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VIEWPOINT

From stem and progenitor cells to neurons in the developing neocortex: key differences among hominids

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Comparing the biology of humans to that of other primates, and notably other hominids, is a useful path to learn more about what makes us human. Some of the most interesting differences among hominids are closely related to brain development and function, for example behaviour and cognition. This makes it particularly interesting to compare the hominid neural cells of the neocortex, a part of the brain that plays central roles in those processes. However, well-preserved tissue from great apes is usually extremely difficult to obtain. A variety of new alternative tools, for example brain organoids, are now beginning to make it possible to search for such differences and analyse their potential biological and biomedical meaning. Here, we present an overview of recent findings from comparisons of the neural stem and progenitor cells (NSPCs) and neurons of hominids. In addition to differences in proliferation and differentiation of NSPCs, and maturation of neurons, we highlight that the regulation of the timing of these processes is emerging as a general foundational difference in the development of the neocortex of hominids.

Introduction

The closest living relatives of humans are other primates, particularly those also in the hominid lineage, the non-human great apes. Among them, the chimpanzees and bonobos are the closest, with our ancestors probably splitting from theirs sometime in the order of around 7 million years ago (mya), from gorillas around 10 mya and from orangutans around 18 mya [1–3]. On an evolutionary scale, this makes us all very close cousins, and we share many anatomical and behavioural similarities. Non-human great apes are, for example, typically considered among the most intelligent animals. Despite our closeness, all non-human great ape species are threatened with extinction, mostly by human activities such as habitat destruction and poaching [4]. This not only threatens those species, but it also severely limits what we could learn from them, especially in their natural condition. In addition, for sound ethical reasons, experimentation with non-human great apes is typically under very strict regulations. Samples are therefore extremely scarce and, when available, usually come with degrees of uncertainty regarding tissue, cell and molecular integrity. In the case of extinct archaic hominids, no samples containing neural tissue remnants have ever

Abbreviations
APs, apical progenitors; aRG, apical radial glia; BPs, basal progenitors; bIPs, basal intermediate progenitors; bRG, basal radial glia; iPSCs, induced pluripotent stem cells; ISVZ and OSVZ, inner and outer SVZ; mya, million years ago; NE, neuroepithelial; NECs, neuroepithelial cells; NSPCs, neural stem and progenitor cells; SVZ, subventricular zone; VZ, ventricular zone.

been found, beyond the fascinating yet nonetheless limited impressions of shape and texture that remain on the inner side of old fossilized skulls, and from which brain endocasts are made. Consequently, we know very little about what neural cell differences may exist among hominids, especially in the context of their native tissue.

Humans have a threefold larger cerebral cortex than chimpanzees [5], and it is estimated to contain twice as many neurons [6] (Figure 1). It is therefore intuitive to hypothesize the existence of certain differences in the neural stem and progenitor cells (NSPCs) of hominids, particularly regarding their capacity to proliferate in tissue and generate a large neocortex. Due to the lack of available samples, the search and analysis of such potential differences has remained largely impossible. One emerging solution has been the recent development of *in vitro* neural tissue assemblies that go beyond the typical 2D monolayer cell cultures and are generated from embryonic stem cells or induced pluripotent stem cells (iPSCs). Brain organoids in particular have opened ways to mimic more closely the 3D architecture and tissue environment, and how they develop over time [7,8]. Organoids still show notorious limitations in how closely they can mimic different brain tissues. For example, reproducibility within and between batches of organoids, and between laboratories, remains challenging. Also, the architecture and cellular composition of the neocortical germinal zones located basally to the ventricular zone (VZ), as well as of the neuronal layers, are still not faithfully reproduced in organoids. Nevertheless, they are progressively becoming powerful tools to study neural cell biology in species where neural tissue is scarce or unavailable [9–12].

Here, we discuss studies that have begun to look for differences between neural cells in the cerebral cortex of hominids, from the proliferation and differentiation characteristics of NSPCs to the maturation of neurons. We suggest that differences in the timing and transitions of cellular and developmental mechanisms constitute a pivotal general aspect of neurogenesis that has the potential to help explain the bases of many functional neural differences observed among hominids. Finally, we look at studies that are beginning to address genomic differences that may play a role in the neurobiology of modern and archaic humans.

Neural stem and progenitor cells in cortical neurogenesis

Considered to be exclusively present in mammals, the neocortex is the latest major type of cerebral tissue to appear during evolution, and participates in diverse neural functions, notably in higher cognitive abilities such as abstract thought and language [13]. The neocortex arises from highly proliferative neural stem cells called neuroepithelial cells (NECs) in the dorsolateral neuroepithelium of the developing forebrain. These NECs have an apical process that contacts the ventricle and a basal process that contacts the pia. NECs gradually change and elongate to become mostly apical radial glia (aRG, also called ventricular radial glia) during early neurogenesis. Collectively, NECs and aRG are called apical progenitors (APs) [14–16].

The progressive transition from NECs to aRG coincides with the growth of the tissue and appearance of additional layers basal to the original neuroepithelium. The neuroepithelium then becomes the VZ of the developing cortical wall and continues to harbour aRG. Of these new layers, the subventricular zone (SVZ) is germinal [17]. In many mammals, including primates, the SVZ gets subdivided into an inner and outer SVZ (ISVZ and OSVZ). These become particularly important for large-brained mammals (Fig. 1), with the OSVZ constituting the major site of embryonic neurogenesis [18,19], and has been proposed to also be important for primate gliogenesis and the gyrification associated with it [20]. Together, ISVZ and OSVZ contain a variety of basal progenitors (BPs) with diverse morphologies, proliferative capacities, and neurogenic and gliogenic potentials (Fig. 1). Eventually, the germinal zones and their NSPCs become almost fully consumed, having given rise to the six neuronal layers of the adult mammalian neocortex [16,21,22]. In the case of primates, neurons in the supragranular layers (layers II and III) are considered to play especially prominent roles in higher cognitive functions [23].

