in the right panel) [69]. **c**, Layer 2/3 UL pyramidal neurons in the human cortex are larger, more complex (increased branching) and have a a longer apical dendrite than those present in mice and other mammals (including other non-human primates)[45-48]. It has been proposed that the longer apical dendrite of UL in human cortex leads to increased dendritic compartmentalization because the the apical tuft is located further away from the soma, although the evidence for this remains controversial (indicated by the ?)[52-55,58]. Human UL PNs also receive more excitatory synapses and inhibitory synapses compared to other mammals[3,50].

Fig. 2| Species-specific features of human cortical development. a, Mouse cortical neurogenesis lasts approximately a week[206,207]. Following amplification of neuroepithelial cells (NEC), radial glial neural progenitors (also known as radial glial cells (RGC) in the ventricular zone (VZ) divide symmetrically to expand their pool (step 1) or divide asymmetrically to generate neurons (step 2). Following the migration of the neurons along the radial glia scaffold, this generates first the

deep layer (DL) neurons destined to reside in layers 5/6 and to project sub-cortically. In later steps of neurogenesis, mainly through the generation of intermediate progenitor cells (IPC) in the subventricular zone (SVZ), additional DL neurons (step 3) and the upper layer (UL) neurons destined to reside in layers 2/3 (step 4) and to form cortico-cortical projections are produced. A specialized type of radial glial progenitors called outer radial glia (oRG), which lose their apical attachment at the ventricular surface but keep their basal endfeet at the pial surface, are found in mouse cortex but are extremely rare. **b**, In human cortex, neurogenesis lasts for approximately 4 months, with a more prolonged period of neuronal generation [72]. oRGC are found in increased numbers in non-human primates, and in particular in the human cortex, contributing to the increased generation of layer 2/3 UL neurons in these species[85]. **c-d**, Comparison of the timeframe of the sequential events that characterize mouse and human corticogenesis [72]. In the human cortex all of the developmental events shown — including neurogenesis, gliogenesis (formation of astrocytes), synaptogenesis and the myelination of axons by oligodendrocytes — are highly neotenic[72]. In human cortex, synaptogenesis (which includes synapse formation and pruning) is not complete until apprixmately 15 years after birth. **e-f**, In xenotransplantation experiments, cortical neurons derived from pluripotent stem cells (PSCs) of various species are transplanted into the neonatal cortex of immunodeficient mice, followed by their analysis in the months following transplantation (**e**). These studies have revealed the intrinsically slow and neotenic features of human induced pluripotent stem (iPS)-cell or embryonic stem (ES)-cell derived pyramidal neurons, compared to those derived from ape or mouse stem cells. When mouse or ape PSC-derived cortical pyramidal neurons are transplanted into the mouse neonatal cortex, they develop mature morphological features in about one month thus following the timeline of mouse cortical neurons [76]. However, when human PSC-derived cortical pyramidal neurons are xenotransplanted into mouse cortex, their differentiation takes place over more than 6-9 months (**f**) mimicking the protracted maturation of cortical neurons in the developing human cortex [75,111]. The schematic chart in panel **g**, illustrates the timeline of dendrite and synapse maturation observed in vivo for the indicated species compared to xenotransplan[111]tated cortical neurons from the corresponding species into mouse cortex[111]. Results from these xenotransplantation experiments indicate that transplanted neurons from each species differentiate at a similar pace to their in vivo equivalents, suggesting that the mechanisms controlling the species-specific timing of development are largely intrinsic to the neurons. CP: cortical plate; E : embryonic day ; GW : gestational week; IZ: intermediate zone; MZ: marginal zone ; ISVZ/OSVZ ; inner and outer subventricular zone. The mouse transplanted neuron image in panel **f** is adapted with permission from [76]. The human transplanted neuron images in panel **f** are adapted, with permission, from [75]. The mouse and human dendritic spine images in panel **f** are adapted, with permission from [111].

