Understanding the human brain: insights from comparative biology

Alex R. DeCasien[1,2,3], Robert A. Barton[4], James P. Higham[1,2]

1. Department of Anthropology, New York University
2. New York Consortium in Evolutionary Primatology (NYCEP)
3. Section on Developmental Neurogenomics, National Institute of Mental Health
4. Evolutionary Anthropology Research Group, Durham University

Correspondence: alex.decasien@nyu.edu (A.R. DeCasien)

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Abstract

Human brains are exceptionally large, support distinctive cognitive processes, and evolved by natural selection to mediate adaptive behavior. Comparative biology situates the human brain in evolutionary context to illuminate how it has been shaped by selection and how its structure relates to evolutionary function, while identifying the developmental and molecular changes that were involved. Recent applications of powerful phylogenetic methods have made new findings, some of which overturn conventional wisdom about how brains evolve. Here, we focus on four long-standing claims about brain evolution, and discuss how new work has either contradicted them or shown them to be much more complicated than previously appreciated. Throughout, we emphasize studies of nonhuman primates and hominins, our recent ancestors and close relatives.

Main body

Updating our beliefs about human brain evolution

The human brain is, in comparative terms, extraordinarily large, particularly among **primates** (see Glossary). It contains almost 90 billion neurons, approximately two-and-a-half times more than the brains of our closest living relatives, the great apes [1]. It also contains hundreds of trillions of synapses, which connect nerve cells to create neural networks of staggering complexity. Altogether, the brain is the quintessence of what Darwin [2] termed “organs of extreme perfection and complication” – a complex biological structure with many interacting parts that together produce a whole greater than the sum of their parts. Comparative biology is key to unlocking the secrets of the brain, as its methods allow us to examine how the ‘experiments’ of **natural selection** gave rise to the brains of living species, including humans. Not only can we test hypotheses about the adaptive significance of neurobiological traits, but we can also identify how human brains conform to, or deviate from, broader evolutionary trends and ‘expectations’ (Box 1). Neuroscience has a deep history of adopting this approach: in the mid-late 1800s, Thomas H. Huxley showed that humans are not unique in possessing a ‘hippocampus minor’, thereby winning the ‘Great Hippocampus Debate’ against Richard Owen and bolstering claims that humans are closely related to other primates [3]. Today, it is recognized that robust comparative analyses must include many species from lineages exhibiting trait variation across a broad **phylogenetic** range (i.e., distantly related species), to

avoid the problem that low statistical power leads to unreliable inferences. Although researchers often encounter a tradeoff between phylogenetic breadth and data precision, ongoing efforts have increased the availability of detailed neuroanatomical and "omics" data from a wider sampling of primate species, facilitating remarkable insights into human brain **evolution**.

We suggest that the broad acceptance and popularity of certain paradigms has infused comparative neurobiology with specific preference biases, influencing researchers' study designs and interpretations for decades. Because of limited data availability, these ideas were often based on analyses of a small number of species. Now, comparative research is increasing the power to robustly detect patterns by incorporating novel data sets, innovative statistical approaches, and explicit phylogenetic modelling. As a result, some long-standing claims about brain evolution have recently been questioned or even contradicted. Here, we focus on four ideas that have guided a large proportion of brain evolution research. Specifically, we address one popular view in the literature, that "social complexity is the primary driver of nonhuman primate and human brain evolution" (Claim 1), instead suggesting at least an equal role for **ecological** factors. Such studies have relied, in part, on the assumption that "brain size has similar effects and cognitive implications across a wide range of species" (Claim 2); however, new work highlights that the significance of brain size variation depends on which mosaic structural changes were involved. A better understanding of these mosaic patterns of evolution can help us evaluate whether certain 'human-specific' traits are the consequence of adaptive specialization or **allometric scaling** [4]. For example, studies that focus on the prefrontal cortex (PFC) as the 'seat of human intelligence' often rely on claims that "the proportionally large human PFC reflects selection on PFC-specific functions" (Claim 3); however, new work suggests that allometry may be a sufficient explanation for human PFC size, and that the importance of other cortical regions and subcortical structures in human cognition have been understimated. Finally, while allometric scaling is important for understanding patterns of covariation among brain regions, recent studies largely reject that "developmental constraints play a major role in the evolution of brain structure" (Claim 4), instead highlighting the role of functional anatomical integration in dictating the coordinated evolution of parts. Throughout, we emphasize recent studies of primates, including humans, **extant** nonhuman primates, and the **hominins** (our **extinct** close relatives and recent ancestors). Not only do primate brains exhibit distinct structural features (Box 2), but extensive socioecological and behavioral variation across species makes them an ideal group for detecting instances of convergent brain evolution across different lineages [e.g., 5,6].