Differences among hominid neural stem and progenitor cells

Mitosis, cell cycle and the time for proliferation vs differentiation

Recent studies have explored potential differences, notably cell biological differences, among hominid NSPCs that could underlie differences in brain size. For example, spindle orientation has long been considered an important determinant of cell fate in NSPCs [24–26] and is therefore a conspicuous candidate to influence brain size and total neuron number among hominids. However, no differences in spindle orientation dynamics were found among the APs in human neocortical tissue ex vivo and human cerebral

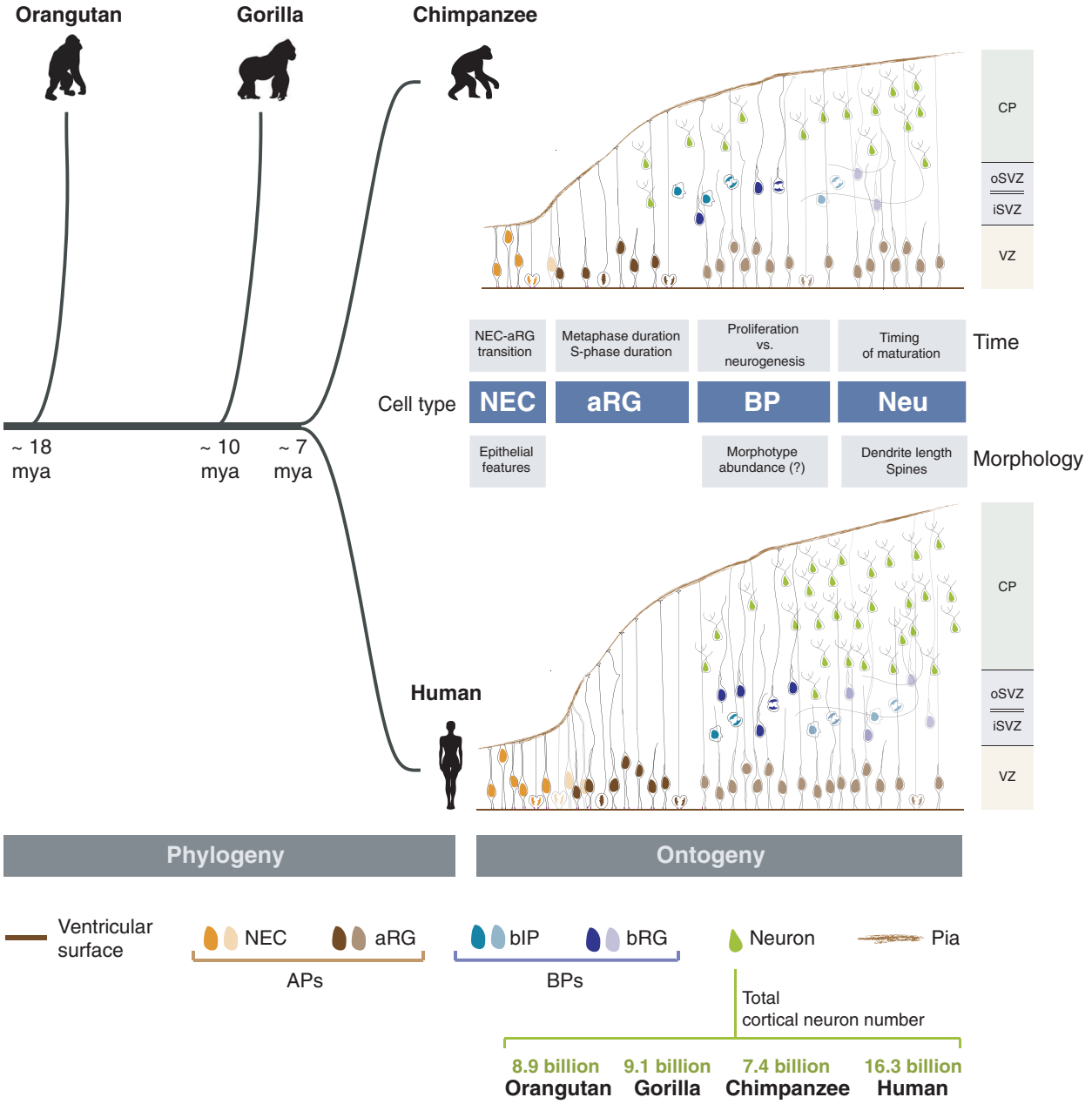

Fig. 1. Illustration of key differences in neocortex development between humans and other great apes. Processes that are subject to a different temporal regulation among these hominids are listed under 'Time', with reference to the cell types concerned (Neu, neuron). Morphological features of the indicated cell types that differ among these hominids are listed under 'Morphology'. Note the greater number of APs, BPs and neurons, and the thicker SVZ and cortical plate (CP), in humans than in the other great apes. For the various cell types and tissue features illustrated, see the key at the bottom; the total number of cortical neurons is indicated [37,60,61]. To illustrate the progression from NECs to aRG to BPs to neurons (from left to right), the nuclei of the various NSPCs are first shown in full colour and then in a pale version of the respective colour.

organoids vs chimpanzee cerebral organoids, neither in metaphase nor in anaphase [27]. Spindle orientation appears therefore unlikely to play a major role in brain size evolution among hominids.

This 'negative' result nevertheless helped to uncover an intriguing cell division difference among hominids. In both human neocortical tissue ex vivo and human cerebral organoids, metaphase duration of APs was

~ 50% longer compared with chimpanzee and orangutan cerebral organoid APs [27]. While a potential function for this AP metaphase prolongation awaits elucidation, it was shown to be absent in the same cultured human iPSCs used to generate the organoids. It then appeared in day 30 (d30) only in the human cerebral organoids, but was then no longer present at d52. This indicates that AP metaphase length is regulated during human neural development and increases in relatively early, more proliferative APs. Consistent with these observations, mouse proliferating APs showed a moderately longer metaphase than mouse neurogenic APs [27]. A longer metaphase is therefore a likely characteristic of early, more proliferative APs, with human APs showing the strongest prolongation.

In macaques, which are 'Old World' monkeys closely related to hominids, the pool of proliferative NSPCs is not only larger than in rodents, but it also remains proliferative for longer. Also, in contrast to rodents, a large AP pool can remain proliferative even when other APs have switched to neurogenesis, by generating mostly basal intermediate progenitors (bIPs) [28–31]. In neural rosettes, the APs of hominids (human and chimpanzee) were in turn shown to remain proliferative for longer than those in macaque rosettes [32], which may contribute to the larger hominid brain. Could there also be differences in NSPC proliferation among hominids? This is indeed suggested by data from cerebral organoids during development, where the proportion of proliferating APs appeared to be depleted more slowly in human than chimpanzee, while the proportion of neurogenic BPs appeared to increase faster in chimpanzee than human. In addition, single-cell transcriptomic data of these organoids were consistent with a higher proliferative potential of human APs than chimpanzee APs [27]. Consistent with this view, neurogenic potential assessed by single-cell transcriptomics was found to increase earlier in chimps than human progenitors [27,33,34]. Taken together, these observations strongly suggest that a longer persistence of proliferative NSPCs in humans than chimpanzee developing neocortex may underlie the larger brain of humans.