Fig. 3| Genetic modifiers of human brain evolution. a, Human-specific base-pair substitutions are often found in regulatory regions (enhancers and promoters), where they can alter spatio-temporal patterns of gene expression. **b**, Another class of human-specific genetic modifiers are non-synonymous base-pair substitutions in exons that result in changes in amino-acid composition in the corresponding protein coding region specifically in the human genome. **c**, Human-specific gene

duplications can lead to the production of new gene paralogs. These duplications can lead to a new (A') nearly identical gene copy that increases gene dosage (redundancy), cause the copied gene to lose its function, becoming a pseudogene (because of a loss in regulatory sequences or transcription start site), or produce a gene that has acquired a new function through truncation or fusion with other coding sequences (neo-functionalization). As illustrated in **Table 1**, a number of genetic modifiers in each of these classes have been shown experimentally to result in alterations in cellular functions in the developing or adult brain.

Fig. 4| Example of a human-specific modifier of cortical development and function

a-b, SRGAP2 (known as SRGAP2A in humans) is a postsynaptic protein that contains three functional domains. SRGAP2 is located at both excitatory and inhibitory synapses in mammalian cortical pyramidal neurons, where it promotes the maturation of the synapses while limiting their density[196.] A human-specific truncated paralog of this protein, SRGAP2C, binds to and inhibits all known functions of SRGAP2A, leading to neotenic synaptic development and increased synapse density (as shown on the right side of the figure) when expressed in mouse pyramidal neurons[196,198]. **c**, The introduction of *SRGAP2C* into mouse layer 2/3 pyramidal neurons drives an increase in the number of excitatory synapses as a result of a specific increase in cortico-cortical (CC) synaptic connections from both feedforward and feedback projections (shown in blue, with increased connections indicated by a thicker line) but not from subcortical inputs (SC, shown in red)[199]. **d**, The changes in circuit architecture induced in mice transgenically expressing *SRGAP2C* in all cortical pyramidal neurons lead to increased reliability of sensory coding, illustrated here as the fraction of action potentials that are induced during sensory stimulations (shown in grey) in layer 2/3 pyramidal neurons. Mice expressing *SRGAP2C* also show improved learning, compared to wild-type (WT) littermates, in a whisker-based sensory discrimination task[199].

Box 1. New experimental paradigms to study human brain development and evolution

The study of human corticogenesis has entered an new era, enabled by the development of new tools and experimental models. Each of these tools and models (illustrated in the figure) has both advantages and limitations, but their successful combination has led to novel insights into mechanisms underlying human-specific features of brain development.

The simplest in vitro models of corticogenesis that have been developed are adherent two-dimensional (2D) cultures of cortical progenitors and neurons, generated from pluripotent stem cells (PSCs; either embryonic stem (ES) cells or induced pluripotent stem cells (iPS cells)) derived from humans, macaques or chimpanzees. Despite their simplicity, these models enable one to faithfully recapitulate many aspects of cortical neurogenesis, including the sequential generation of cortical pyramidal neurons with identities corresponding to those present in the six layers of the human brain [75,76,208]. These models are particularly well suited for high throughput genetic or chemical screening or for single cell experiments, such as those involving live imaging or clonal analyses. However they generate outer radial glia cells and upper layer neurons in a much lower proportion than occurs in vivo and do not allow us to study key cytoarchitecture features such as the ventricular zone (VZ),

outer subventricular zone and cortical plate [75]. Three dimensional (3D) cultures of cortical cells, also known as neural or cortical organoids, leverage the remarkable self-organizing properties of neural cells to recapitulate some of the key aspects of the in vivo spatial organization of cortical progenitors and neurons, including a highly patterned VZ-like structure. However, the generation of properly patterned neuronal layers remains difficult to obtain robustly [209-211]. Organoids are also amenable to functional screening, at least at early stages, and can include assemblies of excitatory and inhibitory neuronal populations (assembloids) that reflect some aspects of cortical circuits [212]. Neural organoids have been maintained in culture for long periods (>1 year); however, it remains challenging to keep differentiated neurons healthy in these relatively large structures and in vitro culture can lead to metabolic stress[213]. PSC-derived (or fetal cortex-derived) cortical cells grown in 2D culture or as organoids can also be studied in vivo thanks to xenotransplantation in the neonatal mouse brain. This enables us to follow neuronal development for months-long periods without metabolic stress and allows the cells to develop into functional neurons that can display higher order properties, such as robust synaptic plasticity and even physiologically tuned responses to sensory stimuli[111]. Xenotransplantation experiments are thus ideally suited to the study of higher order properties of human neurons in vivo; however, they remain a low-throughput method that is time-consuming compared with in vitro approaches.