Claim 1: Social complexity is the primary driver of nonhuman primate and human brain evolution

Perhaps the most widely accepted adaptive hypothesis for the evolution of large brains is the Social Brain Hypothesis (SBH). The SBH posits that social complexity is the primary driver of evolutionary increases in brain size, since large brains facilitate certain cognitive skills (e.g., transitive inference, deception, manipulation) that support more complex social systems [7]. Although the earliest studies proposed that ecological variables (e.g., diet quality) best predict relative brain size across primates (e.g., [8]), ideas focused on social complexity – supported by empirical correlations between social group size and relative brain/neocortex size [7,9] – subsequently dominated the literature for decades. During this period, this idea inspired an enormous amount of research across a diverse set of animal groups and received a large amount of popular media coverage. However, inconsistent and small sample sizes slowly led to the emergence of conflicting results across studies, including: 1) claims that **polygynandrous** species (living in large groups) or monogamous species (living in small family groups) have the largest brains and neocortices [9–12]; and 2) work suggesting that ecological variables are also important within primates (e.g., **cathemeral strepsirrhine** primates have relatively large brains [13]; fruit eating **diurnal haplorrhine** primates relatively large neocortices [14]). New studies have attempted to resolve these inconsistencies by incorporating data from many more species, furnishing greater statistical power and the capacity to test more complex statistical models. These studies concluded that ecology (dietary complexity, home range size, and/or **activity pattern**), rather than sociality, best predicts relative brain and neocortex size across primates [15–18]. In line with these findings, new studies suggest that primate species with relatively larger brains exhibit greater manipulation complexity [19] and technical innovation [20], and computational models suggest that modern human brain and body sizes are most likely obtained when individuals face a combination of ecological and social challenges [21].

Given that the brain and neocortex are structurally and functionally heterogenous, evolutionary changes in the size of these areas are necessarily the result of selection on specific neural systems within these areas (see Claim 2). For example, the suggested link between ecological factors and brain expansion may reflect selection on visual information processing systems specifically, since: 1) the latter comprise a large proportion (over 50%) of the neocortex and, therefore, the brain of some anthropoid primates [22]; 2) the relative sizes of visual brain structures (LGN and V1) explain a large proportion of variance in relative brain size across

species (~35-45%) [22]; 3) brain and neocortex size are predicted by visual specializations (e.g., number of **LGN** parvocellular neurons) across primates [22]; and 4) in multiple primate lineages, visual specializations and fine visuo-motor control are likely to have evolved to facilitate foraging behavior (fruit identification, selection, and manipulation) prior to the emergence of colorful social signals [23]. Accordingly, although most studies of human uniqueness have focused on aspects of human social cognition and behavior (e.g., theory of mind, cooperation, language), perhaps a greater focus should be placed on the sensorimotor and cognitive skills associated with human-specific ecological characteristics (e.g., the high quality diets and costly processing behaviors that comprise the hunter–gatherer ecological niche) [24]. In fact, new work demonstrates that although human hunter-gatherers and horticulturalists spend a similar amount of energy on subsistence as other great apes, humans achieve greater foraging efficiency energy capture per hour [25].

The findings discussed above do not suggest that the impact of sociality on brain evolution has been negligible. Sociality may be related to the evolution of specific neural systems without necessarily impacting overall brain size (see under Claim 2, below). In addition, while hominin evolution involved major ecological innovations (e.g., production and use of tools, fire for cooking) that were necessary to obtain enough calories per day to sustain large brains and prolonged parental investment [26], the knowledge of these skills were, and continue to be, transmitted socially over an extended period of development and require extensive cooperation to meet the costs of extended development (see Box 3 on **life-history** correlates of brain size). However, evolutionary increases in relative brain size must necessarily overcome the associated energy costs through stable increases in energy input and/or reallocation of energy away from body maintenance (e.g., locomotion, other organs) or production (e.g., growth, reproduction) [27]. While it is relatively straightforward to link certain ecological factors (e.g., fruit-eating) to both specific selection pressures relevant to the brain (e.g., visual information processing) and increased energy availability, it is more difficult to do so for various measures of social complexity. For instance, living in either smaller or larger groups may decrease the probability of starvation, since the former experience reduced within-group competition for food while the latter experience a higher probability of winning between-group contests for food [28].

Inconsistencies across the aforementioned studies are likely to, in part, reflect different modelling or data selection approaches and difficulties surrounding how to properly measure ecological or social complexity. For instance, ranging data may better represent species

differences in spatial cognition [29], and non-linear approaches may better capture potential group size effects since new work suggests that mammals living in medium-sized groups experienced more rapid brain size evolution [30]. Accordingly, studies of more specific neuroanatomical and behavioral traits are likely to more precisely capture coevolutionary patterns [31] (see Claim 2), so our interpretations of brain size correlations must be balanced and cautious. Nevertheless, to the extent that the SBH was built upon correlations between relative brain or neocortex size and socioecological variables, and to the extent that large-scale analyses obtain consistent results, the current weight of evidence does not clearly support the SBH.