What could these time-related differences in NSPC proliferation and neurogenesis mean? It is interesting to note that, during neocortex development, the neurogenic period is different not only between rodents and primates, but also among various primates including humans. In fact, mathematical modelling has shown that the neurogenic period is a likely candidate to sufficiently explain differences in cortical neuron numbers among hominids [35]. Consistent with this modelling, which has received experimental support recently [36], the increase in size among primate brains and neocortices, from monkeys to humans, occurs in similar correlation with the increase in neuron numbers [6,37]. Differences in the proliferation of NSPCs among hominids found at specific stages of neocortex development may therefore mostly reflect differences in the onset, dynamics and end of the neurogenic period. It will be key to identify what underlies the differences in neurogenic period length among hominids that allow NSPCs to proliferate for longer.

When deliberating on the different dynamics in neurogenic period regulation, the length of the cell cycle and its various phases are parameters that should be considered. In developing mouse neocortex, the more proliferative APs have a shorter cell cycle than BPs, which are mostly neurogenic bIPs. This shorter cell cycle is primarily due to a shorter G1 [38,39]. However, human and chimpanzee d52 cerebral organoid APs, a stage where progenitor pool differences were found, did not show a major difference in total cell cycle length. Interestingly though, S-phase was found to be longer in human d52 APs than chimpanzee d52 APs [27], raising the possibility that S-phase lengthening may be involved in the longer-lasting human AP proliferation phase. Consistent with this view, S-phase has been found to be longer in proliferative than neurogenic mouse APs [39], and it is prolonged from early to mid-neurogenesis in macaques, but shortens again towards the end of neurogenesis [19,28]. Taken together, these findings suggest that a longer S-phase of APs is indicative of a greater proliferative potential.

By contrast, in APs of transgenic mice with the human orthologue of human-accelerated regulatory enhancer HARE5, both the total cell cycle and the S-phase were reported to be shorter, compared to APs of transgenic mice with the chimpanzee orthologue [40]. In the former APs, human HARE5 increased expression of FZD8, involved in the Wnt pathway, and the transgenic mice with the human HARE5 orthologue also showed a radial extension of the dorsolateral neocortex that included more FoxP1-positive neurons [40]. While this further supports a role for cell cycle regulation in hominid NSPC proliferation, it suggests a complexity in regulation that will benefit from further work, particularly in organoids.

Epithelial morphology and developmental timing

A recent preprint provides further evidence suggesting a fundamental link between developmental timing and NSPC proliferation potential among hominids. Specifically, very early forebrain organoid APs of human remained longer in a more neuroepithelial (NE)-like

state than those of gorilla, as suggested by morphological features [41]. This NE-like morphology included shorter apicobasal processes and a larger apical contact with the surface of the ventricle-like structures of the organoids. The switch to an aRG-like state occurred earlier in gorilla than human organoid APs, being accompanied by a downregulation of certain epithelial features such as lower and more apically restricted levels of the tight junction marker occludin. The transcription factor ZEB2 was proposed as a driver of this switch, as it was expressed earlier in gorilla than human organoid APs, and its overexpression in human organoid APs mimicked the gorilla organoid phenotype [41]. These findings are of interest in light of the notion that NECs exhibit greater proliferative capacity than aRG [14–16]. Another epithelial difference related to developmental timing between NECs and aRG was reported in the mouse and other vertebrates. NECs, but not aRG, can divide the entire length of the cell, from the tip of the basal process to the apical domain. This maintains the full epithelial morphology throughout mitosis in these early proliferating cells [42]. It is conceivable that such transitional differences may also occur among hominids.

These data further support a broad involvement of epithelial components in the NSPC proliferation vs differentiation decision, even among hominids. In this context, the diversity of apicobasal morphologies has been shown to be involved in NSPC proliferation vs differentiation, by providing spatially modulated access to various cell-extrinsic developmental signals distributed from the ventricle to the pia. For example, an apical endfoot is required to access signals in the cerebrospinal fluid, such as diverse growth factors like sonic hedgehog. On the other side, a basal process increases access to signals in more basal compartments, such as extracellular matrix components in the SVZ [16,19,43,44]. Interestingly, macaque BPs display a higher morphological diversity than rodent BPs and include basal radial glia (bRG, also called outer radial glia) with all possible combinations of single apical and/or basal processes, as well as exhibiting different process lengths [30]. Some of this morphological diversity of NSPCs has recently also been found in ferrets, including variability in basal process length and the presence of short processes called lamellate expansions [45,46] and, in addition, BPs with bifurcated basal processes [47]. Furthermore, the abundance of these more complex BP morphotypes is higher in humans [47]. At the cellular level, the expression of human PALMD-Caax, an isoform of the morphoregulatory protein PALMDELPHIN (or paralemmin-like protein), was found to be required for the abundance of BP process complexity and for their proliferative potential, and partially reproduced these phenotypes in mouse and ferret BPs [47].

Given the general relationship that seems to emerge between abundance of complex basal process morphotypes and BP proliferation [43], it is tempting to speculate that non-human great apes may possess a high abundance of process diversity and complexity that is intermediate between that of carnivores and monkeys and that of humans. Also, it would be interesting to investigate whether such BP morphotype abundance differences are somehow related to developmental timing differences among hominids.

Ultimately, genomic differences are likely to provide a basis for the diversity in neocortex size, structure and function among hominids [48,49]. One example of a gene variant specific to humans that has been shown to increase BP proliferation in primate systems is *ARHGAP11B* [50]. Its expression in the foetal marmoset neocortex was associated with an increased number of BPs, notably bRG, and upper-layer neurons, and also with an expanded neocortex [51]. Furthermore, a recent preprint has proposed that chimpanzee organoids forced to express *ARHGAP11B* had a higher number of BPs [52]. At the cellular level, the observed increase in BPs was shown to depend on higher mitochondrial glutaminolysis [53]. This and other gene variants could help to support the extended metabolic requirements faced by BPs that proliferate further during a longer neurogenic period. Combinations of such gene variants involved in NSPC abundance [54–60] likely constitute major genetic underpinnings of the cellular and molecular changes that sustain the dynamics of NSPC proliferation vs differentiation. Differences in the timing and length of these developmental phases appear to have in turn contributed decisively to the changes in neocortex size and complexity among hominids. In addition, specific patterns in the expression of genes that are common to all hominids could also impact the proliferation vs differentiation of NSPCs, as well as the maturation of neurons. Comparative transcriptomic analyses have therefore also emerged as a source for the identification of relevant candidates [27,33,34].

