What makes us human?

Developmental mechanisms of cerebral cortex expansion and folding: evolving towards human uniqueness

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Humans are a unique species in their capacity for dominating and changing their environmental conditions, including growing crops, painting caves, building pyramids or flying space shuttles. This uniqueness comes from our brain, particularly the cerebral cortex, one of a kind among mammals in size and complexity. This unmatched human cerebral cortex is the product of very elaborate and precisely controlled processes of embryonic development, which gradually emerged during evolution as a result of changes in DNA sequence. The appearance of new genes from duplication and modification of previously existing ones, together with the appearance of new ways of regulating the levels and patterns of gene expression, favoured the emergence of new progenitor cell types and germinal layers, as well as their novel regulation and expansion during embryogenesis. This increase in the number and diversity of neurons preceded cortical expansion and folding, key elements in the advent of human uniqueness.

Are humans unique? Although we all have a deep sense of what makes us different from other animals (dogs, cats, horses, blue jays, crows… you name it!), this becomes progressively less obvious as we compare ourselves with evolutionarily closer relatives, such as other apes (i.e. gorillas, orangutans and chimpanzees), aside from the fur. Nevertheless, it is very clear that a distinctive feature of humans is our capacity to change our environment, whether growing crops for feeding, building houses for living or airports for travelling. With the industrial revolution, our ability to alter the environment led us to literally change the world.

The human ability to alter its environment was only possible with the advent of fabrication and use of tools (first a sharpened bone, later a smartphone). This required two major innovations in animal evolution: imagination and a skilful use of our hands. Stephen J. Gould argued extensively and wisely on the importance of our opposing thumbs for tool use and hence as our gate to changing the world. Likewise, bipedalism was fundamental to free our hands from locomotion and allow their specialization for tool handling. But yet a pair of skilful hands still depends completely on a creative brain to imagine, plan and execute a production strategy (i.e. crop growing, cave painting, David sculpting or space shuttle building). For this and other reasons (which, in the interests of space, we will not go into), the brain is the ultimate distinctive feature of human uniqueness.

What is unique about the morphology and development of the human cortex?

Comparison of brain size across mammals shows that the human brain is one of the bigger examples, but is not the largest: elephants and blue whales have larger brains (Figure 1). However, if we compare across mammals the ratio between brain weight and body weight (encephalization), then *Homo sapiens* is the champion in its class (Figure 2). Importantly, differences in brain size across mammals are largely due to variations in the size and surface area of the cerebral cortex. Accordingly, the human cerebral cortex is by far the most complex organ in the animal kingdom. Formed by trillions of excitatory and inhibitory neurons exquisitely interconnected for fast and efficient communication, the cerebral cortex is responsible for processing and combining information from all sensory modalities with previously stored memories. As a result, the cerebral cortex gives rise to all kinds of behaviours, including those most characteristic of humans such as abstract thinking, art and sense of humour. In the human lineage, cortical expansion is particularly prominent, and it therefore seemingly enabled the progressive emergence of the higher cognitive functions associated with human nature. Thus we may consider that human uniqueness ultimately emerged from the extreme size and complexity of its brain, particularly the cerebral cortex.

Abbreviations:
aRGC, apical radial glial cell; bRGC, basal radial glial cell; IPC, intermediate progenitor cell; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; SVZ, subventricular zone; TAP, transit amplifying progenitor; VZ, ventricular zone.
Neurons in the cerebral cortex come in different flavours, and are constantly talking to each other and with the rest of the organism, such that the majority of cortical functions emerge from the precision and co-ordination of this activity. Anatomically, the cerebral cortex is a sheet of tissue where neurons are arranged in six distinct layers (a structure also referred to as the neocortex). At the same time, it is subdivided into multiple functional areas, each with a specific composition of neuron types that establish a specific set of connections within itself and with other cortical areas and brain structures. Cortical areas are histologically and functionally different between species, where humans have a larger number and a greater variety of neuron types than any other primate. Moreover, the human brain displays a pronounced specialization of its two cerebral hemispheres, including differences in the frontal granular cortex (which may be unique to primates) and in the expanded posterior parietal cortex that allows sensory guidance of motor actions and decisions. All of these cortical areas together comprise the neocortical network ultimately responsible for generating the full repertoire of human behaviours.