Claim 2: Brain size has similar effects and cognitive implications across a wide range of species

Some of the main motivations for studies of the socioecological correlates of brain size include observations that: 1) modern humans and (many) hominins stand out among primates in terms of absolute and relative brain size (Figure 1; see Figure 2 on issues with different brain size measures); and 2) evolutionary changes in hominin brain size were particularly rapid (directional and accelerating) relative to other primate lineages [32–35]. However, one major complication in interpreting the significance of evolutionary changes in relative brain size is that variation has been produced differently in different lineages [36]. An evolutionary history of increasing brain size is not unique to humans, as absolute and relative brain size tended to increase in parallel in multiple primate lineages [36]. Decreases also occurred in certain lineages within all major clades, albeit rarely [36]. However, while large relative brain sizes in some lineages (e.g., hominins) reflect faster evolutionary increases in brain than body size [36,37], high **encephalization** in other lineages (e.g., callitrichids) reflects slower brain than body size decreases [36]. These findings suggest that, while brain and body size generally show strongly correlated evolution, brain-body allometry is not constrained to a single stable scaling relationship [36] due to brain and body size-specific selective and genetic mechanisms. These distinct evolutionary histories should therefore be considered when selecting or evaluating model species based on brain size or behavior.

It is tempting to interpret evolutionary increases in brain size as a reflection of some global cognitive benefit of larger brains, a view reinforced by comparative studies linking brain size to various measures of ‘intelligence’. For example, nonhuman primate species with larger brains are reported to perform better on problem-solving tasks measuring self-control [38] and exhibit

higher ‘global cognition’ composite measures (including tool use, learning, discrimination tasks) [39], manipulation complexity measures [19], technical innovation rates [20], and social learning rates [20,40]. However, substantial deviations from these relationships exist, which are particularly apparent in studies of larger taxonomic groups [38,41]. A potential confound is that performance on these tasks may be affected by sensory capacities such as visual acuity or visual motion tracking. In addition, although correlations between overall brain size (or specific regions) and so-called **general intelligence** may be alluring [42,43], biologically meaningful definitions of general intelligence are elusive, and multiple conceptual and methodological issues confound interpretations [44,45]. For example, each functional brain network is likely to influence multiple cognitive/sensorimotor functions, and each of these functions is likely to influence multiple behaviors and performance on multiple tests. Accordingly, observed correlations among performance measures may not reflect a single, “general” cognitive or biological property that is itself subject to selection, but rather multiple, overlapping many-to-many relationships [46]. Additionally, it is inappropriate to implement dimensionality reduction on cognitive performance and interpret the first component as ‘general intelligence’ without confirming a non-random correlation structure [44]. Thus, the idea that general intelligence is a useful construct for understanding cognitive evolution has been challenged

Further undermining the notion that selection operated on some general cognitive capacity, comparative studies have revealed complex patterns of mosaic adaptive evolutionary change in primate brain structure. Within the primate order, there are clear differences in relative brain region sizes, particularly between the sub-orders, strepsirrhines and haplorrhines (e.g., haplorrhines have relatively expanded neocortices [18,47,48]). These differences reflect adaptations to distinct ecological niches, as strepsirrhines are largely nocturnal and haplorrhines predominantly diurnal, resulting in greater investment in olfactory and visual systems, respectively. Haplorrhine visual specializations include differences in the layering of the LGN [49] and larger visual cortices containing more distinct areas [18,49]. Strepsirrhines have visual specializations for increasing photosensitivity in dim light, but their low visual acuity requires less neural tissue. Instead, strepsirrhines exhibit larger olfactory bulbs (possibly reflecting evolutionarily recent size increases [50]) and also retain accessory olfactory bulbs (AOBs), lost in some haplorrhines [18,51]. These differences highlight the role of sensory specialization and mosaic change in brain evolution and exemplify how a focus on overall brain size can conceal the role of adaptive specializations.