Differences among hominid neurons

In addition to the analysis of the cellular and molecular mechanisms influencing NSPCs in the developing hominid neocortex, and the resulting numbers of cortical neurons [37,61,62], increasing attention has been devoted to the study of possible differences among

hominids regarding (a) neuronal structure and (b) the dynamics and timing of neuronal maturation.

Neuronal structure

Insight into differences in neuronal structure among hominids has come from comparative analyses of various brain areas. Originally, the analysis of the size and organization of different brain areas has been tackled in anatomical studies based on histology [63,64]. More recently, the use of magnetic resonance imaging on a high number of human and non-human individuals revealed no obvious differences in the relative size of the frontal lobe in hominids [65]. However, a closer inspection and analysis revealed that specific areas of the frontal lobe, in particular area 10 of the prefrontal cortex, were proportionally bigger in humans compared with other hominids [66]. This observation is intriguing, as area 10 is functionally linked to higher cognitive functions, working memory and planning of future actions.

Area 10 in humans features a higher total neuron number. However, in the supragranular layers, despite their greater neuron number, the neuron density was lower in humans compared with other hominids [66]. This lower density reflects a greater spacing between neuronal cell bodies, which in turn suggests the presence of a greater amount of neuropil in humans compared with hominids [66]. Besides glial cell processes, neuropil comprises dendrites including dendritic spines, axons and synapses. It has therefore been suggested that the neurons in human area 10 might have bigger – or longer – processes and, in general, 'more space available for connections with other higher-order association areas' [66]. In line with these observations, human pyramidal neurons were reported to have (a) longer and more complex dendrites compared with the ones of chimpanzee [67–69] and (b) a higher number of – and longer – dendritic spines [70,71].

In the genomic era, we are witnessing the hunt for gene variants setting human neurons apart from the ones of other hominids. *SRGAP2* variants were identified as candidates driving such a difference. The expression of human-specific *SRGAP2C* in rodent neurons increased dendritic spine density. In addition, a major difference was found to be the timing of maturation, as the 'humanized' spines matured at a slower pace [72,73]. As discussed above for NSPCs, this report elegantly shows that a critical feature setting human neurons apart from those of other hominids might be their behaviour with regard to temporal aspects. Specifically, existing evidence indicates delayed dynamics of neuronal maturation (also called neoteny) at a structural and functional levels.

Dynamics and timing of neuronal maturation

The advent of the iPSC technology and the use of iPSC-derived neurons from human and primates have made it possible to take a closer and more direct look at the dynamics of neuronal maturation in hominids. Human iPSC-derived pyramidal neurons have been found to mature more slowly than their chimpanzee counterparts with regard to both structural and functional aspects [74]. Mature IPSC-derived human neurons had longer dendrites than chimpanzee ones, as revealed upon transplantation into the rodent brain. Also, once fully mature, human IPSC-derived neurons tended to be electrophysiologically more active than chimpanzee ones, although they matured at a slower pace [74]. A slower maturation rate of human neurons was also observed in a recent study when using a direct conversion approach, in which induced cortical and sensory neurons were obtained via forced expression of Ngn2 in iPSCs, bypassing the neural progenitor stage [75]. Induced human neurons were found to mature more slowly in terms of both morphology and function. As to the latter, this was found to be the case especially regarding the sEPSCs (spontaneous excitatory postsynaptic currents), a measure of spontaneous synaptic activity. Interestingly, the delayed synaptic communication was paralleled by a delayed expression of genes encoding synaptic proteins [75]. This experimental pipeline allowed to uncouple the generation of neurons from NPCs (developmental process) from the neuronal maturation processes *per se*, minimized the effects of the local environment, and suggested that neoteny is an intrinsic feature of human excitatory neurons and is not limited to pyramidal neurons.

In line with the slow maturation of human neurons, neoteny appears to also be characteristic of their dendritic spines. When human iPSC-derived neurons were transplanted into the mouse brain, they were reported to retain juvenile-like dendritic spine dynamics and to mature over a wider time window [76]. These findings are in line with previous studies that suggest that the human brain develops and matures more slowly than that of closely related primates.

Several questions are still open: what are the reasons for human neoteny, and what are its possible consequences? Is there a causative relation between time and the complexity of neuronal structure, or is it simply that the growth of neurons to a given size scales with time? If the latter were to be the case, then one

would expect the duration of neuronal maturation in humans to last for longer, and/or the actual rate of maturation to be slower, as described for human neuronal neoteny. One can then ask: what may be the advantage of human neurons maturing at a slower pace? One possibility is related to cellular energetics and metabolism. It is known that the exo- and endocytosis cycles of synaptic vesicles are an energetically very expensive process. Hence, a delayed neuronal and synaptic maturation in humans may provide an energetic and/or metabolic advantage, as it would allow the neuron to save energy per unit time that can be invested into other cellular activities.

As for the consequences of human neuronal neoteny, one should consider that synaptic maturation in humans spans a wide time window and extends to childhood. A fascinating hypothesis is that a prolonged neuronal maturation might result in enhanced social learning and therefore substantially contribute to the evolution of cultural behaviours. In line with this, synaptic maturation was found to be faster in macaque compared with chimpanzee and humans [71]. However, a prolonged neuronal maturation could be a two-sided coin, as on the one hand it would provide the cellular basis for a more plastic, adaptable brain, but on the other hand would keep the brain vulnerable to external damage during the longer critical periods of development and maturation. It would also be interesting to understand whether the prolonged window of maturation is making the human brain more prone to mental illness.

