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A molecular and cellular perspective on human brain evolution and tempo

Feline W. Lindhout[1 ✉], Fenna M. Krienen[2], Katherine S. Pollard[3,4,5] & Madeline A. Lancaster[1 ✉]

The evolution of the modern human brain was accompanied by distinct molecular and cellular specializations, which underpin our diverse cognitive abilities but also increase our susceptibility to neurological diseases. These features, some specific to humans and others shared with related species, manifest during different stages of brain development. In this multi-stage process, neural stem cells proliferate to produce a large and diverse progenitor pool, giving rise to excitatory or inhibitory neurons that integrate into circuits during further maturation. This process unfolds over varying time scales across species and has progressively become slower in the human lineage, with differences in tempo correlating with differences in brain size, cell number and diversity, and connectivity. Here we introduce the terms 'bradychrony' and 'tachychrony' to describe slowed and accelerated developmental tempos, respectively. We review how recent technical advances across disciplines, including advanced engineering of in vitro models, functional comparative genetics and high-throughput single-cell profiling, are leading to a deeper understanding of how specializations of the human brain arise during bradychronic neurodevelopment. Emerging insights point to a central role for genetics, gene-regulatory networks, cellular innovations and developmental tempo, which together contribute to the establishment of human specializations during various stages of neurodevelopment and at different points in evolution.

The age-old question of what sets the human brain apart from those of other species touches on diverse fields including evolutionary biology, anthropology, psychology and neuropathology. There is profound interest in understanding the distinct molecular and cellular structures of the human brain, as well as how these come about during neurodevelopment, in order to ultimately link these to our broad range of cognitive abilities. Many of these abilities have often been touted as being unique to our species; however, recent reinvestigations have revealed that other animals exhibit cognitive abilities that were previously considered to be human-specific, such as communication, theory of mind and self-awareness[1,2]. Similarly, animals have evolved characteristic cognitive abilities that humans lack—for example, long-distance navigational memory[3]. Thus, rather than a singular skill, it appears that the diversity and degree of cognitive abilities—for example, advanced language skills, complex problem solving and rich social and cultural societies[2,4,5]— is characteristic in humans, which also seems to make our species more prone to neuropsychiatric diseases[6,7].

Similar to our cognitive abilities, most of the genetic, cellular and structural features of the human brain are shared with our relatives[8–10], indicating that many aspects of human neurodevelopment and function can be extrapolated from other species. Indeed, important insights from a broad spectrum of model organisms have laid the foundation for our current understanding of human brain development and function. However, the human brain also exhibits distinct features at the functional, cellular and molecular levels that manifest during brain development. Thus, there is a keen interest in understanding exactly what these specializations are and how human neurodevelopment diverges to give rise to them. Investigating the neurobiological basis of human-specific specializations has long been challenging and has until recently relied on snapshot views of the human brain compared with model organisms, as well as limited in-depth molecular profiling. Technical advances in in vitro models, comparative genetics and single-cell profiling are now providing an unprecedented opportunity to explore human-specific features across a wide range of neurodevelopmental stages and at high molecular resolution[11–17]. Recent studies have begun to provide a deeper mechanistic understanding of the molecular and cellular innovations of the human brain, with a central role for genetics and gene-regulatory networks, as well as developmental tempo, one of the most notable developmental differences in human brain evolution. Although the field is still nascent, insights into specializations that arise at various points along the human lineage, from distant relatives such as rodents to our closest hominin ancestors, as well as a mechanistic understanding of how they come about during development, are emerging.

Distinct features of the human brain

The adult human brain exhibits certain features that are likely to be evolutionary adaptations (Box 1) that arose at different times during

[1]MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK. [2]Princeton Neuroscience Institute, Princeton University, Princeton, NJ, USA. [3]Gladstone Institutes, San Francisco, CA, USA. [4]Chan Zuckerberg Biohub, San Francisco, CA, USA. [5]Department of Epidemiology & Biostatistics, Institute for Computational Health Sciences, and Institute for Human Genetics, University of California San Francisco, San Francisco, CA, USA. ✉e-mail: flindhout@mrc-lmb.cam.ac.uk; madeline.lancaster@mrc-lmb.cam.ac.uk

Adaptations in the context of human brain evolution

Teasing apart which traits of human brain specializations and functions are adaptive and which are simply divergent characteristics can be difficult. Indeed, many traits that differ can arise as by-products of other unrelated processes or constraints, passenger effects and genetic drift, and many features may in fact be exaptations or co-opted traits (Box 2). Adaptation is a process that involves genetic change leading to a specific phenotypic outcome that provides a fitness advantage[238]. Certain traits show compelling evidence for adaptation, such as an effect on cognition that may be expected to increase fitness, and its emergence on a very rapid evolutionary time frame. These include overall brain size, neuron number and more elaborate connectivity. Functional studies are necessary to link genotype to phenotype and so even the most compelling cases for human adaptive traits have relied upon correlative evidence, but this is beginning to change.

evolution. These can be unique to humans (human-specific), or shared with other great apes (hominid-specific), primates (primate-specific) or mammals (mammal-specific) (Fig. 1a). One of the most distinct anatomical specializations of the human brain is its notable relative size (Fig. 1b,c). Although primates exhibit a high degree of encephalization (a metric for brain size relative to body mass), this feature is more pronounced in humans[18]. The human brain is about three times larger than those of our closest living relatives—chimpanzees, gorillas and bonobos[19]. A similar increase is observed when comparing modern humans to *Australopithecus*, an archaic ancestor of humans with a brain size of around 450 cm^3, based on endocranial volumes of endocast fossils[20]. However, brain size began to increase with the birth of the genus *Homo* two million years ago, where a slight increase in endocranial volume (to around 600 cm^3) is first visible, followed by a more marked increase to approximately 1,000 cm^3 in *Homo erectus* and finally the jump to 1,400–1,500 cm^3 in *Homo sapiens* and *Homo neanderthalensis*[21]. Thus, the evolutionary expansion of the human brain was a gradual one involving stepwise enlargement, suggesting multiple evolutionary events.

Expansion of brain regions
The evolutionary expansion of the brain was not entirely uniform, and specific regions, such as the neocortex, cerebellum and white matter structures, have undergone increased expansion (Fig. 1b,c). Although most of this increase aligns with expected allometric scaling patterns, a fraction remains that is unaccounted for[22]. This has sparked discussions of whether some regions expanded merely owing to an overall increase in brain size[23] or if they could also have evolved independently[24]. Within these debates regarding the target of selection, there is consensus that these brain regions, particularly the neocortex, are integral for our distinct cognitive abilities.

