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Molecular insights into human brain evolution

Robert Sean Hill[1] & Christopher A. Walsh[1]

Rapidly advancing knowledge of genome structure and sequence enables new means for the analysis of specific DNA changes associated with the differences between the human brain and that of other mammals. Recent studies implicate evolutionary changes in messenger RNA and protein expression levels, as well as DNA changes that alter amino acid sequences. We can anticipate having a systematic catalogue of DNA changes in the lineage leading to humans, but an ongoing challenge will be relating these changes to the anatomical and functional differences between our brain and that of our ancient and more recent ancestors.

Santiago Ramon y Cajal, widely regarded as the founder of modern neuroscience, recognized as early as the turn of the twentieth century that the human brain was not just larger than that of our ancestors, but it differed in its circuitry as well. Over the course of the last century these differences have been extensively studied at a histological level, although specifying the exact changes that distinguish the human brain has been elusive.

> "The opinion generally accepted at that time that the differences between the brain of [non-human] mammals (cat, dog, monkey, etc) and that of man are only quantitative, seemed to me unlikely and even a little offensive to the human dignity… My investigations showed that the functional superiority of the human brain is intimately bound up with the prodigious abundance and unusual wealth of forms of the so-called neurons with short axon." (Ref. 1, translated by J. DeFelipe).

Comparative differences in brain structure

Understanding the genetic changes that distinguish our brain from that of our ancestors starts with defining the key structural and functional differences between the human brain and that of other primates. Our brain is roughly three times the size of the chimpanzee brain, our nearest living relative, from which we diverged 7–8 million years ago, and about twice the size of pre-human hominids living as recently as 2.5 million years ago[2]. The increased size particularly affects the cerebral cortex, the largest brain structure and seat of most higher cognitive functions. The cortex is a multi-layered sheet that is smooth in rodents, but folded in mammals with larger cortices (Fig. 1), allowing more cortex to squeeze into the limited volume of the head.

The enlarged cortex of great apes reflects a longer period of neuronal formation during pre-natal development, so that each dividing progenitor cell undergoes more cell cycles before stopping cell division[3]. Cortical progenitors undergo 11 rounds of cell division in mice[4], at least 28 in the macaque[3], and probably far more in human. In addition to making a larger cortex, the longer period of neurogenesis adds novel neurons to the cortex, so that the cortical circuit diagram differs between primates and other mammals (Fig. 1). Upper cortical layers, generated late in neurogenesis, are over-represented in the primate cerebral cortex, especially in humans[5]. Additionally, special cell types, such as spindle cells (specialized, deep-layer neurons[6]), are unique to primates. The upper-layer neurons that are so unusually common in great apes represent either locally projecting neurons—the "neurons with short axon" of Cajal—or neurons that connect the cortex to itself, but do not project out of the cortex (Fig. 1).

The cerebral cortex shows remarkable local specialization, reflected as functionally distinct cortical 'areas' that are essentially a map of the behaviours and capabilities most essential to each species. For example, whereas rodents show relatively larger areas that respond to odours and sensation from the whiskers, they have small areas subserving their limited vision. In contrast, primates are highly visual, with more than a dozen distinct functional areas analysing various features of a visual scene. Recent work has compared functionally homologous visual regions between humans and macaques, suggesting that some areas are quite similar, whereas other visual areas have been either added or greatly modified during the course of evolution[7]. Primates also have particularly large areas of the frontal lobes anterior to the motor cortex (prefrontal cortex), whereas prefrontal cortex is tiny in non-primates. Prefrontal areas regulate many social behaviours and are preferentially enlarged in great apes. Although it has long been thought that prefrontal cortex is especially enlarged in humans, recent work suggests that other great apes may have equivalent proportions of prefrontal cortex[8].

The human cerebral cortex also shows functional asymmetries, with most of us being right handed and having language function preferentially localized in the left hemisphere. Chimpanzees do not show such strong asymmetry in handedness[9], although their brains show some asymmetries in frontal and temporal lobes (which correspond to language areas in humans)[10]. Recent evidence suggests that the left–right asymmetries of the human cerebral cortex are accompanied by asymmetric gene expression during early fetal development[11], although it is not known whether asymmetries of gene expression are seen in non-human primates. There is some evidence from fossil skulls for cortical asymmetry in human predecessors as well[12].