Differences within the human lineage

In addition to differences between modern humans and other living hominids, it is also interesting to consider potential neural cell differences between modern and extinct archaic humans, for example Neanderthals and Denisovans, and the role such differences may play in brain development and function. Regarding brain expansion, the modern human brain is not considered to be larger than that of Neanderthals [77]. However, analysis of fossil skull endocasts has shown that the brain of Neanderthals was more elongated, that is less globular, than the one of modern humans, suggesting differences in their respective developmental pathways [78–80]. Interestingly, the level of brain globularity in modern humans was found to be associated with introgressed archaic haplotypes related to myelination and neurogenesis [81], suggesting that these two processes have influenced the evolution of human brain shape and function.

Regarding the genetic makeup of different humans, the sequencing of complete Neanderthal [2,82–85] and Denisovan [86] genomes has helped to identify genomic changes that may be mostly specific to either modern or archaic humans and that are potentially involved in brain development and other processes [87]. *In vitro* studies have begun to probe the potential neurobiological impact of small differences in genomic loci between modern and archaic humans, for example in regulating the binding and activity of a transcription factor potentially involved in speech development [88,89], and in the activity of a sodium channel involved in pain sensitivity [90]. Recently, cortical organoids were used to probe the effects of a single amino acid difference in the gene *NOVA1* (neuro-oncological ventral antigen 1) between archaic and modern humans. Organoids with the ancestral amino acid variant were reported to have a more uneven surface and be smaller, possibly related to the observed higher apoptosis, and differences in protein expression were also seen, including in synaptic marker levels [91].

In addition, other studies have begun to investigate the potential impact of remnants of archaic DNA introgression into modern human genomes [92–95], for example in altitude adaptation [96]; cognitive abilities and craniofacial morphology related to William-Beuren syndrome [97], brain connectivity [98] and even COVID-19 susceptibility [99], a zoonotic disease were neurological consequences are being closely investigated, and which can affect not only humans but also other great apes [100–102]

Outlook

Notable cell biological differences among hominid neocortical NSPCs identified so far not only pertain to key proliferation vs differentiation mechanisms, such as mitosis [27], and regulation of epithelial morphology [41], but they also share a dependence on time. Intriguingly, similar developmental/maturation timing differences have also been found among hominid neocortical neurons [74,75] (Fig. 1). This suggests that a comprehensive characterization of neural cell differences among hominids, and perhaps across broader taxa, requires taking into account the timing of each characteristic process throughout development. With the advent of brain organoids, a need has emerged to not only compare equal times of *in vitro* development (e.g. 'organoid days'), but also include more developmentally equivalent stages whenever possible, and even explore stages that may be a priori considered different. This has the potential to reveal that features

previously thought to be unique for a given organism or taxon can also be observed in others.

In addition to this variety of exciting advances in the comparison of humans to other living great apes, it is likely that emerging studies focused on comparisons with extinct archaic hominins will support a new direction of research, the comparative experimental neurobiology of modern and archaic brains, or experimental palaeoneurobiology.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

FM-B conceived the topic and outline, wrote the paper and edited the paper. ET conceived the figure, wrote the paper and edited the paper. WBH wrote the paper and edited the paper.

References

1 Scally A, Dutheil JY, Hillier LW, Jordan GE, Goodhead I, Herrero J, Hobolth A, Lappalainen T, Mailund T, Marques-Bonet T *et al.* (2012) Insights into hominid evolution from the gorilla genome sequence. *Nature* **483**, 169–175.

2 Prüfer K, Racimo F, Patterson N, Jay F, Sankararaman S, Sawyer S, Heinze A, Renaud G, Sudmant PH, de Filippo C *et al.* (2014) The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43–49.

3 Mailund T, Munch K & Schierup MH (2014) Lineage sorting in apes. *Annu Rev Genet* **48**, 519–535.

4 IUCN (2020) The IUCN Red List of Threatened Species. International Union for Conservation of Nature. https://www.iucnredlist.org/

5 Donahue CJ, Glasser MF, Preuss TM, Rilling JK & Van Essen DC (2018) Quantitative assessment of prefrontal cortex in humans relative to nonhuman primates. *Proc Natl Acad Sci USA* **115**, E5183–E5192.

6 Herculano-Houzel S, Collins CE, Wong P & Kaas JH (2007) Cellular scaling rules for primate brains. *Proc Natl Acad Sci USA* **104**, 3562–3567.

7 Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP & Knoblich JA (2013) Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379.

8 Kadoshima T, Sakaguchi H, Nakano T, Soen M, Ando S, Eiraku M & Sasai Y (2013) Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci USA* **110**, 20284–20289.

9 Kelava I & Lancaster MA (2016) Stem cell models of human brain development. *Cell Stem Cell* **18**, 736–748.

10 Heide M, Huttner WB & Mora-Bermúdez F (2018) Brain organoids as models to study human neocortex development and evolution. *Curr Opin Cell Biol* **55**, 8–16.

11 Arlotta P & Pasca SP (2019) Cell diversity in the human cerebral cortex: from the embryo to brain organoids. *Curr Opin Neurobiol* **56**, 194–198.

12 Chiaradia I & Lancaster MA (2020) Brain organoids for the study of human neurobiology at the interface of *in vitro* and *in vivo*. *Nat Neurosci* **23**, 1496–1508.

13 Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci* **10**, 724–735.

14 Götz M & Barde YA (2005) Radial glial cells: defined and major intermediates between embryonic stem cells and CNS neurons. *Neuron* **46**, 369–372.

15 Götz M & Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* **6**, 777–788.

16 Taverna E, Götz M & Huttner WB (2014) The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. *Annu Rev Cell Dev Biol* **30**, 465–502.

17 Bystron I, Blakemore C & Rakic P (2008) Development of the human cerebral cortex: Boulder Committee revisited. *Nat Rev Neurosci* **9**, 110–122.

18 Smart IH, Dehay C, Giroud P, Berland M & Kennedy H (2002) Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb Cortex* **12**, 37–53.

19 Dehay C, Kennedy H & Kosik KS (2015) The outer subventricular zone and primate-specific cortical complexification. *Neuron* **85**, 683–694.

20 Rash BG, Duque A, Morozov YM, Arellano JI, Micali N & Rakic P (2019) Gliogenesis in the outer subventricular zone promotes enlargement and gyrification of the primate cerebrum. *Proc Natl Acad Sci USA* **116**, 7089–7094.

21 Mitchell C & Silver DL (2018) Enhancing our brains: genomic mechanisms underlying cortical evolution. *Semin Cell Dev Biol* **76**, 23–32.