Indeed, the expansion and specialization of the neocortex has been a focal point of recent research owing to its link with complex cognitive functions and related pathologies. Compared with other primates, the human neocortex has undergone 50% more expansion relative to the rest of the brain[25], with an accompanying increase in gyri and sulci. Accordingly, following allometric scaling, humans have the largest number of cortical neurons compared to close and more distant relatives[26–28]. Compared to chimpanzees, humans display a reduced genetic heritability in cortical anatomy and size, particularly in the association cortices, suggesting greater environmental influences on

our brain development[29]. Parts of the association cortices, including the prefrontal, temporal and parietal lobes, show additional selective enlargement in humans[30]. Of these, the prefrontal cortex has attracted substantial attention, as this region in the frontal lobe is key for flexible integration of various functional modalities and is more susceptible to neuropsychiatric disorders, including schizophrenia, bipolar disorder and post-traumatic stress disorder[31–33]. Differences in the frontal lobe in modern humans have been suggested to have evolved more recently than initially thought, as a fossil study reported that this reorganization occurred around the time of the initial migrations of *Homo* from Africa[34]. Thus, the expansion and macrostructural remodelling of the neocortex, particularly the association cortices, have evolved along the human lineage, with a notable human-specific increase.

Another brain region that has shown relatively increased expansion in humans, yet remains relatively less explored, is the cerebellum[35–38] (Fig. 2b,d). Humans exhibit further selective enlargements of cerebellar regions connected to the frontal and parietal association cortices compared with chimpanzees[35–38]. This regional expansion is disproportionate when compared with the expansion of cerebellar cortical motor areas, which has led to the emerging hypothesis that the cerebellum, in addition to its well-known role in motor functions, may contribute to specialized cognitive functions in humans[37].

In parallel with the evolutionary expansion of cortical and cerebellar grey matter, which harbour somatodendritic structures and local axon projections, there is an even greater increase in white matter volume along the human lineage (Fig. 2b,c). This is mostly attributable to the long-range axon projections in the white matter, which must cover greater distances in larger brains. Indeed, the disproportionate increase in white matter volume in different mammalian species is largely explained by a universal power law, suggesting that animals from different lineages with large brains share global mechanisms that drive these changes[39]. Additionally, there is further regional expansion of white matter structures that is human-specific and could underlie human-specific differences in connectivity. For instance, cross-species analyses in humans, great apes and other primates show that the expansion of the prefrontal cortex is mostly explained by an increase in white matter structures rather than grey matter structures[40,41], indicating a human-specific increase in connectivity of the prefrontal cortex with other brain structures.

Cellular conservation and divergence
Despite large species-specific variations in the size of brains and certain brain regions, the overall tissue architecture is shared across mammals, indicating that the basic ontogenetic plan is developmentally and genetically conserved. Overall homology of neuronal and non-neuronal subtypes is also largely similar across mammals. The mammalian neocortex exhibits a six-layered cytoarchitecture, with pyramidal neurons, the predominant glutamatergic excitatory subtypes, categorized by their layer-specific localizations. These include infragranular neurons (layer V and VI), which largely project to subcortical regions, supragranular neurons (layer II and III), which mainly project to other cortical regions, and layer IV neurons that innervate local structures while receiving input from the thalamus and other regions (Figs. 2e and 3a). The cortex displays regional variations in overall thickness, as well as differences in layering and thickness of individual layers[42]. Granular cortical regions contain all six layers, whereas agranular areas lack layer IV[42]. The granular prefrontal cortex has received particular attention in the context of human brain evolution, as it emerged initially in early primates and further diversified in simians, and its evolutionary expansion occurred in concert with other association areas[43].

Although overall cortical cytoarchitecture is shared across mammals, the structure is expanded and more elaborate in primates, exhibiting thickened supragranular layers throughout the cortex compared with other mammals[44] (Fig. 1e). The expansion of supragranular layers extends to apes, with a further thickened cortical grey matter exhibiting
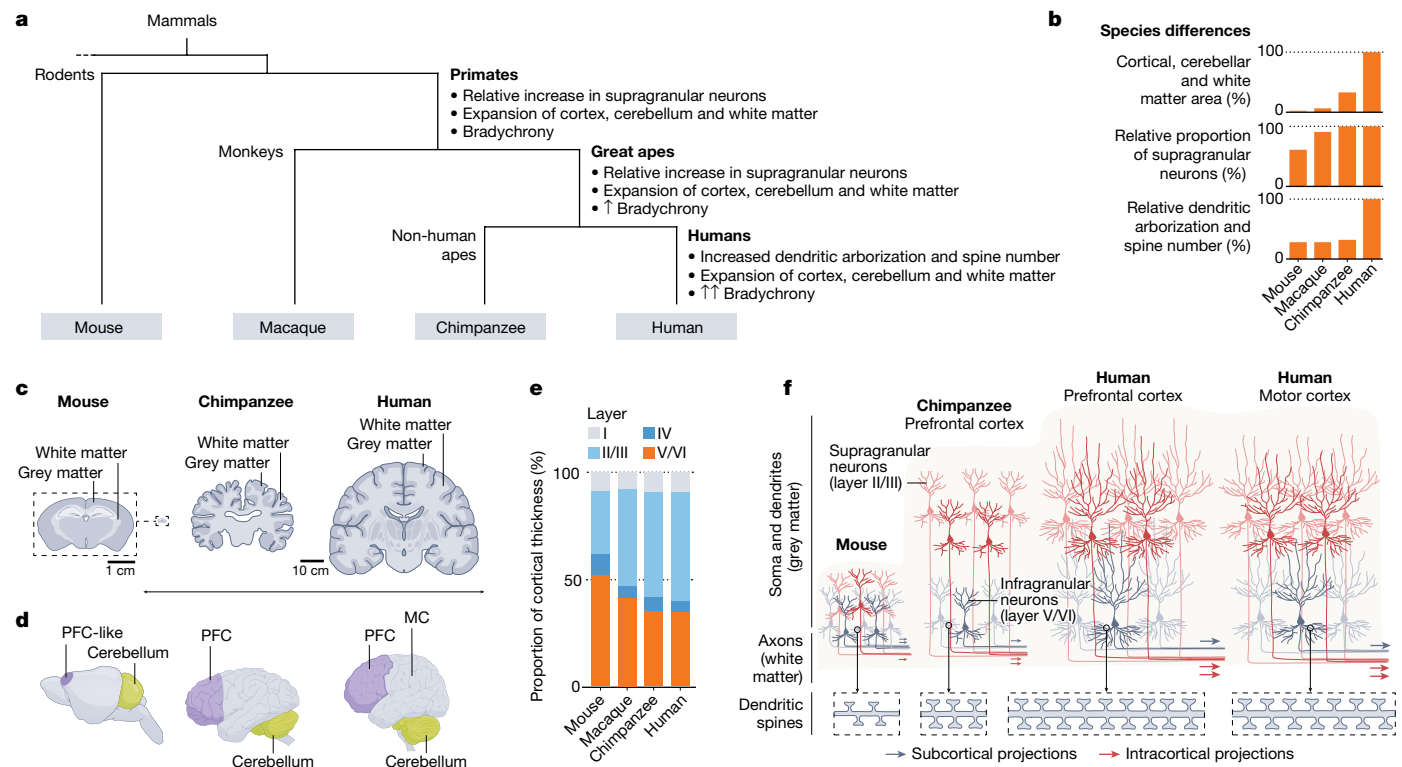
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Fig. 1 | A comparative overview of evolutionary specializations of the human brain. a, Phylogenetic tree of the human lineage, highlighting brain specializations that have evolved at each point (see **b**–**f** for details and references). **b**, Comparative analysis across species of specializations emerging at different times during human evolution, including the expansion of cortical, cerebellar and white matter structures[38,44,235] (top), the relative increase of supragranular neurons[44] (middle) and the increase in neuronal spine number and dendritic arborization[66,67,71,236] (bottom). **c**, Scaled representation of a coronal brain section of mouse[237], chimpanzee and human (modified with permission from Welker and Johnson Comparative Brain Collection and http://brainmuseum.org). White and grey matter regions are highlighted, indicating a relative increased evolutionary expansion of white matter. **d**, Schematic representation of the mouse, chimpanzee and human brain displayed at similar sizes, highlighting regions that show increased relative expansion during evolution. MC, motor cortex; PFC, prefrontal cortex. **e**, Cross-species analysis of the proportion of different cortical layers relative to the total cortical thickness[44]. **f**, Schematic representation of cortices of mouse, chimpanzee (prefrontal cortex) and human (prefrontal cortex and motor cortex), with a magnified representation of a dendritic shaft with spines. Illustrations display species-specific and regional differences in cortical thickness[44], relative proportion of supragranular and infragranular neurons[44], and dendritic arborization and spine number[66,67,71,236].