Evolutionary mechanisms

What sorts of genetic changes underlie diverse brain shape and size? Approaches to this question have come increasingly into focus, although the answers themselves await further work. Three major mechanisms of evolutionary changes include: (1) addition or subtraction of entire genes to or from the genome; (2) alterations in levels or patterns of gene expression; and (3) alterations in the coding sequence of genes. Recent evidence suggests roles for all of these mechanisms.

The recent completion of sequencing the chimpanzee genome emphasizes the highly similar composition of the human and chimpanzee genomes[13]. There is evidence for inactivation of genes, especially many olfactory receptor genes, by their conversion into

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pseudogenes[14]. However, there is currently little evidence to suggest that the addition of novel genes is a major mechanism in human brain evolution[13].

Recent studies suggest that human brain evolution is associated with changes in gene expression specifically within the brain as opposed to other tissues such as liver. A few studies suggest more-accelerated gene expression changes in the brain along the human lineage compared with the chimpanzee lineage[15]. Although the studies differ in design and principal conclusions, they share support for an increase in expression level in a subset of brain-expressed genes in the lineage leading to humans[16,17].

There is also accumulating evidence that some neural genes underwent important changes in their coding sequence over the course of recent brain evolution, although the proportion of neural genes that were targets of positive selection is still in debate. Genes strongly influenced by natural selection can be identified by comparing DNA changes that occur in different, closely related species, for example in different primate species. Synonymous DNA substitutions do not alter the amino acid sequence because they occur at degenerate sites in the codon (such as a CGT to CGG change, as both codons encode arginine). Because synonymous changes do not alter the biochemical properties of the encoded protein, they are usually