Recent comparative studies have illuminated additional links between mosaic brain structure and socioecology in primates. For instance, larger social groups and higher quality diets produced either expanded olfactory or visual systems, depending on whether the lineage was nocturnal or diurnal, respectively [18,51]. This may reflect that fruit eating requires visual or olfactory detection and discrimination, and that complex sociality relies on social communication via visual or olfactory signaling, depending on whether a species is active in a high/low light environment. Similarly, species with more frequent alloparental care exhibit a higher relative proportion of neuropil in facial nucleus of the brainstem, which may reflect increased facial dexterity to facilitate nonverbal communication between infants and caregivers and/or between caregivers [52]. Furthermore, the AOB, involved in pheromonal communication, is smaller in pair-living compared to group-living or dispersed species, which may reflect chemosignal mediated inter- and/or intra-sexual competition in group-living species and enhanced pheromone detection in dispersed species. Additionally, solitary primate species have expanded hippocampi, which may reflect the demands of locating dispersed mates [18]. Nutritionally higher quality diets are negatively correlated with hippocampus and schizocortex size, which may reflect that insectivorous primates hunt their unpredictably distributed prey opportunistically, rather than using spatial memory [18]. New work on closely related species also suggests a link between diet and spatial cognition. For example, the most frugivorous lemur species exhibits more robust spatial memory than the most folivorous species [53], and, relative to bonobos, chimpanzees are both more dependent on patchy food sources and have more accurate spatial memory [54]. Within the hippocampal complex, cornu ammonis 1 (CA1) volume is negatively and fascia dentata volume is positively correlated with home range size [55,56]. Interestingly, humans exhibit a unique combination of hippocampal and neocortical traits [48] and there may have been particularly large shifts in hippocampal size and organization in the human lineage [55]. This body of work demonstrates that mosaic patterns of evolution are not only relevant to understanding brain size evolution but are also critical for understanding structural evolution within major brain regions.

Changes to overall brain shape are likely to reflect alterations in underlying brain region sizes, so we can also identify mosaic aspects of human brain evolution using hominin **endocasts**. These types of data are critical complements to comparative analyses of extant species since they provide direct evidence of evolutionary events. For example, while modern humans and **Neanderthals** exhibit similar brain sizes, new work has confirmed that the former have more globular endocrania with wider, longer parietal and larger cerebellar regions [57,58]. This

human-typical endocranial shape emerges early in development [59] and may have occurred recently in human evolution [60,61].

One reason for the focus on brain size is that, compared to other neurobiological measures, comparative studies of brain size are often more feasible. Although brain size is an interestingly variable biological trait, with variation that is related in some way to cognitive capacities, the link is in no way simple. Accordingly, while brain size has traditionally been considered a reflection of general computational capacity and, therefore, a potential target of selection, new studies continue to illuminate how brain size emerges from mosaic evolution and reflects different sorts of specializations in different evolutionary lineages. This is in line with evidence that certain cognitive skills evolve independently from each another in response to specific physical and social environments [62].

Claim 3: The proportionally large human PFC reflects selection on PFC-specific functions

Allometric scaling patterns vary greatly among brain components, leading different regions to represent larger, smaller, or similar proportions of overall brain volume as brain size increases. One result of these patterns is that human brains exhibit the highest proportion of neocortex among primates, contributing to a long-standing bias focusing research on this area. However, recent evidence accounting for both phylogenetic and allometric effects identified only one shift to larger neocortex size during primate evolution, at the origin of haplorrhines, suggesting that the human neocortex is not exceptionally large among this group [33]. Furthermore, this region exhibits correlated evolution with the cerebellum and the structures connecting them [47,63,64], suggesting that wider cortico-sub-cortical circuits were a major target of selection. This pattern of correlated expansion characterizes mammalian brain evolution more widely [64], and in primates, appears to reflect elaboration of visuo-motor systems [64]. Recent studies also suggest that among primates, apes exhibit a distinct pattern of cortico-cerebellar coevolution: the ape cerebellum, especially the lateral cerebellum, is larger than in other anthropoid primates [65], there was an evolutionary shift to larger cerebellar volume in apes [33], rates of cerebellar versus neocortical expansion were 3-4 times higher within the great ape and hominin clades compared to other haplorrhines [66], and apes converge with pinnipeds and cetaceans in having large lateral relative to medial cerebella [67]. New work also shows that during ape evolution, genes involved in cerebellum development were more likely to be targets of positive selection than genes involved in neocortical development, whereas on the rest of the primate

phylogenetic tree, changes in cerebellar and neocortical genes were equally likely [68]. Hence, the cortico-centric bias of much comparative research appears to be unwarranted, and to neglect important patterns of correlated evolution among cortical and subcortical regions.