subdivision of layers II and III into as many as four sublayers in certain regions[45] that has remained unchanged in humans compared with other apes[44,46] (Fig. 1e). This expansion in cytoarchitecture is also reflected in the wider diversification of glutamatergic neuron types in humans compared to mice, which is observed at the morphological, biophysical and transcriptomic levels[47].

In mice, the cortical neuronal population is made up of approximately 15% GABAergic inhibitory neurons, a proportion that is largely invariant across neocortical areas[48,49]. In primates, however, these proportions vary by neocortical area and generally favour higher proportions of interneurons, reaching as much as 30–40%, particularly in higher-order association areas[48–51]. Ferrets and other non-rodent mammals also have higher proportions of interneurons than mice, suggesting that laboratory mice may be an outlier[51]. However, in the motor cortex humans exhibit an even larger number of interneurons than marmosets, suggesting that an additional change was acquired at some point in our more recent lineage[49]. Thus, these various neuronal populations have undergone substantial changes in their diversification at various points in human evolution.

The mammalian cerebellar cortex also follows a shared ontogenetic plan, comprising molecular, Purkinje cell and granule cell layers, with each layer containing specific neuronal subtypes including interneurons[52]. Although the cerebellar cortex has been described as a uniform sheet of invariant circuits, recent cellular profiling in mouse cerebellar cortex has revealed subtle regional and functional heterogeneity in each of these neuronal classes[53]. The developing human cerebellum shows features that are not seen in other species, including macaques, which in particular involve its protracted development and the appearance of a progenitor pool in the posterior lobe[54]. It is presently unknown whether cortical cerebellar cell types have further diversified during human evolution, highlighting an area in need of further research.

Evolutionary diversification and specialization have also been described for non-neuronal brain cells, which include oligodendrocytes, astrocytes, microglia, vascular and leptomeningeal types, ependymal cells, and choroid plexus epithelial cells, which together have wide-ranging functions in neuronal transmission, immune responses, providing barriers and homeostasis. A cross-species transcriptomic and morphological analysis demonstrated a greater diversification of subtype diversity and abundance of astrocytes, microglia and oligodendrocytes in humans compared with other primates and mammals[55–58]. In an analysis of single-nucleus RNA sequencing from the middle temporal cortex of five primates, including humans and other great apes, microglia showed the greatest transcriptional divergence with evolutionary distance[50]. Moreover, differentially expressed genes in human non-neuronal types were more likely to be associated with regions of the genome exhibiting signatures of evolutionary selection such as human-accelerated regions (HARs) or human deletions in conserved regions[50] (hCONDELs). Cortical astrocytes also show distinct features in humans compared with rodents, marked by their larger cell
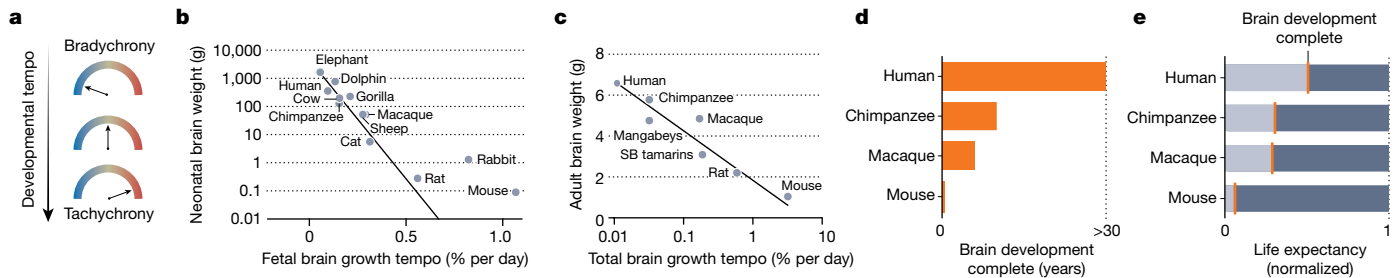
Fig. 2 | Neurodevelopmental tempo has become progressively slower, or bradychronic, in human evolution. a, Schematic illustration of bradychrony and tachychrony, which describe slowed and accelerated developmental timing, respectively. **b**, Relation between neonatal brain weight and fetal brain growth tempo across species. Data are presented in Supplementary Table 1. Nonlinear regression, $R^2 = 0.86$, with 10 degrees of freedom. **c**, Relation between adult brain weight and adult brain growth tempo across species. Data are presented in Supplementary Table 2. Nonlinear regression, $R^2 = 0.96$, with 6 degrees of freedom. SB tamarins, saddle-back tamarins. **d**, Comparative analysis across species of the time window of total brain development, from the start of gastrulation until the end of myelination. Data are presented in Supplementary Table 3. **e**, Comparative analysis across species of the relative time of total brain development (as shown in **c**) compared to the average life expectancy in captivity. The red line indicates complete brain development (as shown in **d**). Data are presented in Supplementary Table 3.

shapes with more complex processes, and their notably faster propagation of Ca^{2+} waves[59]. Additionally, oligodendrocytes exhibit greater gene expression divergence and acceleration compared with neurons[58], with a human-specific increase in oligodendrocyte progenitor cells compared with mature oligodendrocytes[60], and they are enriched for psychiatric disorder variants[58].

Most of the identified evolutionary changes within the human brain seem to come from more subtle shifts in homologous cell-type counterparts, such as differences in cell number, diversification, transcriptomic expression profiles and chromatin accessibility[49,61,62], which together may have marked consequences in terms of overall cell numbers and brain size. Although changes in cell number and diversification of cellular homologues are prevalent, recent reports have also identified a number of important qualitative differences, including the emergence of unique cell types, specific loss of certain cells or distinct reallocations of homologous cell types. For instance, various unique interneuron types have recently been identified in the human striatum that are specific for humans or primates[51,63]. One specific inhibitory neuron subtype, the 'ivy cell', is abundantly present in cortical and hippocampal regions in primates, but appears in only the hippocampus in mice and ferrets[51]. Another example of reallocation of conserved types is found in a population of lateral ganglionic eminence-derived interneurons that migrate primarily to the olfactory bulb in mice. In humans and other primates, this type instead becomes abundant in the deep cortical white matter along the cingulate cortex, suggesting divergence in migration between primates and rodents[64].