If human uniqueness is primarily due to our outstanding brain and in particular the cerebral cortex, what makes the human cortex so unique? The Russian evolutionary biologist Christian Theodosius Dobzhansky said in 1973, “nothing in biology makes sense except in the light of evolution”; in fact, this notion has been extended by saying that “nothing in evolution makes sense except in the light of development”. Indeed, major neuroanatomical features (such as brain size) can only evolve as the result of significant changes in their development. Taking advantage of this fact, we may reach an approximate understanding of cortical evolution by identifying the genetic and morphogenic events that differ during embryonic development across extant mammals.

Multiple differences distinguish neocortical development between mice and humans: the human neocortex is over 1000-fold larger than that of mouse, the period dedicated to cortical neurogenesis is 20-fold longer, the transient subplate is several times larger and more compartmentalized, the duration of the cell cycle is 3–4-fold longer, cortical areas are much more abundant and diverse in humans compared with mice (humans have more than 50 distinct cytoarchitectural areas), and the postnatal maturation of neocortical circuits lasts for much longer. Importantly, the human neocortex is highly folded on to itself (gyrated or gyrencephalic), whereas the mouse neocortex is smooth (lissencephalic).

As expected, the processes involved in the development of the cerebral cortex also differ significantly.
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Figure 3. Interspecific variation in neural progenitor cells. Schematic diagram of the main progenitor cell types and their lineage relationships in the cerebral cortex of mammals. Very significant differences exist between the developing mouse cortex and that of ferrets and humans. Black arrows indicate lineage relationships demonstrated by timelapse imaging and/or by retroviral lineage tracing. Red arrows indicate hypothetical or assumed lineage relationships, but currently these have not been demonstrated. aRGC, apical radial glial cell; bRGC, basal radial glial cell; CP/IZ, cortical plate/intermediate zone; IPC, intermediate progenitor cell; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; VZ, ventricular zone.

between mice and humans. In mice, neurons of the cerebral cortex are generated by progenitor cells organized in two germinal layers: the ventricular zone (VZ) containing apical radial glial cells (aRGCs) and a relatively small subventricular zone (SVZ), containing mostly neurogenic intermediate progenitor cells (IPCs). In contrast, the human SVZ is severalfold thicker and subdivided into multiple sublayers including inner (ISVZ) and outer (OSVZ) SVZ, which contain a much greater abundance of IPCs than mouse and a large proportion of basal radial glial cells (bRGCs) (Figure 3).

These differences in progenitor cell types and abundance, which are dramatic between mice and humans, but also significant across mammals, are believed to underlie the differences in neuron number and neocortical size and folding across phylogeny.

Other significant differences between cortical development in humans and mice include the sources of inhibitory interneurons (although this remains controversial); the existence of a subpial granular layer in human embryos, which is absent from mice; the molecular specification of a much larger diversity of neuron subtypes in humans; and the genetic regulation of stem and progenitor cells by protein-coding and non-coding RNAs.

Cellular mechanisms: new progenitor cell types or new regulation?

The neocortex develops by the selective expansion of the rostral-most domain of the neural tube, the telencephalon. Differences in size of the cerebral cortex between mammals (i.e. mice compared with humans) emerge from variations in this expansive process, mainly due to the combined effect of three main parameters: (i) size of the initial pool of neural stem cells before the onset of neurogenesis; (ii) rate of self-amplification and self-renewal of radial glial cells and basal neurogenic progenitors during neurogenesis; and (iii) duration of the neurogenic period. All of these parameters have been directly related to the outstanding increase in neuron number and expansion of the primate neocortex compared with that of mice. Specifically, differences in the proliferative activity, abundance and lineage relationships of particular types of neural progenitor cells appear more prone to underlie cortical expansion. In the developing mammalian neocortex, we can distinguish three principal classes of progenitors: apical, basal and subapical progenitors. Apical progenitors divide at the ventricular surface of the telencephalon and include neuroepithelial cells (NECs), which are multipotent, have a high rate of amplification and are typical of the very early stages of cortical development, aRGCs and apical intermediate progenitors (AIPs). Basal progenitors undergo mitosis at basal positions with respect to the ventricular surface, and essentially include IPCs with various capacities of self-amplification (those which are highly amplificative are also named transit amplifying progenitors, or TAPs) and bRGCs (initially also called oRG cells or intermediate radial glia cells by some authors).