Given that allometry-related differences in proportional region size may be functionally equivalent across species [69], attempts to identify adaptive neuroanatomical changes underlying distinctively human abilities have focused on species differences in relative region size (i.e., departures from predicted allometric relationships). Certain neocortical regions have been widely assumed to be relatively expanded in humans, including the frontal lobe, in particular the PFC, or part of the PFC (e.g., [70–72]). However, some studies of several independent datasets and scaling regions report that the human PFC does not depart from allometric expectations or exhibit outstanding rates of evolution [73], and recent evidence suggests that human brains do not contain more PFC neurons than expected [74]. Accordingly, while the human PFC represents a large proportion of total cortical volume [72], this may reflect general allometric scaling laws related to conserving the functional properties of large-scale networks rather than selection on PFC function specifically [4]. Put another way, the PFC is likely to be a critical part of multiple networks that facilitate distinctive human abilities; however, it is unjustified to focus on the PFC specifically, rather than on these extended networks, if the PFC has expanded together with its connected regions rather than independently. Conflicting results across studies are likely to reflect low sample sizes and different statistical methods, data sets, demarcation methods, comparison groups, and scaling regions [4]. For example, while human PFC appears large relative to some regions (e.g., V1, which is relatively small in humans) [70,71], studies using different scaling variables suggest that humans are not outliers or that non-human species are outliers [73]. Until clear criteria can be agreed upon and enough data are collected to allow robust statistical inference, the issue of relative PFC expansion in humans will remain unresolved. In addition, studies using more detailed neurobiological measures (albeit, with more limited species sample sizes) have also highlighted possible ‘human brain-specific’ traits outside the PFC (Box 4). Overall, new work suggests that emphasis on the human neocortex (in particular, the PFC) has been excessive and has distracted attention from the importance of wider neural networks as the basis of human neuro-cognitive specializations.

Claim 4: Developmental constraints play a major role in the evolution of brain structure

Two hypotheses have dominated the discussion about the underlying cause of allometric scaling among brain components. The developmental constraints hypothesis explains correlated evolutionary change across brain regions as a consequence of strongly coupled developmental processes [75] (i.e., allometric patterns do not necessarily reflect adaptive co-functionality). One aspect is the so-called 'late equals large' hypothesis, which suggests that late-maturing structures (e.g., neocortex) become disproportionately large as brain size increases through relatively prolonged neurogenesis. The functional constraints hypothesis instead assumes that brain regions evolve together due to selection acting on the functional systems that connect those regions, and that allometries reflect the ways in which relative size changes maintain functional equivalence [47,76,77]. It predicts more complex, 'mosaic' patterns of change at the network level, since brain structure should evolve adaptively and in response to changing environments. It also suggests that 'concerted' patterns of brain evolution do not represent conclusive evidence for developmental constraints, since allometric relationships between brain areas may result from selection to maintain functional connectivity. This is supported by recent computational modeling work [78], which also suggests that the value of mosaic or concerted patterns may fluctuate through time in a variable environment, and that developmental coupling may not be a strong evolutionary constraint. Hence, the concept of concerted evolution can be decoupled from that of developmental constraints.

In line with this, recent neuroanatomical studies suggest that instances of mosaic evolution occur against a background of concerted evolution. For example, in songbirds, the sizes of most brain nuclei co-vary with one another [79]; however, inter-regional pairwise size correlations are higher within functional systems than between systems [79]. Similarly, in primates, fish, and dragon lizards, overall size explains most internal brain structure variation [80–82]; however, remaining variation is associated with species- or lineage-specific adaptations (e.g., relatively large cerebella in mormyroid fish with electrosensory systems) [80–82]. Interestingly, artificial selection experiments in guppies suggest that selection on a specific brain region can produce changes in the relative size of that region (without changes to other regions) in just a few generations [83]. While patterns of both concerted and mosaic evolution are consistent with the functional constraints hypothesis, mosaic evolution precludes a strong developmental constraints hypothesis [76,78].

In further support of the functional constraints hypothesis, new genetic studies suggest a lack of genetic co-variation between brain components. For example, quantitative trait loci (QTLs) are

brain region-specific in hybrid chickens [84] and nine-spined sticklebacks [85]. Similarly, genetic correlations for relative brain region size are low in three-spined sticklebacks [86]. Finally, human **GWAS** and twin studies suggest that: 1) genetic variants tend to show brain region-specific volumetric effects [87]; 2) there are substantial region-specific genetic contributions to the heritability of various subcortical region volumes [88]; 3) genetic influences on cortical versus subcortical brain structures tend to be particularly distinct [89]; and 4) genetic effects on cortical thickness are largely region-specific [90].

Overall, new work suggests that neuroanatomical changes in response to selection are not highly constrained by a conserved developmental program (or pleiotropy). This is likely to reflect the fact that there are multiple developmental mechanisms that contribute to species differences in relative brain region size (timing/onset of neurogenesis [75], tissue allocation (i.e., gene expression) during brain regionalization, and cell cycle rates [91]), each of which may evolve independently across regions and species. In essence, developmental linkages evolve in response to selection, rather than constraining the response to selection.

Concluding Remarks

Many researchers who study primate brain evolution aim to increase our knowledge of the human brain. This has led to an overemphasis on certain regions (e.g., the PFC) as the critical sites of importance. Observed patterns of mosaic brain evolution suggest that single-factor grand theories may be inappropriate and divert attention from the manifold neurocognitive adaptations that occurred at different times, in response to different selection pressures, on different parts of the tree of life. New work continues to reveal this complexity, creating fertile ground for future studies of brain evolution.