Specializations in neuronal connectivity

The human neocortex displays structural, functional and subcellular differences in neuronal connectivity when compared to close relatives, as well as between distinct structures within the human brain. Most of these connectivity-related features are typically not shared with other great apes, emphasizing their specific emergence within the human lineage. For instance, humans display higher connectivity between association areas and lower interhemispheric connectivity compared with chimpanzees[65]. Subcellular differences associated with connectivity, including changes in synaptic connections and dendritic arborization, have also been observed in humans when compared with closely and more distantly related species. Despite constraints imposed by inevitable small sample sizes, these studies appear to show consistent trends across species and cortical regions. For instance, compared to chimpanzees, layer III pyramidal neurons in various human cortical areas contain notably longer and more branched dendrites with relatively more spines, and less profound differences are

also observed in spine density[66] (Fig. 1b,f). By contrast, differences in spine number, spine densities and dendritic complexity are less profound between macaques and rodents than between humans and macaques, despite their considerably larger evolutionary distance[66–71] (Fig. 1b,f). In humans and other great apes, connectivity-related changes are also detected across cortical regions; for instance, the prefrontal and temporal cortex exhibit relative increases in spine number and dendritic arborization compared with primary sensory cortices[66–71] (Fig. 1b,f).

Whilst comprehensive profiling of connectivity-related differences in humans compared with other great apes is technically and ethically challenging, comparisons with other primates and mammals have yielded valuable insights. For instance, proteomic analysis of synaptosomes from the human, macaque and mouse motor cortex revealed a relative enrichment of presynaptic components and microtubules[72]. This points to variations in molecular composition at individual synapses, which are likely to contribute to human-specific differences in connectivity[72]. Indeed, functional assays have revealed distinct biophysical properties of both glutamatergic neurons[73–76] and GABAergic (γ-aminobutyric-acid-producing) neurons[77–79], resulting in differences in transmission capabilities in neural circuits in humans. Moreover, a human-specific modulator of neuronal excitability, encoded by the hominid-specific gene *LRRC37B*, was recently identified and functions by inhibiting voltage-gated sodium channels[80].

The cerebellum in humans also exhibits notable changes in neural connections compared with other species. Cerebellar Purkinje cells in humans exhibit increased dendritic complexity and spine numbers compared with rodents, leading to enhanced cellular computational capacity[81]. However, it remains to be determined whether these changes are also evident when comparing humans with more closely related species.

Distinct features of neurodevelopment in humans

Specializations of the human brain arise during distinct stages of neurodevelopment, a spatiotemporally controlled process whose ontogeny is generally shared between mammals. Neurodevelopment starts with the proliferation of neuroepithelial cells that convert into different populations of neural progenitors (Fig. 3a). These progenitors give rise to various neuronal subtypes that migrate to specific brain regions. In the cortex, the first progenitors that arise are apical radial glia, which continue to expand and produce additional amplifying progenitor types, such as intermediate progenitors and basal or outer radial glia. This pool of cortical progenitors eventually gives rise to cortical neurons, which then migrate towards the outer surface and
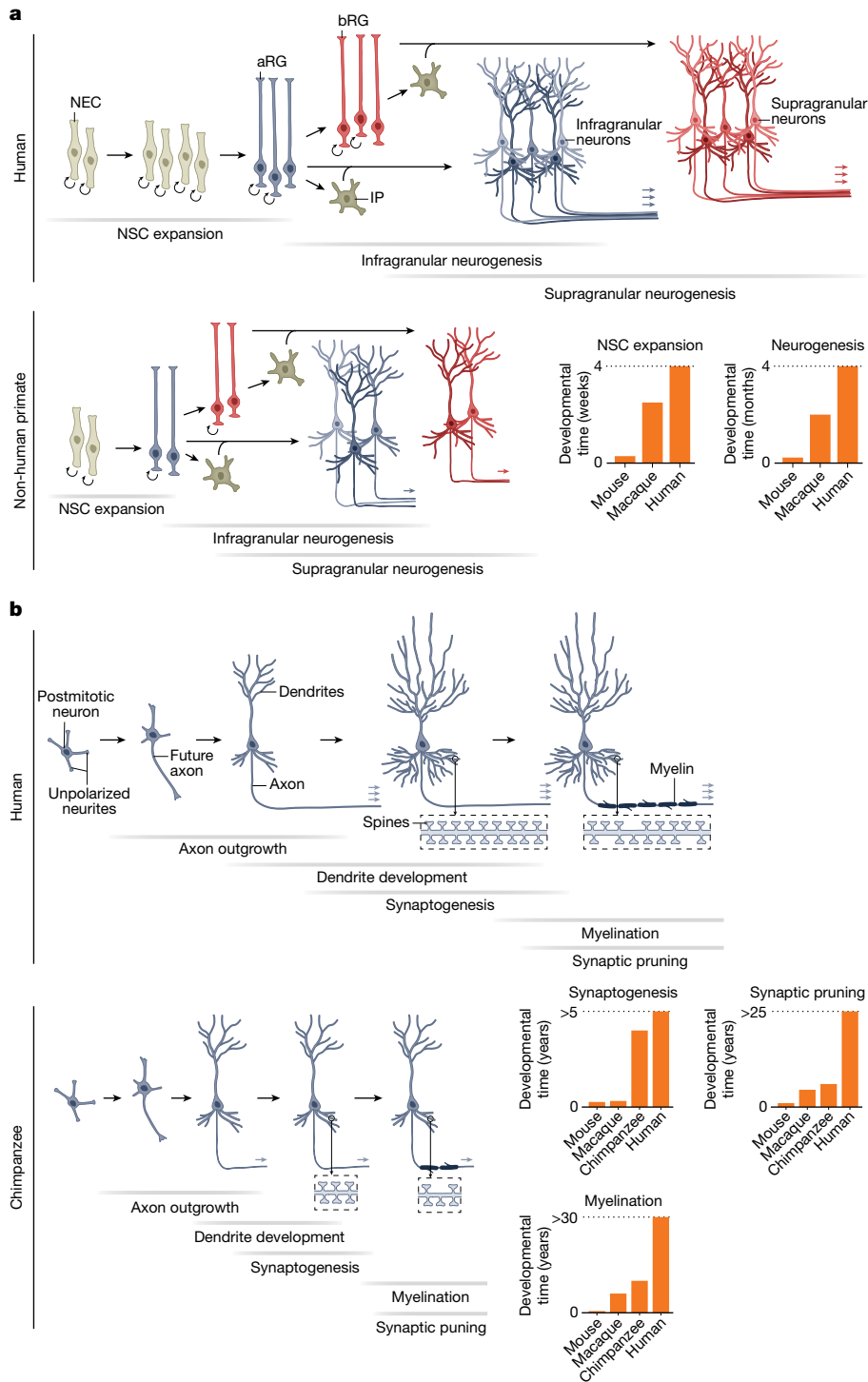
Fig. 3 | Neurodevelopment in human and non-human primates. a, Left, schematic illustration of prolonged neural stem cell expansion, increased progenitor proliferation and delayed supragranular neurogenesis in humans compared with non-human primates[95,97,100]. Bottom right, scaled cross-species analysis of the timing of neural stem cell expansion[91,93,102–105] and neurogenesis[91–93]. aRG, apical radial glia; bRG, basal radial glia; IP, intermediate progenitor; NEC, neuroepithelium cell; NSC, neural stem cell. **b**, Left, schematic illustration of neuronal maturation stages in humans and chimpanzees. Humans develop relatively longer axons, more arborized dendrites and more spines per neuron. Bottom right, scaled cross-species analysis of the timing of synaptogenesis[132–134,145], synaptic pruning[132,133,139,145] and myelination[135–138], showing that later maturation stages are more protracted than early maturation stages in humans compared with chimpanzees.