22 Molnar Z, Clowry GJ, Sestan N, Alzu'bi A, Bakken T, Hevner RF, Huppi PS, Kostovic I, Rakic P, Anton ES *et al.* (2019) New insights into the development of the human cerebral cortex. *J Anatomy* **235**, 432–451.

23 Sousa AMM, Meyer KA, Santpere G, Gulden FO & Sestan N (2017) Evolution of the human nervous system function, structure, and development. *Cell* **170**, 226–247.
24 Homem CC, Repic M & Knoblich JA (2015) Proliferation control in neural stem and progenitor cells. *Nat Rev Neurosci* **16**, 647–659.
25 Mora-Bermúdez F & Huttner WB (2015) Novel insights into mammalian embryonic neural stem cell division: focus on microtubules. *Mol Biol Cell* **26**, 4302–4306.
26 Nano M & Basto R (2017) Consequences of centrosome dysfunction during brain development. *Adv Exp Med Biol* **1002**, 19–45.
27 Mora-Bermúdez F, Badsha F, Kanton S, Camp JG, Vernot B, Kohler K, Voigt B, Okita K, Maricic T, He Z *et al.* (2016) Differences and similarities between human and chimpanzee neural progenitors during cerebral cortex development. *eLife* **5**, e18683.
28 Kornack DR & Rakic P (1998) Changes in cell-cycle kinetics during the development and evolution of primate neocortex. *Proc Natl Acad Sci USA* **95**, 1242–1246.
29 Lukaszewicz A, Savatier P, Cortay V, Giroud P, Huissoud C, Berland M, Kennedy H & Dehay C (2005) G1 phase regulation, area-specific cell cycle control, and cytoarchitectonics in the primate cortex. *Neuron* **47**, 353–364.
30 Betizeau M, Cortay V, Patti D, Pfister S, Gautier E, Bellemin-Ménard A, Afanassieff M, Huissoud C, Douglas RJ, Kennedy H *et al.* (2013) Precursor diversity and complexity of lineage relationships in the outer subventricular zone of the primate. *Neuron* **80**, 442–457.
31 Betizeau M & Dehay C (2016) From stem cells to comparative corticogenesis: a bridge too far? *Stem Cell Investig* **3**, 39.
32 Otani T, Marchetto MC, Gage FH, Simons BD & Livesey FJ (2016) 2D and 3D stem cell models of primate cortical development identify species-specific differences in progenitor behavior contributing to brain size. *Cell Stem Cell* **18**, 467–480.
33 Pollen AA, Bhaduri A, Andrews MG, Nowakowski TJ, Meyerson OS, Mostajo-Radji MA, Di Lullo E, Alvarado B, Bedolli M, Dougherty ML *et al.* (2019) Establishing cerebral organoids as models of human-specific brain evolution. *Cell* **176**, 743–756.
34 Kanton S, Boyle MJ, He ZS, Santel M, Weigert A, Sanchis-Calleja F, Guijarro P, Sidow L, Fleck JS, Han DD *et al.* (2019) Organoid single-cell genomic atlas uncovers human-specific features of brain development. *Nature* **574**, 418–422.
35 Lewitus E, Kelava I, Kalinka AT, Tomancak P & Huttner WB (2014) An adaptive threshold in mammalian neocortical evolution. *PLoS Biol* **12**, e1002000.
36 Stepien BK, Naumann R, Holtz A, Helppi J, Huttner WB & Vaid S (2020) Lengthening neurogenic period during neocortical development causes a hallmark of neocortex expansion. *Curr Biol* **30**, 4227–4237.
37 Herculano-Houzel S (2012) The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. *Proc Natl Acad Sci USA* **109** (Suppl 1), 10661–10668.
38 Dehay C & Kennedy H (2007) Cell-cycle control and cortical development. *Nat Rev Neurosci* **8**, 438–450.
39 Arai Y, Pulvers JN, Haffner C, Schilling B, Nüsslein I, Calegari F & Huttner WB (2011) Neural stem and progenitor cells shorten S-phase on commitment to neuron production. *Nat Commun* **2**, 154.
40 Boyd JL, Skove SL, Rouanet JP, Pilaz LJ, Bepler T, Gordan R, Wray GA & Silver DL (2015) Human-chimpanzee differences in a FZD8 enhancer alter cell-cycle dynamics in the developing neocortex. *Curr Biol* **25**, 772–779.
41 Benito-Kwiecinski S, Giandomenico S, Sutcliffe M, Riis ES, Freire-Pritchett P, Kelava I, Wunderlich S, Martin U, Wray G & Lancaster MA (2020) An early cell shape transition drives evolutionary expansion of the human forebrain. *bioRxiv* [PREPRINT].
42 Kosodo Y, Toida K, Dubreuil V, Alexandre P, Schenk J, Kiyokage E, Attardo A, Mora-Bermúdez F, Arii T, Clarke JDW *et al.* (2008) Cytokinesis of neuroepithelial cells can divide their basal process before anaphase. *The EMBO Journal* **27**(23), 3151–3163. https://doi.org/10.1038/emboj.2008.227.
43 Kalebic N & Huttner WB (2020) Basal progenitor morphology and neocortex evolution. *Trends Neurosci* **43**, 843–853.
44 Amin S & Borrell V (2020) The extracellular matrix in the evolution of cortical development and folding. *Front Cell Dev Biol* **8**, 604448.
45 Rakic P (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. *J Comp Neurol* **145**, 61–83.
46 Reillo I, de Juan Romero C, Cardenas A, Clasca F, Martinez-Martinez MA & Borrell V (2017) A complex code of extrinsic influences on cortical progenitor cells of higher mammals. *Cereb Cortex* **27**, 4586–4606.
47 Kalebic N, Gilardi C, Stepien B, Wilsch-Bräuninger M, Long KR, Namba T, Florio M, Langen B, Lombardot B, Shevchenko A *et al.* (2019) Neocortical expansion due to increased proliferation of basal progenitors is linked to changes in their morphology. *Cell Stem Cell* **24**, 535–550.e9.
48 Geschwind DH & Rakic P (2013) Cortical evolution: judge the brain by its cover. *Neuron* **80**, 633–647.

49 Dennis MY & Eichler EE (2016) Human adaptation and evolution by segmental duplication. *Curr Opin Genet Dev* **41**, 44–52.

50 Florio M, Albert M, Taverna E, Namba T, Brandl H, Lewitus E, Haffner C, Sykes A, Wong FK, Peters J *et al.* (2015) Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. *Science* **347**, 1465–1470.