The mouse is the main in vivo tool for the study of cortical development, because of its associated genetic and embryology toolbox (including transgenesis and in utero electroporation) that can be used to study in vivo the effects of human genes or regulatory elements. One major caveat of this approach, however, is that the genes are studied in a cellular context that may lack key species-specific features. Other mammalian models (such as the ferrret, marmoset or macaque) are much less versatile than the mouse but enable us to study human-specific genes in a context that is closer to the human. In addition, non-mammalian models (ranging from invertebrates such as drosophila to vertebrates such as reptiles, fish and birds) are crucial for the identification of new molecular, cellular and developmental mechanisms relevant for human brain evolution.

Access to ex vivo samples of fetal and adult human cortex and the ability to perform not only molecular but also cellular and physiological experiments on these preparations provides opportunities to study the human cortex, despite the scarcity of the available material. Finally, human genetics and comparative genomics enable the identification of candidate genes and genomic elements linked to cortical development and evolution as well as mutations leading to neurodevelopmental disorders [214]. These candidate genomic elements can then be tested, alone or in combination, for their cellular and molecular impact in cortical development and function, using a combination of in vivo animal models, in vitro human cellular models or xenotransplantation.

Box 2. Human cortex evolution and disease.

Many neurological and psychiatric diseases alter cognitive and social brain functions that are particularly enhanced in humans, while the human brain seems particularly sensitive to specific neurological conditions. Moreover, many of these diseases appear to have a developmental origin,

suggesting the fascinating possibility that their pathogenic mechanisms involve human-specific genomic and developmental mechanisms.

Some neurodevelopmental disorders appear to be directly caused by genetic disruption of human-specific genes or mechanisms. These include mutations in human accelerated regions (HARs)[124] and also mutations in human-specific gene duplicates, many of which are found within loci that are the locations of pathogenic copy number variants (CNVs)[215]. Among these, the 1q21.1 locus is enriched for many human-specific genes, including the *NOTCH2NL* human-specific paralogs. This suggests that the phenotypic expression of neurodevelopmental disorders arising from mutations in this region (such as 1q21.1 deletion) might be, in part, human-specific. Microdeletions in the 1q21.1 locus are associated with microcephaly and schizophrenia, while microduplication of this region is associated with macrocephaly and autism spectrum disorders (ASD)[216]. Notably, some of the CNV breakpoints within the 1q21.1 locus are provided by the *NOTCH2NL* genes, which can then be deleted or duplicated[139,217]. *NOTCH2NL* gene increased expression and copy number amplification have also been associated with increased proliferative properties of gliobastoma cells[218], while mutations in these genes were found in cases of neurodegeneration[219,220].

Conversely, some well-conserved disease genes may exert their pathogenic effects through human-specific mechanisms. For instance, the protein encoded by the causative gene in fragile X syndrome, FMRP, was found to bind to differents sets of mRNAs in the human compared to other species [221,222]. Among the human-specific targets of FMRP is *NOS1* and protein levels of NOS1 are affected by the mutation that causes fragile X syndrome in human but not mouse neurons[221,222].

It is also possible that human corticogenesis is more sensitive to specific diseases than that of other animals because the pathophysiological mechanisms underlying these disorders are rooted in developmental events that display human-specific features. These include the prolonged neotenic timeline of cortical development, which could be particularly relevant for ASD and schizophrenia (two neurodevelopmental disorders that affect critical periods of development and plasticity)[223]. At least some forms of ASD could be linked to accelerated neurogenesis or neuronal development. Synapse and dendritic spine formation are increased precociously in individuals with ASD[224], while early postnatal brain overgrowth is found in many forms of ASD[101,102]. Moreover, transcriptome analysis identified genes displaying delayed 'neotenic' expression in the human vs non-human primates, and showed that this pattern appears to be accelerated in some individuals with ASD[225]. Studies using pluripotent stem cell models suggest that there are temporal shifts in cells derived from individuals with ASD, with these cells displaying accelerated neuronal maturation (which could be primed in neural progenitor states[226]) and differentiation[225].