Future work will continue to provide new insights through the generation of new neuroanatomical and “omics” data. Thus far, most comparative studies searching for ‘human-specific’ traits have focused on two or three species (e.g., human versus mouse; the human-chimpanzee-macaque triad); however, we have outlined multiple examples in which results changed after including more **outgroups** and/or individuals, suggesting that broader phylogenetic and sampling approaches are required to make inferences about the directionality and ‘uniqueness’ of human trait evolution. The need for increased sample sizes not only pertains to broadening species sampling, but also increasing within species samples so that we

might better understand the patterns and drivers of intraspecific neurobiological variation. Recent work by multiple teams [e.g., 92–96] represent exciting efforts to expand species and individual sample sizes for brain and cognition data, and new open data resources (e.g., [97]) are facilitating collaboration and sharing of primate brain data.

Efforts to develop novel analytical methods and to gather more detailed neurobiological measures (e.g., neuron counts, synaptic density, connectivity, 'omics') will allow us to better understand the relationship between gross morphological measures and function, test new hypotheses, and evaluate ideas that may be narrowing the scope of scientific inquiry. While sampling within comparative genomics will be facilitated by cheaper and better sequencing technologies, comparisons of neurodevelopmental mechanisms across taxa will remain practically and ethically difficult. The use of induced pluripotent stem cells (iPSCs) and brain organoids – as long as these are not exclusively focused on the neocortex - can help overcome some of these challenges and may provide insight into the developmental mechanisms underlying species differences in brain composition. This would improve our understanding of brain structure evolution by helping us distinguish between instances of **homology** versus **homoplasy**. Nevertheless, these tools alone cannot illuminate the cognitive and behavioral variation produced by evolutionary changes to genomic and developmental mechanisms, bolstering the need for comparative studies of these traits. An exciting possibility is that studies using these tools could generate evolutionary hypotheses that can be tested by comparative studies, creating a virtuous circle between experimental and phylogenetic approaches. Additionally, further developing and increasing the accessibility of relevant causal modelling approaches (e.g., phylogenetic path analysis [98]) will allow researchers to move beyond the purely correlative evidence provided by many comparative approaches.

We summarize future directions and remaining questions for comparative neurobiology in the Outstanding Questions Box. Expanding the types of questions that we can answer about human brain evolution will require researchers to move beyond cortico-centric ideas and increase neurobiological data availability. Prior to new data becoming available, we encourage comparative biologists to integrate existing datasets to test new hypotheses. We also encourage comparative neurobiologists to consider relevant paleoanthropological and archaeological data when interpreting potential 'human brain-specific' features. Finally, we urge researchers who use model organisms to study human-specific conditions to consider

perspectives and findings from comparative biology, as they can gain valuable insights that may inform their selection of model species.

Text Boxes

Box 1: Comparative methods

Closely related species are more likely to be similar to each other than distantly related ones, so they represent dependent data points. Comparative analyses use phylogenetic models that incorporate species' evolutionary relationships. Note: the correlative nature of many comparative methods cannot distinguish correlation from causation.

Modelling trait evolution

Evolutionary models describe patterns of trait evolution (direction, timing, rate of change). Popular models for continuous traits include Brownian motion (BM) and Ornstein–Uhlenbeck (OU) models. BM is the simplest (the least parameters) model in which variance accumulates at a constant rate in random directions. The OU model also fits central tendencies ('optimal trait values') and trends for variance to accumulate toward these central tendencies ('strength of selection').

Sample questions:

Which species exhibit convergent cerebellum morphology (i.e., have the same trait optima)?

When did the rate of brain size increase accelerate during primate evolution?

Reconstructing trait evolutionary histories

Ancestral character estimations recreate the evolutionary histories of traits. Traits may be discrete (e.g., olfactory bulb presence/absence) or continuous (e.g., neocortex size). Inputs include: 1) known trait values from a species sample; 2) a phylogenetic tree; and 3) a model of trait evolution. Reconstruction is accomplished by estimating trait values for all internal nodes of the tree. Convergent evolution events (repeated evolution of similar phenotypes in different lineages in response to similar socioecological variables) provide independent replicates for evolutionary 'experiments', allowing researchers to avoid "just so" storytelling [99].

Sample questions:

When did modern human brain size emerge?

What was the gyrification index for the earliest primates?

Inferring selective pressures, constraints, and co-evolutionary relationships

We can test for evolutionary associations between two biological traits (e.g., brain and body size; the size of two brain areas; an environmental variable and cognitive test performance). The most popular method is phylogenetic generalized least squares (PGLS) regression – a type of weighted regression. In a standard regression, all data points are independent and equally influence regression line estimation. PGLS “down-weights” data points from closely related species by incorporating a variance-covariance (VCV) matrix, which describes the expected similarity among species based on their degree of relatedness [100]. The inferences that result from such analyses are often based on the assumptions that natural selection is responsible for driving the observed association and that species average values appropriately capture adaptative changes.