form the distinct layers of the cortex in an inside-out order. The subsequent development of various neuron types is characterized by a series of drastic morphological changes and the assembly of specialized subcellular structures, a process collectively referred to as neuronal maturation. First, during neuronal migration, neurons grow out a single axon, which bundles with other axons to form distinct tracts projecting to specific brain regions (Fig. 3b). Axon outgrowth is followed by the sequential processes of dendrite formation, synaptogenesis, synaptic pruning and axon myelination, which together enable the establishment and refinement of neuronal circuits.

Variations in neurodevelopmental tempo

Although the sequence of neurodevelopmental events is shared amongst mammals, the tempo at which these events unfold greatly varies between species. The protraction of these developmental stages becomes increasingly pronounced from primates to great apes, culminating in a further delayed progression in humans compared with close relatives. At birth, the human brain is 28% of the adult size, compared with 40% in chimpanzees[82]. A similar trend is observed within hominins, where this proportion has gradually decreased from 38% in Australopithecus to 28% in middle pleistocene pre-modern *Homo*[83]. During subsequent postnatal development, humans reach adult brain size later than chimpanzees and the white matter of the prefrontal cortex develops more slowly[82,84].

Such developmental delays have often been described as neoteny. This term, derived from Greek meaning 'extended youth', was originally used to describe how certain amphibians, such as the axolotl, retain larval traits into adulthood[85]. Although the term has been extended to also indicate other developmental delays, its varied usage may lead to confusion. Indeed, in humans neoteny is often referred to as a retention of juvenile characteristics, due to our rather immature physical features, such as fine body hair and delicate craniofacial structures, features that also distinguish us from other apes and primates. However, protracted development is already present in primates compared with the next closest mammalian clade made up of rodents and lagomorphs, and apes exhibit a further protraction, yet these species do not exhibit such juvenile features. Moreover, developmental delay is not limited to pre-adult traits persisting into adulthood, but occurs throughout embryonic and postnatal development. Thus, we propose the term 'bradychrony', meaning slowed time, to describe developmental delay (Fig. 2a and Box 2). Similarly, certain mammals, such as mice, exhibit accelerated development, which we term 'tachychrony' (Fig. 2a and Box 2), probably owing to their unique environmental and seasonal pressures[86]. Because the degree of bradychrony has progressively increased in human evolutionary history, whereas neotenic features have appeared rather suddenly in our recent history, the mechanisms underlying the two may be distinct.

This divergence in developmental timing may provide the extended window that is necessary for distinct specializations to take shape in the developing human brain. Consistent with this idea, brain growth tempo—defined as the relative rate of brain development to reach neonatal or adult brain weight—is slower in humans compared with our close relatives (Fig. 2b,c and Supplementary Table 1 and 2). The degree of bradychrony of brain growth tempo aligns with what is expected for our brain size, indicating that allometric scaling in primate evolution applies not only to brain size and neuron number[26–28], but also to the phase in which this is achieved. Nevertheless, it is worth noting that the human brain continues to mature and refine neural circuits until about 30 years of age, with no increase in brain weight after 22 years of age. These later stages of neurodevelopment are particularly extended in humans, potentially leading to a more pronounced bradychrony in overall brain development. Indeed, in humans it takes 30 years to complete brain development, from gastrulation to completion of myelination, which is nearly half our average lifetime, compared with around 30% of the lifespan in chimpanzees and macaque, and around 5% in mouse (Fig. 2d,e and Supplementary Table 3). This delayed maturity until well after birth also enables increased environmental influence on brain development, thereby providing opportunities for enhanced behavioural plasticity and cultural transmission[87]. Studies on more species are needed to determine whether this profound bradychrony of these late maturation stages also follows allometric scaling or is slower than expected for our species.

Divergent aspects of neurogenesis

How humans obtain their increased cortical neuron numbers, diversification of homologous cell types and unique cell types is also a key question. Over the past decade, many studies have pinpointed various neurodevelopmental mechanisms that distinguish humans from other mammals, such as rodents, and progress has been made in understanding differences between humans and mice during glutamatergic neurogenesis[13,88–90]. However, human and primate-specific differences that deviate from other mammals are only now beginning to emerge. Additionally, whereas glutamatergic neurogenesis has been the subject of much research, attention is also turning to the less well-explored GABAergic neurogenesis.

Investigating human-specific aspects of brain development, such as exploring the timing of embryonic neurodevelopmental stages, is challenging owing to the limited accessibility of fetal tissues of great apes. Compared with other primates, the in vivo neurogenic time window in humans is bradychronic, lasting about four months, which is about twice as long as in macaques and ten times longer than in mice[91–93] (Fig. 3a). Although the exact duration of non-human ape neurogenesis remains uncertain, ultrasound imaging has revealed a similar rate of brain growth in chimpanzees to humans. However, humans exhibit both a longer phase of growth and a larger brain at the earliest investigated time point of 15 weeks of gestation[94], suggesting that the human brain is larger even before the majority of neurons have been produced.

Whereas it is challenging to compare the developing human brain with those of other apes, in vitro models provide an alternative window and have begun to uncover ape and human-specific delays in neurogenic transitions. Two- and three-dimensional differentiation of human and chimpanzee cortical neurons revealed a prolonged proliferative state of progenitors followed by a delayed switch to supragranular identities compared with macaque[95] (Fig. 3a). The same study reported no difference in the timing of onset of supragranular neurogenesis between human and chimpanzee[95], consistent with the ape-acquired elaboration of laminar architecture seen in vivo that is unchanged in humans. This is also in line with in vivo reports showing that the proliferative phase of basal progenitors is bradychronic in humans compared with rodents, similarly resulting in increased numbers of supragranular neurons[91,96,97].

Organoids—in vitro self-organizing neural tissues—also revealed a switch to gliogenesis earlier in chimpanzee than human[98], as well as a difference in cell cycle that relates to neurogenic versus proliferative divisions[99]. Such heterochrony also appears to be present at the earliest stages of neuroepithelial expansion, with human compared to non-human apes showing enhanced proliferative capacity and bradychrony in the switch from symmetrically expanding neuroepithelial cells to radial glia[100] (Fig. 3a). Although there are no in vivo data on non-human apes during these early stages, comparisons between humans, macaque and mice show that the time window of tangential cortical expansion, which encompasses the proliferation of neuroepithelial cells and symmetrically dividing apical radial glia—collectively

termed neural stem cells[101]—is bradychronic in humans. Specifically, this period, spanning from neural tube closure until neurogenic onset, takes about 4 weeks in humans[91,102], compared to 2.5 weeks in macaque[103,104] and 2 days in mouse[93,105] (Fig. 3a). Together, the expansion of these neural stem cells results in an increased founder neurogenic progenitor pool and—consistent with in vivo observations and the radial unit hypothesis[103]—helps explain how human-specific tangential cortical expansion can arise without a change in cortical thickness or relative proportions of cortical layers compared with other apes.