Finally, the prolonged development of human cortical circuits makes the human brain particularly sensitive to postnatal disruptions, whether of intrinsic or environmental origin[32]. For instance, schizophrenia has been long associated with excessive synapse pruning, leading to decreased connectivity at adolescent or young adult stages (when symptoms typically arise[227]), perhaps as a

result of the human neotenic pattern of synaptic pruning. On the other hand, even diseases occuring at much later stages, such as Alzheimer disease, could display human-specificity linked to neuronal development. For instance, human (but not mouse) cortical neurons are sensitive to amyloid plaque toxicity following transplantation in mouse models of Alzheimer disease[228].

Glossary terms

Association areas

Class of cortical areas defined by their opposition to primary areas (cortical regions receiving direct inputs from the dorsal thalamus). Association areas are where different sensory and/or motor modalities combine and where complex cognitive processes such as attention, planning and memories are encoded.

Cell fate mapping

Range of techniques aimed at genetically labelling the progeny of individual classes of progenitors thereby reconstructing the lineage linking dividing progenitors and all the cells they generate.

Chromatin loops

Describes the situation in which stretches of genomic sequence that lie on the same chromosome (configured in *cis*) are in closer physical proximity to each other than they are to intervening sequences.

Cis regulatory element

Proximal portion of the regulatory region of a gene containing the promoter and other regulatory elements controlling the transcription of a gene

Comparative genomics

Subfield of biology involving the analysis of DNA sequences divergence and conservation between different organisms.

Connectome

Description of all the synaptic connections between neurons found within a brain region or the entire nervous system of an organism.

Cortical organoids

Self-organized 3-dimensional multicellular structures that can be patterned to mimic the neocortex.

Dendritic processing

Describes the receipt, integration and and processing of many synaptic inputs by dendrites. This processing takes the form of changes in membrane potential which can - depending on the density and distribution of passive or active ionotropic channels - differentially affect the generation of action potentials at the level of the soma.

Dendritic spines

Micron long protrusions present in specific neuronal subtypes, such as cortical pyramidal neurons, at the tip of which is located an excitatory synapse. Spines play important roles in electrically and biochemically isolating the postsynaptic compartment from the dendrite shaft. Experimentally, morphologically identified dendritic spines represents a close approximation to measuring number or density of excitatory synapses received by a neuron.

Epigenetic profiling

Molecular biology technique combined with biochemistry to monitor the posttranslational modifications and physical interaction of DNA and chromatin and how they impact gene expression in cells.

Heterochrony

Changes in the relative timing of a developmental event when comparing different species.

Large segmental genomic duplications

Large (>1kB) segements of the genome that have been duplicated in another position in the genome.

Long non-coding RNAs (lncRNAs)

RNAs longer than 200bp that are not translated into protein.

Massively parallel reporter assays (MPRA)

A molecular biology technique used to simultaneously test the activity of multiple candidate genetic regulatory elements in a high throughput manner.

Morphogen

Class of extracellular cues that can act at distance from its source and regulate gene expression in receiving cells and tissues and thereby play a central role in cell type specification or tissue patterning.

Neurogenesis

The generation of postmitotic neurons by specialized classes of dividing progenitors.

Single cell RNA sequencing

High-throughput sequencing technique used to determine the sequences of messenger RNA expressed in single cells, also referred to as the transcriptional profile of single cells.

Topology associated domains (TADs)

Large (~1Mb) self-interacting genomic regions physically interacting inside the nucleus. This level of chromatin organization plays key roles in regulating temporal and spatial patterns of gene expression in gene families such as the Hox cluster.

Xenotransplantation

Transplantation of cells from one species into a different species.

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