Sample questions:

Why do some primate species have relatively large brains?

Which primate lineages exhibit exceptional rates of neocortex size evolution?

Box 2: The primate brain

Primate brains are larger than expected relative to body size [101], and recent work has confirmed that this difference emerges prenatally due to relatively slower fetal body growth [102]. Slow somatic growth rates may reflect relatively low total energy expenditure in primates [103] and are likely to represent an evolutionary strategy to direct limited fetal resources to brain growth. These relatively large primate brains are comprised of relatively larger neocortices, which are particularly large relative to the size of the dorsal thalamus compared to other mammals [104]. Neuronal density decreases as brain size increases across primates [105], but this effect may be less marked in primate compared to other mammalian brains [106]. Additionally, compared to other mammals, primates have more cortical upper layer neurons and increased cross-cortical integration [107], and higher interlaminar astrocyte density and complexity [108].

Primates also possess brain areas not found in other taxa and exhibit distinctive organization of certain regions. For example, the dorsolateral PFC of primates, involved in working memory [109], possesses an evolutionarily novel granular layer 4. Primates also possess a unique thalamic subregion, the dorsal pulvinar, which may play a role in spatial selective attention [49]. Additional primate-specific brain areas may include the ventral premotor area (which facilitates visually guided control of manual and orofacial grasping), the ventral somatosensory area, and

a posterior cingulate area (area 23; [49,110]). Furthermore, primates exhibit numerous specializations related to visual information processing. These include unique patterns of lamination of the LGN of the thalamus, more segregated retinopic organization of the superior colliculus of the midbrain, and the presence of many visual areas (e.g., V3) and so-called visual cortex “blobs” (features that evolved independently in some carnivores) [49,110]. Notably, visual areas are organized into two, distinct functional systems, the dorsal and ventral pathways, which are involved in the spatial location and identification of objects, respectively [111]. In addition, the middle temporal (MT) visual area, which processes stimulus orientation and direction of motion, may be unique to primates [112], and primates uniquely exhibit a relatively large posterior parietal cortex, a portion of which receives inputs from higher visual areas [110]. Finally, new work suggests that primates possess a unique striatal interneuron subtype [113]. Overall, these primate-specific features make primate comparative biology particularly relevant to the study of human brain evolution.

Box 3: Life-history correlates of brain size

Numerous comparative studies have found that longer-lived primates have larger brains (e.g., [e.g., 40]) and more cortical neurons [114]. The most well-known adaptive hypothesis for this relationship is the Cognitive Buffer Hypothesis (CBH), which posits that larger brains provide behavioral flexibility to respond to ecological challenges (e.g., predation), leading to reduced extrinsic mortality and longer lifespans. In support of this idea, relative brain size predicts a proxy of cognitive buffering (i.e., the difference between environmental and experienced seasonality) across primates [115,116]. This relationship does not hold in strepsirrhines, which may reflect a larger proportion of basal metabolism devoted to brain maintenance [117]. Similarly, there is a negative relationship between brain size and the coefficient of variation in body mass in primates, which may reflect alternative strategies to deal with periods of food scarcity – either by fat storage or cognitive buffering [118]. Finally, ancestral state reconstructions suggest that relatively large brained, long-lived primate species evolved from species that already had relatively large brains, consistent with the CBH [119].

Other studies have suggested that the observed relationship between brain size and lifespan is simply a side effect of extended neurodevelopment and does not provide evidence of cognitive buffering. The Developmental Costs Hypothesis (DCH) posits that a longer period of maternal investment is necessary to support large-brained offspring, leading to slower life-history and a

longer lifespan [120]. Across mammals, prenatal brain growth correlates with gestation length, postnatal brain growth correlates with lactation, and adult brain size correlates strongly with the total period of maternal investment [120]. In primates, some studies find that the correlation between brain size and lifespan does not hold after controlling for maternal investment [40,121,122]; however, the appropriate criteria to confirm or deny a remaining association is not always clear. Nevertheless, a recent study showed that neocortex size, the growth of which is largely complete by birth, is predicted by gestation length, while cerebellum size, the growth of which continues postnatally, is predicted by juvenile period length and lifespan. Since ape life-history has a distinctive extended juvenile period, this may reflect the developmental cost of evolving a large cerebellum [122].

Given that these adaptive and developmental explanations are not mutually exclusive, current evidence suggests that our large brains may have contributed to our lengthy lifespans through both extended maternal investment and cognitive buffering of environmental challenges.