51 Heide M, Haffner C, Murayama A, Kurotaki Y, Shinohara H, Okano H, Sasaki E & Huttner WB (2020) Human-specific ARHGAP11B increases size and folding of primate neocortex in the fetal marmoset. *Science* **369**, 546–550.

52 Fischer J, Peters J, Namba J, Huttner WB & Heide M (2020) Human-specific ARHGAP11B is necessary and sufficient for human-type basal progenitor levels in primate brain organoids. *bioRxiv* [PREPRINT].

53 Namba T, Doczi J, Pinson A, Xing L, Kalebic N, Wilsch-Brauninger M, Long KR, Vaid S, Lauer J, Bogdanova A *et al.* (2020) Human-specific ARHGAP11B acts in mitochondria to expand neocortical progenitors by glutaminolysis. *Neuron* **105**, 867–881.

54 Dumas L & Sikela JM (2009) DUF1220 domains, cognitive disease, and human brain evolution. *Cold Spring Harb Symp Quant Biol* **74**, 375–382.

55 Vaid S & Huttner WB (2020) Transcriptional regulators and human-specific/primate-specific genes in neocortical neurogenesis. *Int J Mol Sci* **21**, 4614–4633.

56 Ju XC, Hou QQ, Sheng AL, Wu KY, Zhou Y, Jin Y, Wen T, Yang Z, Wang X & Luo ZG (2016) The hominoid-specific gene TBC1D3 promotes generation of basal neural progenitors and induces cortical folding in mice. *Elife* **5**, e18197.

57 Florio M, Heide M, Pinson A, Brandl H, Albert M, Winkler S, Wimberger P, Huttner WB & Hiller M (2018) Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex. *eLife* **7**, e32332.

58 Fiddes IT, Lodewijk GA, Mooring M, Bosworth CM, Ewing AD, Mantalas GL, Novak AM, van den Bout A, Bishara A, Rosenkrantz JL *et al.* (2018) Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis. *Cell* **173**, 1356–1369.e22.

59 Suzuki IK, Gacquer D, Van Heurck R, Kumar D, Wojno M, Bilheu A, Herpoel A, Lambert N, Cheron J, Polleux F *et al.* (2018) Human-specific NOTCH2NL genes expand cortical neurogenesis through Delta/Notch regulation. *Cell* **173**, 1370–1384.e16.

60 Heft IE, Mostovoy Y, Levy-Sakin M, Ma W, Stevens AJ, Pastor S, McCaffrey J, Boffelli D, Martin DI, Xiao M *et al.* (2020) The driver of extreme human-specific olduvai repeat expansion remains highly active in the human genome. *Genetics* **214**, 179–191.

61 Herculano-Houzel S & Kaas JH (2011) Gorilla and orangutan brains conform to the primate cellular scaling rules: implications for human evolution. *Brain Behav Evol* **77**, 33–44.

62 Collins CE, Turner EC, Sawyer EK, Reed JL, Young NA, Flaherty DK & Kaas JH (2016) Cortical cell and neuron density estimates in one chimpanzee hemisphere. *Proc Natl Acad Sci USA* **113**, 740–745.

63 Stephan H, Frahm H & Baron G (1981) New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol* **35**, 1–29.

64 Deacon T (1988) Human brain evolution: II. Embryology and brain allometry. In Intelligence and Evolutionary Biology (Jerison HJ & Jerison I, eds), pp. 383–415, Springer-Verlag, London.

65 Semendeferi K & Damasio H (2000) The brain and its main anatomical subdivisions in living hominoids using magnetic resonance imaging. *J Hum Evol* **38**, 317–332.

66 Semendeferi K, Armstrong E, Schleicher A, Zilles K & Van Hoesen GW (2001) Prefrontal cortex in humans and apes: a comparative study of area 10. *Am J Phys Anthropol* **114**, 224–241.

67 Bianchi S, Stimpson CD, Bauernfeind AL, Schapiro SJ, Baze WB, McArthur MJ, Bronson E, Hopkins WD, Semendeferi K, Jacobs B *et al.* (2013) Dendritic morphology of pyramidal neurons in the chimpanzee neocortex: regional specializations and comparison to humans. *Cereb Cortex* **23**, 2429–2436.

68 Elston GN, Benavides-Piccione R & DeFelipe J (2001) The pyramidal cell in cognition: a comparative study in human and monkey. *J Neurosci* **21**, RC163.

69 Hrvoj-Mihic B, Marchetto MC, Gage FH, Semendeferi K & Muotri AR (2014) Novel tools, classic techniques: evolutionary studies using primate pluripotent stem cells. *Biol Psychiatry* **75**, 929–935.

70 Defelipe J (2011) The evolution of the brain, the human nature of cortical circuits, and intellectual creativity. *Front Neuroanat* **5**, 29.

71 Bianchi S, Stimpson CD, Duka T, Larsen MD, Janssen WG, Collins Z, Bauernfeind AL, Schapiro SJ, Baze WB, McArthur MJ *et al.* (2013) Synaptogenesis and development of pyramidal neuron dendritic morphology in the chimpanzee neocortex resembles humans. *Proc Natl Acad Sci USA* **110** (Suppl 2), 10395–10401.

72 Fossati M, Pizzarelli R, Schmidt ER, Kupferman JV, Stroebel D, Polleux F & Charrier C (2016) SRGAP2 and its human-specific paralog co-regulate the development of excitatory and inhibitory synapses. *Neuron* **91**, 356–369.