Although GABAergic progenitors and neuron differences in humans compared with other great apes are less well explored, comparisons with other primates and rodents reveal notable specializations that have evolved in the human lineage. Single-cell epigenomic analysis reveals an impressive diversification amongst neural progenitors across brain regions in humans[106]. Although less is known about the proliferative dynamics of human and other primate telencephalic GABAergic progenitors, the relative proportions of neuron types in the adult neocortex suggests that population dynamics of inhibitory and excitatory neurons are coordinated. For example, primate V1 has vastly higher neuronal density than elsewhere in the neocortex[107]. The expansion of the progenitor-rich outer subventricular zone proximal to presumptive V1 during mid-gestation could account for increased excitatory neuron numbers[108]. Interneuron populations also markedly increase in absolute numbers in primate V1[109], resulting in similar overall proportions of inhibitory to excitatory neuron ratios to other cortical areas[51]. Whether this population matching is controlled by differential rates of interneuron neurogenesis timing, migration or cell death is not yet known.

Similar to other neural progenitors and cell types, progenitor classes within the ganglionic eminences, which give rise to telencephalic GABAergic neurons, appear generally well conserved between primates and rodents[64]. The emergence of human-specific cell types or brain structures is likely to be exceptionally rare, yet some primate- or human-specific features may arise from novel or specialized progenitor domains. For example, a recently-discovered striatal GABAergic interneuron type[51] may arise from a specialized progenitor domain in the ganglionic eminences in primates[64]. Whereas mice have a single detectable initial class for medial ganglionic eminence-derived interneurons destined for striatum, in primates this class diverges early (after conception day 65) into two distinct postmitotic classes, one of which goes on to mature into a specialized *TAC3*+ population in primates[64].

One intriguing evolutionary modification to primate neocortical progenitors entails the possibility that dorsal cortical progenitors give rise to both glutamatergic and GABAergic neurons, rather than exclusively glutamatergic neurons (as is found in rodents). Whereas past evidence for multipotency of primate dorsal progenitors has been mixed[110–114], recent in vitro lineage tracing in human organotypic cortical slice cultures has found that a substantial proportion of cortical interneurons and glutamatergic neurons share a common lineage[115]. This multipotency is relatively rare early in human cortical development and becomes more prevalent at mid-gestational timepoints[110,114,116]. No clonal relationships between cortical GABAergic and glutamatergic neurons were found when using the same lineage-tracing approach in mice in vivo[115,117]. Furthermore, recent examination of intrinsic genetic mosaicism revealed a subtype of *DLX1*+ inhibitory neurons in the human brain that share an origin with excitatory neurons[118], suggesting that local proliferation of cortical GABAergic types could be one potential route by which cortical interneuron abundances increase in primates within particular cortical regions.

While novel primate- or human-specific cell types are fascinating, changes in cell proportions, gene expression programmes and spatial reallocations of conserved types are much more prevalent. For example, whereas rodent thalamic nuclei are dominated by glutamatergic neurons, primate thalamus contains high proportions of GABAergic neurons[119]—in general, GABAergic neuron composition of dorsal thalamic nuclei appears to scale with species complexity[120]. Although thalamic structures largely derive from the diencephalon, there appears to be some recruitment of GABAergic neurons from other developmental compartments in rodents and non-human primates[121,122]. Human organotypic slice culture experiments show that streams of GABAergic neurons of telencephalic origin migrate into thalamic nuclei and populate higher-order associative thalamic nuclei[123]. The developmental origin and cellular composition of the medial pulvinar—another associative thalamic nucleus—remain to be determined, but there is some evidence that it may be initially formed from diencephalic progenitors along the third ventricle, after which there is contribution from a second wave of telencephalic-origin progenitors[124]. These examples reveal that the cell-type-specific developmental processes that control proliferation and response to guidance cues are likely to be critical for understanding human specializations of cellular diversity.

Divergent aspects of neuronal maturation

As previously highlighted, many connectivity-related specializations in humans are absent in other great apes, emphasizing their relevance in understanding distinct aspects of human brain function. Thus there is growing interest in deciphering how these differences in neuronal connectivity, all of which arise during neuronal maturation stages, come about during development[125,126] (Fig. 3b). Technical advancements, many of which have emerged in the past few years, have begun to enable in vitro modelling of these later stages, thereby paving the way for more mechanistic insights[127–129]. Together with in vivo data, a picture is emerging of evolutionary divergence in neuronal maturation—a process that spans from mid-fetal development to early adolescence and appears to be particularly distinctive in humans. A comprehensive comparative transcriptomic analysis in human and macaque samples from early prenatal stage to adulthood reported the most notable differences that occur during maturation stages[130]. Specifically, the transcriptome was most divergent during mid-fetal development, aligning with axon outgrowth, dendritogenesis and the onset of synaptogenesis, and during early adolescence, when synaptic pruning and circuitry refinement occur[130]. Another recent study on proteomic analysis of postsynaptic densities in human, macaque and mouse reported that morphologic and functional synapse maturation is bradychronic in humans, mediated by Rho guanine nucleotide exchange factor signalling[131].

Moreover, the degree of bradychrony during neuronal maturation appears to be most distinct in humans, with later maturation stages becoming increasingly protracted in humans compared with earlier stages. For instance, initial synaptogenesis spans approximately five years in humans, four years in chimpanzees and three months in macaques[132–134] (Fig. 3b), which is notably more bradychronic than the neurogenic stages (Fig. 3a). Late neuronal maturation events, such as synaptic pruning and axon tract myelination, take 30 years in humans, and are increasingly more delayed compared with the 10 years in chimpanzees and 4.5 years in macaques[132,133,135–139] (Fig. 3b). Further, this pattern shows that the increased degree of slowing during late neuronal maturation is especially marked in humans when set against other great apes, whereas the differences between non-human apes and other primates and mammals is less profound (Fig. 3b). This suggests that the degree of bradychrony during neuronal maturation in humans may be the result of a relatively recent evolutionary event. Moreover, a recent report found that the timing of synaptogenesis in mice and macaques was remarkably similar, contradicting the long-held belief that the timing of these processes is primarily related to a species' lifespan[132]. It should be noted, however, that different timeframes for synaptogenesis in mice have been documented in another study[140].

Although the appearance of dendritic and synaptic specializations are relatively more explored, humans also develop extended axonal projections as reflected by increased white matter volume[40,41]. In human neuron cultures, axon formation was found to be bradychronic[141,142]

and to proceed through an additional developmental stage involving an increase in microtubule remodelling and relocation of axon initial segment proteins from the distal axon (classified as stage 3a) to the proximal axon (stage 3b, corresponding to the classic stage 3[143]), which has not been reported in other species[142]. Another study in human neuron cultures revealed that the onset of axon development is mediated by centrosomes, with the axon initial segment protein TRIM46 localizing to centrosomes in human cells, but not in rodent and monkey cells, before it relocates to the axon as it develops[144]. Further research is needed to shed light on divergent aspects of axon development that underlie related specializations in the human brain.