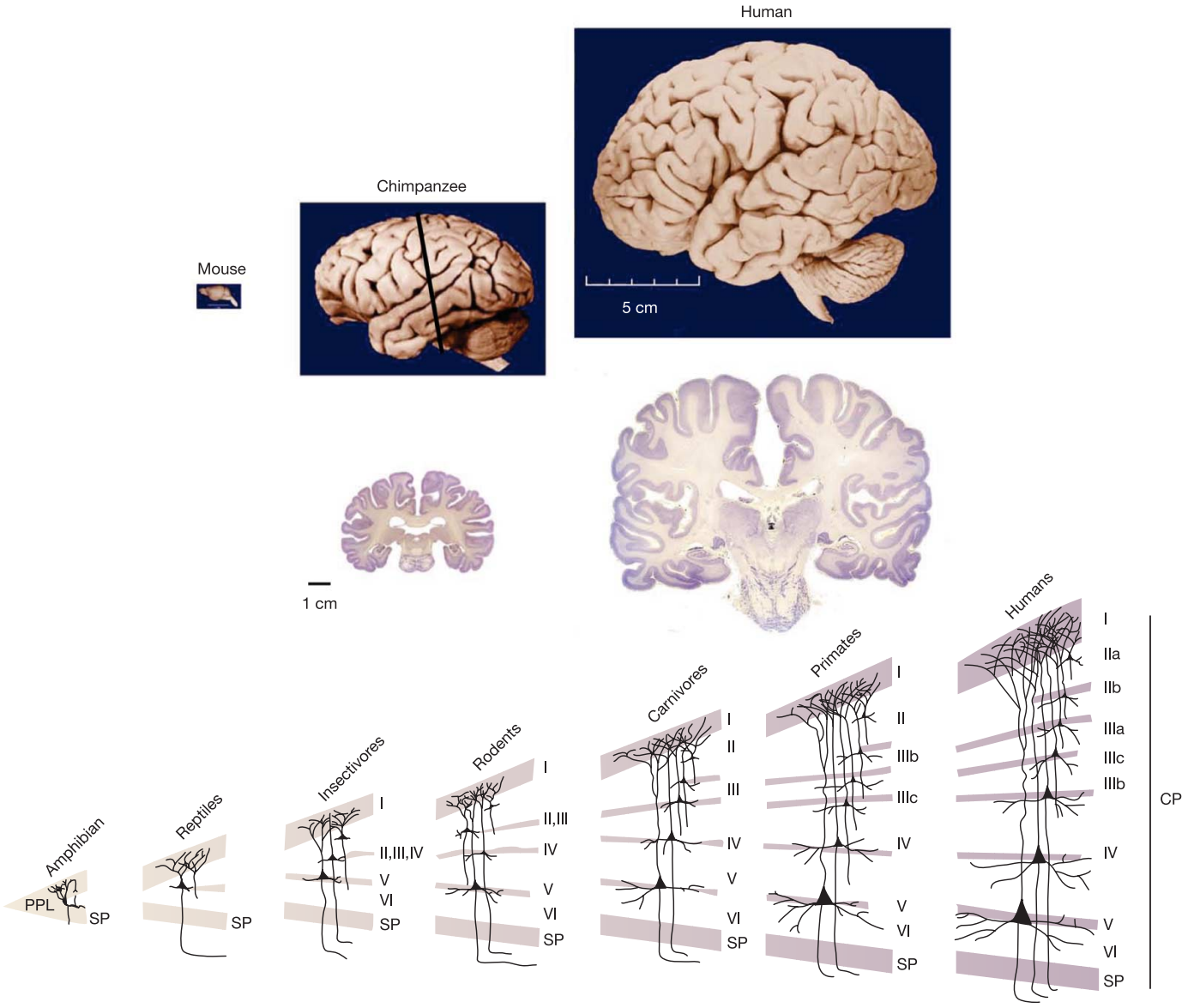

Figure 1 | Differences in cerebral cortical size are associated with differences in the cerebral cortex circuit diagram. The top panel shows side views of the brain of a rodent (mouse), a chimpanzee and a human to show relative sizes. The middle panel shows a cross-section of a human and chimpanzee brain, with the cellular composition of the cortex illustrated in the bottom panel (adapted from ref. 5). The cerebral cortex derives from two developmental cell populations: the primordial plexiform layer (PPL) and the cortical plate (CP). The primordial plexiform layer seems to be homologous to simple cortical structures in Amphibia and Reptilia, and appears first temporally during mammalian brain development. The cortical plate develops as a second population that splits the primordial plexiform layer into two layers (layer I at the top and the subplate (SP) at the bottom; numbering follows the scheme of ref. 31). Cortical-plate-derived cortical layers are added developmentally from deeper first (VI, V) to more superficial (III, II) last. Cortical-plate-derived cortical layers are progressively elaborated in mammals with larger brains (for example, insectivores have a single layer II/III/IV that is progressively subdivided into II, III, IV, then IIa, IIb, and so on), so that humans have a larger proportion of these late-derived neurons, which project locally or elsewhere within the cortex. Images from the top and middle panels are from the Comparative Brain Atlas (http://www.brainmuseum.org).

evolutionarily neutral. In contrast, non-synonymous DNA changes alter the amino acid sequence. The vast majority of non-synonymous DNA changes represent disabling mutations that cause disease, hence decreasing the fitness of the organism, and so most non-synonymous DNA changes are subject to negative, or purifying, selection. In contrast, on rare occasions non-synonymous DNA changes might make the protein work slightly better, hence increasing the fitness of the organism and becoming subject to positive selection (that is, advantageous changes propagated to future generations). A ratio of non-synonymous (K_A) to synonymous (K_S) changes $\ll 1$ is typical of most proteins where change is detrimental[18]; rare proteins show $K_A/K_S > 1$, which can indicate positive selection.