The relative abundance of each of these neural progenitor subtypes varies between different neocortical regions, developmental stages and, most prominently, species. bRGCs were originally described in gyrencephalic species (including human and ferret), and, although they were first identified in the OSVZ, other studies found that bRGCs are more commonplace than initially suspected. For example, bRGCs are also abundant in the ISVZ of gyrencephalic ferrets and macaque monkeys; they also exist in lissencephalic primates (i.e. marmoset monkeys) as well as in species without an OSVZ (i.e. mice), albeit at a much lower abundance. In fact, the SVZ in mice is composed mainly of neurogenic IPCs with very limited self-renewing capacity (~90% of basal progenitors) and a very small fraction of bRGCs (~5%) and proliferative IPCs (~5%). In contrast, these proportions are dramatically different in humans and macaques, where bRGCs become the most abundant type of basal progenitor (~75%) and the remaining basal progenitors are mostly TAPs. Furthermore, whereas bRGCs in the lissencephalic mouse essentially undergo neurogenic self-consuming divisions, they undergo multiple rounds of self-amplification in gyrencephalic species, prominently in primates. This exponential increase in relative bRGC abundance and amplification capacity through evolution towards primates and humans suggests a developmental specialization relevant to the emergence of human uniqueness.

The initial phase of OSVZ expansion in the human cortex does not seem to occur at the expense of progenitor cells in the VZ, as this remains remarkably large. A potential alternative is that the OSVZ may be significantly expanded by self-amplification rather than by continued production from VZ progenitors. This possibility is consistent with the self-amplification of bRGCs in ferrets and humans (albeit at different rates). Expansion of the population of progenitors locally in a basal germinal zone has been proposed as a developmental strategy to greatly increase neuron production, and it may therefore be highly relevant for building large brains.
Genetic mechanisms: new genes or new gene regulation?

The hominin fossil records provide some evidence for the sequence of changes in human brain evolution. In addition, the DNA sequence from extinct human ancestors and close relatives provides another window to understand human evolution\(^14\). For example, of the entire human genome, a very small number of genes are specific to humans (as compared with Neanderthals)\(^15\). However, the effect of genetic variation on the timing, sequence and level of gene expression translate into very significant functional differences. One example of these evolutionary mechanisms is the large amount of primate-specific non-protein coding miRNAs, many of which regulate cell-cycle-related genes involved in brain growth\(^8\). Another example is the emergence and specialization of alternative transcripts or splice isoforms, as is evident by recently improved RNA sequencing studies.

For instance, GPR56 is a gene whose mutation leads to malformations of cortical development in humans (namely polymicrogyria, or an excess of cortical folds) accompanied by intellectual disability and epilepsy. The levels of GPR56 expression seem to modulate proliferation of progenitors in the mouse neocortex, as the loss of GPR56 expression impairs neurogenesis and its overexpression enhances proliferation and progenitor cell number\(^16\). Recently identified mutations in this gene reveal many alternative splicing isoforms of a major enhancer region, which results in the emergence of different expression patterns in the developing embryonic cortex. Accordingly, variations in the sequence of these enhancer regions across mammals led to significant changes in its levels and pattern of expression during embryonic development\(^16\). Therefore evolutionary changes in the exact DNA sequence of this enhancer of GPR56 may have contributed significantly to drive the dramatic changes in brain size and folding in gyrencephalic and large-brained mammals, especially humans.

In addition to modifications in existing genes and enhancer/promoter regions, at least 30 gene families show de novo duplications in Homo sapiens\(^17\). Duplications allow the creation of new genes or new functions that might have also been fundamental for brain evolution. For example, a partial duplication of the gene ARHGAP11A in the hominid lineage led to the formation of the human-specific new gene ARHGAP11B, importantly, overexpression of this new gene in the embryo of the naturally smooth neocortex of mice promotes progenitor amplification and cortical folding\(^17\). Similarly, duplication of the SRGAP2 gene may have driven a dramatic increase in the total number of excitatory synapses, producing the emergence of novel neural activity during human cortical evolution\(^18\).

These are just some examples of how genomic screens and functional studies of genetic variations between modern humans and our ancestors (and their Neanderthal contemporaries) are beginning to reveal unique aspects that may have distinguished brain function in ancient humans from others, potentially providing us with a differential advantage for survival and evolutionary success\(^19\).

In summary, a combination of genetic and cellular mechanisms involved in key events of brain development probably drove the expansion and complexification of the mammalian cerebral cortex during evolution, culminating in the uniquely large and complex human brain.

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