In addition to species-specific variations, spatial differences in neuronal maturation tempo within the human brain are also evident, and are likely to contribute to regional differences in neuronal connectivity. Indeed, neuronal maturation is more bradychronic in neocortical regions that have more specialized functions and higher synaptic densities[133]. For instance, development in the prefrontal cortex is particularly slow, with synaptogenesis peaking 15 months after birth and synaptic pruning continuing up to 30 years, probably contributing to the cognitive and behavioural changes that are observed during childhood and adolescence[133]. By comparison, the auditory cortex has a faster tempo, with synaptogenesis peaking 3 months after birth and synaptic pruning extending until 12 years of age[133]. These regional differences in neuronal maturation tempo contrast with chimpanzees and macaques, in which synaptogenesis is more synchronized across cortical regions[133,145]. However, similar to humans, chimpanzees do exhibit relatively bradychronic dendritic development in the prefrontal cortex compared with other brain regions[145].

Thus, the degree of bradychrony, either across brain regions or between different species, is associated with increased connectivity and cognitive functions, sparking an emerging interest in exploring the mechanisms underlying developmental tempo differences. An important insight is that differences in timing appear to be driven by cell-intrinsic mechanisms, as the species-specific tempo of developing cells is maintained when human cortical neurons are xenografted into the mouse brain or co-cultured with non-human primate neurons[95,129,141]. While the nature of differences in timing between primates is still unclear, recent comparison of human and mouse motor neuron differentiation revealed a correlation with protein stability[146], and comparative studies of the segmentation clock have similarly demonstrated a correlation with protein turnover[147,148]. Besides global timing mechanisms, the varying degree of bradychrony of neuronal maturation compared with other developmental stages suggests the existence of stage-specific factors influencing tempo. Although the exact mechanism of human-specific bradychrony of neuronal maturation is unknown, a recent study comparing human and mouse revealed a slower pattern of mitochondria development and metabolism in human neurons, with neuronal maturation being accelerated upon stimulation of mitochondrial metabolic activity[149]. Whether this mechanism is specific for humans or shared with other primates is still unclear. As discussed below, epigenetic changes may also drive the pace of neuronal maturation[150]. Further investigations are needed to understand global and stage-specific mechanisms that drive the tempo of neurodevelopment, as well as to what extent these contribute to human-specific specializations versus those shared with other primates.

Molecular mechanisms of human features

The human genome differs from those of other primates owing to nucleotide substitutions, short insertions and deletions (indels), and larger structural variants (losses, gains, inversions, translocations and rearrangements of DNA) that arise through processes such as transposon activity, recombination, replication and repair[151–156]. With the generation of telomere-to-telomere genome assemblies[157], we are approaching a complete list of all fixed differences between modern humans and our closest relatives. Research is also shedding light on how these genetic changes alter protein function and gene-regulatory networks, which is key in establishing their roles in human-specific aspects of neurodevelopment and neurological disease.

Divergent proteins

Both gene duplications and modifications to coding sequences have altered the human proteome. Amino acid substitutions and indels have altered about one third of proteins in humans and a similar number in chimpanzees since our lineages split[156]. Further proteome differences have arisen through gene birth[158,159], duplications, fusions and truncations[155,160,161]. In several cases, these protein-coding changes have been linked to distinct characteristics of the human brain. For instance, a single lysine-to-arginine substitution in transketolase-like1 (TKTL1)—which arose after the divergence of modern humans from Neanderthals—increases neural production by basal radial glia in human cerebral organoids and in mice, potentially through greater production of fatty acids required for the outgrowth of processes on radial glia[162]. Radial glial biology has also been modified by three human-specific paralogues of the NOTCH2 receptor (NOTCH2NL), which promote clonal expansion of neural progenitors and delay their differentiation via inhibition of Delta–Notch interactions and activation of the Notch pathway[163,164]. NOTCH2NL genes serve as breakpoints for human polymorphic structural variants that are associated with variation in brain size and autism, supporting the hypothesis that NOTCH2NL contributed to cortical expansion during human evolution.

Another set of gene duplication events with effects on brain development is SRGAP2. SRGAP2C evolved from gene duplication before the major evolutionary increase of brain size in humans[165]. Mice expressing the human-specific SRGAP2C showed enhanced excitatory synaptogenesis in upper layer neurons, increased local and long-range cortico-cortical connections and improved learning abilities of cortex-dependent tasks[165]. Furthermore, the human-specific paralogues SRGAP2B and SRGAP2C slow synaptic maturation by antagonizing and downregulating the ancestral protein encoded by SRGAP2A. This results in prolonged upregulation of postsynaptic SYNGAP1[166], modulating neuronal spine formation and promoting bradychronic maturation[161,167–169]. Another example is Rho GTPase-activating protein 11B (ARHGAP11B), a human-specific gene that enlarges the neocortex and induces gyrification when expressed in mice and marmosets[170,171]. Thus, although most of the proteome is identical between humans and chimpanzees, the search for genes underlying human neurodevelopmental specializations has yielded a number of variants and gene duplications that modify intracellular signalling in the human cortex with effects on downstream metabolic and transcriptional processes, anatomy and developmental timing.

Divergent gene-regulatory networks

Human genetic variants also affect non-coding DNA that contains regulatory elements such as enhancers, repressors and insulators[172–175]. For example, during early and mid-fetal human development, there is a spatially restricted patterning of retinoic acid in the prefrontal cortex that is important for promoting enhanced intra-prefrontal cortex dendritic synaptogenesis and prefrontal cortex–mediodorsal thalamus connectivity[176]. The onset of synaptogenesis in the prefrontal cortex during mid-fetal development aligns with the local, transient and laminar-specific upregulation of CBLN2, NRXN and GRID in humans, an expression pattern that is not observed in macaque[177]. Increased levels of CBLN2 in the prefrontal cortex promote spine formation, a regulatory effect that is shared between human and chimpanzees, driven by a hominin-specific deletion in SOX5-binding sites within a retinoic acid-responsive CBLN2 enhancer[177]. Thousands of other human-derived regulatory elements have been proposed on the basis of cross-species differences in gene expression, chromatin accessibility and histone modifications in post mortem brain tissues[178–186], neuronal cell lines[187]

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and cerebral organoids[17,98]. Many of these elements have human variants that may explain their differential function in the human genome—for example, with changes in binding of transcription factors or chromatin modifiers[172].

The fastest-evolving regulatory elements are HARs, conserved non-coding sequences with an excess of nucleotide changes in the human genome[188–190]. Many HARs function as gene-regulatory enhancers in neurodevelopment[191]. Using massively parallel reporter assays, hundreds of HARs have been validated as enhancers where the modern human sequences show differential activity[188,192–196], and dozens show differential enhancer activity in mice[191,197]. In the case of HARE5, a human-accelerated enhancer of the WNT receptor FRIZZLED8, differential activity has been linked to human brain features. Compared to the chimpanzee sequence, human HARE5 accelerates the cell cycle in neural progenitors and increases brain size in transgenic mice[198]. Thus, innovations in human brain development are now being linked not only to variants affecting proteins, but also to variants in gene-regulatory elements.