In order to test whether genes expressed in the brain were frequent targets of positive selection in primates, one study[19] analysed 200 brain-expressed genes, comparing them to 200 widely expressed genes. They compared K_A/K_S ratios between rats and mice and between humans and macaque monkeys. They concluded that genes involved in brain development or function had a higher tendency to be under positive selection between macaques and humans than between mice and rats. In contrast, systematic surveys of K_A/K_S ratios across much larger numbers of genes between chimpanzees and humans failed to show that neural genes, as a group, have higher K_A/K_S ratios than genes expressed outside of the brain between these two species[20,21]. Analysis of the top 50 genes with the highest K_A/K_S ratios showed surprisingly few with known essential roles in the brain[20]. Analysis of the chimpanzee genome confirms that neural genes, as a group, have much lower average K_A/K_S ratios than genes expressed outside of the brain[13]. However, the more recent study suggested that a substantial fraction of the genes with the highest K_A/K_S ratios had roles in brain development or function[13]. These studies are most easily reconciled by suggesting that a small subset of neural genes may be targets for positive selection (see below), whereas neural genes as a whole are subject to intense negative selection due to the severe disadvantages conferred by mutations that disrupt brain function.

Correlation of genetic evolution with human brain function

Whereas genome-wide analyses systematically highlight targets of positive genetic selection in the human lineage, there has been great interest in a subset of human genes that show positive evolutionary selection, and for which correlations between evolutionary patterns and gene function in humans are possible. For example, mutant alleles of *FOXP2* cause a severe disorder of articulation and speech in humans, yet subtle differences in *FOXP2* sequence between humans and non-humans show evidence of positive evolutionary selection by K_A/K_S ratio. Its involvement in speech production suggests that changes in *FOXP2* may have been important in the evolution of language[22,23]. Furthermore, analysis of *FOXP2*'s DNA sequence in diverse human populations suggests that the gene shows unusually low sequence diversity—that is, many human populations share a common ancestral sequence at the *FOXP2* locus. This evidence for a 'selective sweep' (explained in detail in several recent reviews[2,24]) within humans suggests that evolutionary selection on this gene may have occurred very recently in human evolution; that is, after the appearance of *Homo sapiens*.

Two genes that cause microcephaly (small cerebral cortex) also show strong evidence for positive evolutionary selection. Microcephaly reduces the human brain to 50% or less of its normal mass; that is, to about the size of the brain of chimpanzees or our pre-human ancestors. Whereas marked mutations in abnormal spindle microcephaly (encoded by the *ASPM* locus) and microcephalin (encoded by the *MCPH1* locus) cause microcephaly, both genes show strong evidence that subtler sequence changes were subject to positive selection in the lineage leading to humans (manifested by a high K_A/K_S ratio)[25–29]. Although the precise functions of the two genes are unknown, both are highly expressed in dividing neural precursor cells in the cerebral cortex, and available evidence suggests roles in cell proliferation. Notably, just as neurons in the upper layers of the cerebral cortex (Fig. 1) are added last during development, and are most highly elaborated in humans and great apes, these upper-layer neurons are preferentially lost in many cases of microcephaly, supporting a requirement for microcephaly genes in the formation of the upper cortical layers.

AHI1, which is essential for axon pathfinding from the cortex to the spinal cord (and hence for normal coordination and gait), is another gene that causes a neurological disease when mutated, but for which subtler changes between primate species suggest positive evolutionary selection in the lineage leading to humans[30]. Patients with *AHI1* mutations not only show mental retardation, but can also show symptoms characteristic of autism, such as antisocial behaviour. This raises the intriguing possibility that evolutionary differences in *AHI1* may relate not only to human patterns of gait, but potentially species-specific social behaviour.

The linkage of studies of gene function in humans with evolutionary analysis is just beginning, and is limited mainly by the rate at which the essential functional roles of genes in the human brain are elucidated. As a population, humans show many mutant alleles for every gene that has been extensively studied, so that the human population is likely to represent, to a first approximation, saturation mutagenesis, such that for each gene in the genome there is a human carrying a mutated allele for that gene. Many neurological diseases affect the very processes that define us evolutionarily as human: intelligence (mental retardation), social organization (autism and attention deficit disorder) and higher-order language (dyslexia). As the genes for these uniquely human disorders are characterized, they may give us new insight into our recent evolutionary history.

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