73 Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, Jin WL, Vanderhaeghen P, Ghosh A, Sassa T *et al.* (2012) Inhibition of SRGAP2 function by its human-specific paralogs

induces neoteny during spine maturation. *Cell* **149**, 923–935.
74 Marchetto MC, Hrvoj-Mihic B, Kerman BE, Yu DX, Vadodaria KC, Linker SB, Narvaiza I, Santos R, Denli AM, Mendes AP *et al.* (2019) Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. *Elife* **8**, e37527.
75 Schornig M, Ju X, Fast L, Ebert S, Weigert A, Kanton S, Schaffer T, Nadif Kasri N, Treutlein B, Peter BM *et al.* (2021) Comparison of induced neurons reveals slower structural and functional maturation in humans than in apes. *Elife* **10**, e59323.
76 Linaro D, Vermaercke B, Iwata R, Ramaswamy A, Libe-Philippot B, Boubakar L, Davis BA, Wierda K, Davie K, Poovathingal S *et al.* (2019) Xenotransplanted human cortical neurons reveal species-specific development and functional integration into mouse visual circuits. *Neuron* **104**, 972–986.e6.
77 Hublin JJ, Neubauer S & Gunz P (2015) Brain ontogeny and life history in Pleistocene hominins. *Philos Trans R Soc Lond B Biol Sci* **370**, 20140062.
78 Bruner E, Manzi G & Arsuaga JL (2003) Encephalization and allometric trajectories in the genus Homo: evidence from the Neandertal and modern lineages. *Proc Natl Acad Sci USA* **100**, 15335–15340.
79 Gunz P, Neubauer S, Golovanova L, Doronichev V, Maureille B & Hublin JJ (2012) A uniquely modern human pattern of endocranial development. Insights from a new cranial reconstruction of the Neandertal newborn from Mezmaiskaya. *J Hum Evol* **62**, 300–313.
80 Neubauer S, Hublin JJ & Gunz P (2018) The evolution of modern human brain shape. *Sci Adv* **4**, eaao5961.
81 Gunz P, Tilot AK, Wittfeld K, Teumer A, Shapland CY, van Erp TGM, Dannemann M, Vernot B, Neubauer S, Guadalupe T *et al.* (2019) Neandertal introgression sheds light on modern human endocranial globularity. *Curr Biol* **29**, 120–127.e5.
82 Prüfer K, de Filippo C, Grote S, Mafessoni F, Korlevic P, Hajdinjak M, Vernot B, Skov L, Hsieh P, Peyregne S *et al.* (2017) A high-coverage Neandertal genome from Vindija Cave in Croatia. *Science* **358**, 655–658.
83 Hajdinjak M, Fu Q, Hubner A, Petr M, Mafessoni F, Grote S, Skoglund P, Narasimham V, Rougier H, Crevecoeur I *et al.* (2018) Reconstructing the genetic history of late Neanderthals. *Nature* **555**, 652–656.
84 Mafessoni F, Grote S, de Filippo C, Slon V, Kolobova KA, Viola B, Markin SV, Chintalapati M, Peyregne S, Skov L *et al.* (2020) A high-coverage Neandertal genome from Chagyrskaya Cave. *Proc Natl Acad Sci USA* **117**, 15132–15136.
85 Petr M, Hajdinjak M, Fu Q, Essel E, Rougier H, Crevecoeur I, Semal P, Golovanova LV, Doronichev VB, Lalueza-Fox C *et al.* (2020) The evolutionary history of Neanderthal and Denisovan Y chromosomes. *Science* **369**, 1653–1656.
86 Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, Schraiber JG, Jay F, Prüfer K, de Filippo C *et al.* (2012) A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226.
87 Pääbo S (2014) The human condition-a molecular approach. *Cell* **157**, 216–226.
88 Maricic T, Gunther V, Georgiev O, Gehre S, Curlin M, Schreiweis C, Naumann R, Burbano HA, Meyer M, Lalueza-Fox C *et al.* (2013) A recent evolutionary change affects a regulatory element in the human FOXP2 gene. *Mol Biol Evol* **30**, 844–852.
89 Enard W, Przeworski M, Fisher SE, Lai CS, Wiebe V, Kitano T, Monaco AP & Pääbo S (2002) Molecular evolution of FOXP2, a gene involved in speech and language. *Nature* **418**, 869–872.
90 Zeberg H, Dannemann M, Sahlholm K, Tsuo K, Maricic T, Wiebe V, Hevers W, Robinson HPC, Kelso J & Pääbo S (2020) A Neanderthal sodium channel increases pain sensitivity in present-day humans. *Curr Biol* **30**, 3465–3469.e4.
91 Trujillo CA, Rice ES, Schaefer NK, Chaim IA, Wheeler EC, Madrigal AA, Buchanan J, Preissl S, Wang A, Negraes PD *et al.* (2021) Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment. *Science* **371**(6530), eaax2537. https://doi.org/10.1126/science.aax2537.
92 Sankararaman S, Mallick S, Dannemann M, Prufer K, Kelso J, Paabo S, Patterson N & Reich D (2014) The genomic landscape of Neanderthal ancestry in present-day humans. *Nature* **507**, 354–357.
93 Dannemann M, Prufer K & Kelso J (2017) Functional implications of Neandertal introgression in modern humans. *Genome Biol* **18**, 61.
94 Dannemann M, He Z, Heide C, Vernot B, Sidow L, Kanton S, Weigert A, Treutlein B, Pääbo S, Kelso J *et al.* (2020) Human stem cell resources are an inroad to Neandertal DNA functions. *Stem Cell Reports* **15**, 214–225.
95 Vernot B & Akey JM (2014) Resurrecting surviving Neandertal lineages from modern human genomes. *Science* **343**, 1017–1021.
96 Huerta-Sanchez E, Jin X, Asan, Bianba Z, Peter BM, Vinckenbosch N, Liang Y, Yi X, He M, Somel M *et al.*(2014) Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature* **512**, 194–197.
97 Zanella M, Vitriolo A, Andirko A, Martins PT, Sturm S, O'Rourke T, Laugsch M, Malerba N, Skaros A, Trattaro S *et al.* (2019) Dosage analysis of the 7q11.23 Williams region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication. *Sci Adv* **5**, eaaw7908.

98 Gregory MD, Kippenhan JS, Kohn P, Eisenberg DP, Callicott JH, Kolachana B & Berman KF (2021) Neanderthal-derived genetic variation is associated with functional connectivity in the brains of living humans. *Brain Connect* **11**, 38–44.
99 Zeberg H & Pääbo S (2020) The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. *Nature* **587**, 610–612.
100 Damas J, Hughes GM, Keough KC, Painter CA, Persky NS, Corbo M, Hiller M, Koepfli KP, Pfenning AR, Zhao H *et al*. (2020) Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proc Natl Acad Sci USA* **117**, 22311–22322.
101 Melin AD, Janiak MC, Marrone F 3rd, Arora PS & Higham JP (2020) Comparative ACE2 variation and primate COVID-19 risk. *Commun Biol* **3**, 641.
102 Animal and Plant Health Inspection Service, U. S. D. o. A. (2021) Confirmation of COVID-19 in Gorillas at a California Zoo. https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2021/sa-01/ca-gorillas-sars-cov-2