Box 4: Human-brain specific traits (beyond? the PFC)

Studies of gene regulation, gene expression, and neurochemicals have highlighted potential 'human brain-specific' traits that are in non-PFC brain regions. Here, 'potential' is specifically used to highlight the limited species sample sizes currently available for these types of data. Such findings include: 1) compared to chimpanzees and macaques, human brains exhibit more complex gene regulatory mechanisms not only in the PFC, but also the cerebellum and visual cortex [123]; 2) compared to chimpanzees, bonobos, and macaques, an excess of human-specific gene expression differences is found not only in the PFC, but also other neocortical areas, hypothalamus, internal capsule, and cerebellum [124]; 3) compared to chimpanzees, gorillas, gibbons, and macaques, most human-specific gene expression differences reflect increased expression of hippocampal neuronal and astrocytic markers [125]; and 4) compared to capuchins, macaques, baboons, gorillas, and chimpanzees, the human striatum exhibits a unique neurochemical profile that might promote social cooperation [126]. Additionally, although PFC areas exhibit the highest transcriptional divergence between prenatal human and macaque brains, this divergence is driven by cell proliferation genes and is likely to reflect size-related differences in progenitor cell proportion [127]. Greater insights will continue to be provided by studies with larger species sample sizes. For instance, new work has generated transcriptomic data from four brain regions across an unprecedented 18 primate species, and suggests that

human brains show altered expression of semaphorin genes (which aid in axon guidance) in the cerebellum specifically [128].

Figure legends

Figure 1 | Relative brain size varies greatly across primate species

Phylogeny of primates with brain size ('Br'), body size ('Bo') and relative brain size ('Resid'; residuals from an interspecific regression of log brain size on log body size) represented by circle size. Grey boxes highlights hominin values. Brain data, body data, phylogeny were taken from Miller and colleagues [33]. One representative species for each of the available non-hominin primate genera was included (for visualization purposes). Images were obtained from phylopic.org

Figure 2 | Potential issues with different measures of absolute/relative brain or brain region size

Notes: 1) From Stephan and colleagues [129] ; 2) EQ = encephalization quotient (derived from interspecific regressions) from Jerison [101]; 3) Cognitive brain measure that uses 'the slope of cognitive equivalence' (derived from intraspecific regressions) van Schaik and colleagues [130]
*expected values may be derived from an allometric exponent of 0.67, corresponding to the surface to volume ratio of 'idealized bodies'
**when the response variable (region size) comprises a relatively large fraction of the predictor variable (brain size), this produces a statistical bias towards isometry [131]

Glossary

Activity pattern: the period during which an animal is most active (diurnal=daytime; nocturnal=nighttime; cathemeral=daytime and nighttime)
Allometric scaling: change in the size of one physical attribute relative to another
Cathemeral: activity pattern in which animals are active intermittently across the 24-hour cycle
Ecological: relating to the relationships between living organisms and their physical environment
Encephalization: an evolutionary increase in the size of the brain relative to the body
Endocasts: natural or artificial replicas of the internal surface of the bony braincase

Evolution: a change in allele frequencies and their associated **phenotypes** from one generation to the next

Extant: a species with living members

Extinct: a species with no living members

General intelligence: a concept that describes observed correlations in performance across contexts

GWAS: genome-wide association study, designed to identify links between genetic variants and certain diseases

Haplorrhines: a suborder of primates containing apes and American and African monkeys (see Fig 1)

Hominins: all species on or off the lineage leading to humans since our last common ancestor with the lineage that led to chimpanzees and bonobos

Homology: evolution in which a similarity among organisms was inherited from the common ancestor of those organisms

Homoplasy: evolution in which a similarity among organisms was not inherited from the common ancestor of those organisms

LGN: lateral geniculate nucleus of the thalamus, relay center for the visual pathway

Life-history: the series of events primarily related to maturation, survival, and reproduction, undergone by an organism during its lifetime from birth to death

Natural selection: one mechanism of evolution whereby individuals who express traits that make them better adapted to their environment tend to survive and produce more offspring (relative to other conspecifics)

Neanderthals: *Homo neanderthalensis*, closest extinct relative of humans (see Fig 1)

Outgroup: organism(s) not belonging to the group being investigated

Phenotype: observable features of an individual resulting from the interaction between its genotype and the environment

Phylogenic: pertaining to the evolutionary histories and patterns of relatedness between organisms

Phylogenetic tree: branching diagram showing the evolutionary relationships between species

Polygynandry: a mating system in which both males and females have multiple mating partners

Primates: eutherian mammals within the taxonomic order Primates, usually characterized by a suite of arboreal adaptations, such as grasping hands and feet, stereoscopic vision, the presence of a postorbital bar, and nails (instead of claws)

Strepsirrhines: a suborder of primates containing lemurs and lorises (see Fig 1)

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