Beyond point mutations, structural variants have translocated, duplicated, and deleted thousands of regulatory elements during human evolution[199,200]. Not surprisingly, these large-scale changes in regulatory information can lead to developmental phenotypes. For instance, loss of a hCONDEL that regulates *GADD45G* is correlated with expansion of specific brain regions in humans[199]. Structural variants also alter the relationship between nearby genes and regulatory elements by reorganizing and modifying the linear genome[161,201]. Furthermore, indels and substitutions affecting chromatin boundaries can reorganize three-dimensional chromatin without major changes in the genomic distance between regulatory elements and promoters. These disruptions to three-dimensional genome folding can separate regulatory elements from their target genes, thereby reducing their ability to control gene expression, and conversely they may bring together gene promoters with regulatory elements from which they were previously insulated (enhancer hijacking). Indeed, HARs are enriched in loci with structural variants that alter genome folding[202], suggesting that their accelerated evolution could be attributed, in part, to their being hijacked enhancers.

Other mechanisms that could explain regulatory elements that lack *cis* sequence variation yet show epigenetic differences in the human brain include genetic variants in upstream transcription factors[203], altered cell-type specificity of these factors[204], and differences in the chemical environment inside human cells. A role for epigenetics in controlling neurodevelopmental timing in humans[150] as well as more distant species[205] is emerging. A study using in vitro human neuron cultures identified an epigenetic barrier that controls timing of neuronal maturation, which is mediated by the gradual release of the epigenetic factors EHZ2, EHMT1, EHMT2 and DOT1L, which are otherwise retained in neuronal precursor cells[150]. Post-transcriptional regulation has also contributed to evolution of the human brain[206–208]. In sum, changes to regulatory element sequences, *trans* factors and genome organization all have roles in the evolution of human gene-regulatory networks.

Elucidating the functional effects of this re-wiring on neurodevelopment is an active area of current research. An important future direction is studying regulatory elements in their endogenous genomic context, as opposed to probing them via reporter assays. This will become easier as genome-editing tools with nucleotide resolution and high-throughput capabilities continue to be developed. The demonstration that deletion of SOX5-binding sites within a *CBLN2* enhancer causally contribute to its human-specific upregulation in the prefrontal cortex[177] exemplifies the discovery potential of endogenous editing.

Outlook

Various specializations of the human brain have been identified in recent years. Some of these features are likely to be adaptive, such as the vast increase in neuron numbers, providing a fitness advantage and enabling more complex cognitive and social abilities. Many of these adaptations also appear to be shared with other primates, such as the increase in neuronal density and the relative increase of supragranular layers, which may lead to advanced cognitive functions that are already evident in primates. Other specializations involve those that appear early in evolution but show a profound divergence in the human lineage, including neocortical and cerebellar expansion emerging during neurogenesis, and the increased dendritic arborization, synapse number and axon projections established during neuronal maturation. The human-specific features set during neuronal maturation stages remain relatively unknown, largely owing to technical hurdles in modelling these stages. Emerging evidence is highlighting the role of specific gene-regulatory networks and developmental tempo in driving variations in synapse numbers. Further research is needed to further explore the downstream intracellular programmes that they act on, as well as how evolutionary differences in axon projections and dendritic arborization come about during development. An exciting avenue for future investigations would be to examine the possible influence of neuronal activity during these developmental processes, because in non-primates, neuronal activity in established circuits is known to feedback by modulating gene transcription and subsequent neurodevelopment[209–213].

In addition to specializations with a profound divergence in the human lineage, there is an accompanying marked difference in the degree of bradychrony in humans compared with our close relatives. Although the increased bradychrony in humans may imply that an extended timeframe is important for developing a more expanded brain with divergent features, more experimental evidence is needed to support this. Methodologies to perturb species-specific timing are being developed[214], and will be instrumental in gaining functional insights. Thus, further research is needed to identify mechanisms that modulate developmental timing, as well as their relevance for establishing the different human-specific specializations.

The presence of specializations in non-cortical structures in the human brain is becoming increasingly apparent, and offers another intriguing direction for future research. Notably, modifications to subcortical structures are pronounced in regions that are coupled to distributed networks of higher-order association cortex, suggesting coordinated evolution. For example, non-motor and non-sensory structures in the human cerebellum have expanded in conjunction with the expansion of human higher-order neocortical networks[36,215]. Similarly, the associative mediodorsal and medial pulvinar nuclei of the thalamus have expanded in primate evolution[216], a feat that may in part have been enabled by adaptations to the expansion and migration properties of telencephalic progenitors[124]. Applying cell-type resolved lineage-tracing technologies[115,217] to developing primates in vivo could help to establish how and when primate-specific adaptations to progenitors arise, which could then be further modelled in vitro. Furthermore, technologies that enable cell-type-resolved readouts of synaptic connectivity[218] or projection patterns[219] in primates will be key for understanding the relationship between expanded neuronal populations and changes in connectivity properties. Moreover, investigating features that greatly diverge in humans and rodents, such as the CA1 region of the hippocampus—which is more packed with pyramidal neurons of distinct morphologies in humans—in other primates could enhance our understanding of the evolutionary context of these features[220].

The emerging mechanistic understanding of human brain evolution largely stems from various technical advances. Much progress has been made in developing human in vitro models, including neural cultures and brain organoids, with varying degrees of complexity. A growing body of literature using these methods has consistently demonstrated that these models recapitulate key stages of neurodevelopment, from early proliferation to neurogenesis and gliogenesis, and that they successfully model the anticipated timing differences across species. The

latest developments in this field include in vitro systems that model later stages of human neurodevelopment, including optimized methods for long-term brain cultures, in vivo transplantations and assembloids, as well as the modelling of more brain structures, such as choroid plexus and cerebellum[127–129,221–224]. Future advances in in vitro models may include functional vascularization of organoids, recapitulating long-range axon tracts and modelling more brain structures. As these tools become more commonplace, further comparative studies would also benefit from more individuals per species to increase confidence that any identified differences represent species-specific features rather than technical artefacts.

Additionally, important advances in high-throughput single-cell profiling techniques, such as single-cell RNA sequencing, ATAC (assay for transposase-accessible chromatin) sequencing and proteomics, have enabled scaling up cell numbers and reads per cell, improved quality of readouts, the integration of multiple modalities and spatiotemporal reconstructions[225–227]. This has led to a large number of detailed maps of genetic, gene accessibility and transcriptomic patterns at different neurodevelopmental stages across species[98,228,229], as well as the increased identification of human-specific genomic and gene-regulatory changes during neurodevelopment. Although some have been functionally explored, further investigations are needed to uncover how these changes are mechanistically linked to human-specific specializations arising during neurodevelopment. Moreover, recent technical developments in imaging and microscopy, such as three-dimensional live imaging and clearing methods, have begun to unravel the subcellular structures and temporal dynamics of developing human neurons[230–234]. This multidisciplinary application of new techniques will continue to be at the foundation of understanding the distinct aspects of human brain development and function.

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