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If you please, draw me a neuron — linking evolutionary tinkering with human neuron evolution

Baptiste Libé-Philippot[1,2,3,*], Franck Polleux[4,5,*] and Pierre Vanderhaeghen[1,2,*]

Animal speciation often involves novel behavioral features that rely on nervous system evolution. Human-specific brain features have been proposed to underlie specialized cognitive functions and to be linked, at least in part, to the evolution of synapses, neurons, and circuits of the cerebral cortex. Here, we review recent results showing that, while the human cortex is composed of a repertoire of cells that appears to be largely similar to the one found in other mammals, human cortical neurons do display specialized features at many levels, from gene expression to intrinsic physiological properties. The molecular mechanisms underlying human species-specific neuronal features remain largely unknown but implicate hominid-specific gene duplicates that encode novel molecular modifiers of neuronal function. The identification of human-specific genetic modifiers of neuronal function brings novel insights on brain evolution and function and, could also provide new insights on human species-specific vulnerabilities to brain disorders.

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Introduction

"*If you please — draw me a sheep*" ("*S'il te plait, dessine-moi un mouton*"), asked the Little Prince to the airplane pilot Antoine de Saint-Exupéry, lost in the desert (1943). What could we offer to the Little Prince if he were to ask us today to draw a *human* neuron? The answer would rely on *how* human neurons differ from their counterparts found in the brain of other species. Tackling this question requires cross-species comparisons [1], including closely related hominids (e.g. chimpanzees), simians (e.g. macaques), primates (e.g. marmosets), more distant mammals (e.g. mice), and other amniotes and vertebrates. The evolution of the human brain was discussed in the 1970s by the biologist François Jacob in his essay 'Evolution and Tinkering' [2], where he described the notion of molecular tinkering: "*it works on what already exists, either transforming a system to give it new functions or combining several systems to produce a more elaborate one*". But at the time, human brain evolution was a speculative and observational field of investigation, largely inaccessible to experimental manipulations at the molecular and cellular levels. This has changed radically over the past decade, thanks to rapid progress in comparative genomics, human genetics, and neurobiology.

Human brain evolution at the neuronal level

Speciation involves the emergence of behavioral features, which rely on the evolution of the development, structure, and function of neural circuits [3,4]. Human species-specific brain properties, which underlie the specialized cognitive functions of our species, are thought to be linked mostly to the evolution of synapses, neurons, and circuits of the cerebral cortex [5]. Comparative analyses of the mammalian cortex have pointed to multiple substrates for such evolution, including divergence in cytoarchitecture and cell type composition: more cortical neurons and expansion of some layers and areas, the appearance of new cell types (for instance, more diverse subtypes of cortical pyramidal neurons [CPNs] populating cortical upper layers) [6–8], and patterns of cortical neuronal connectivity (for instance, increase in associative cortical areas and of cortico-cortical projections) [5].

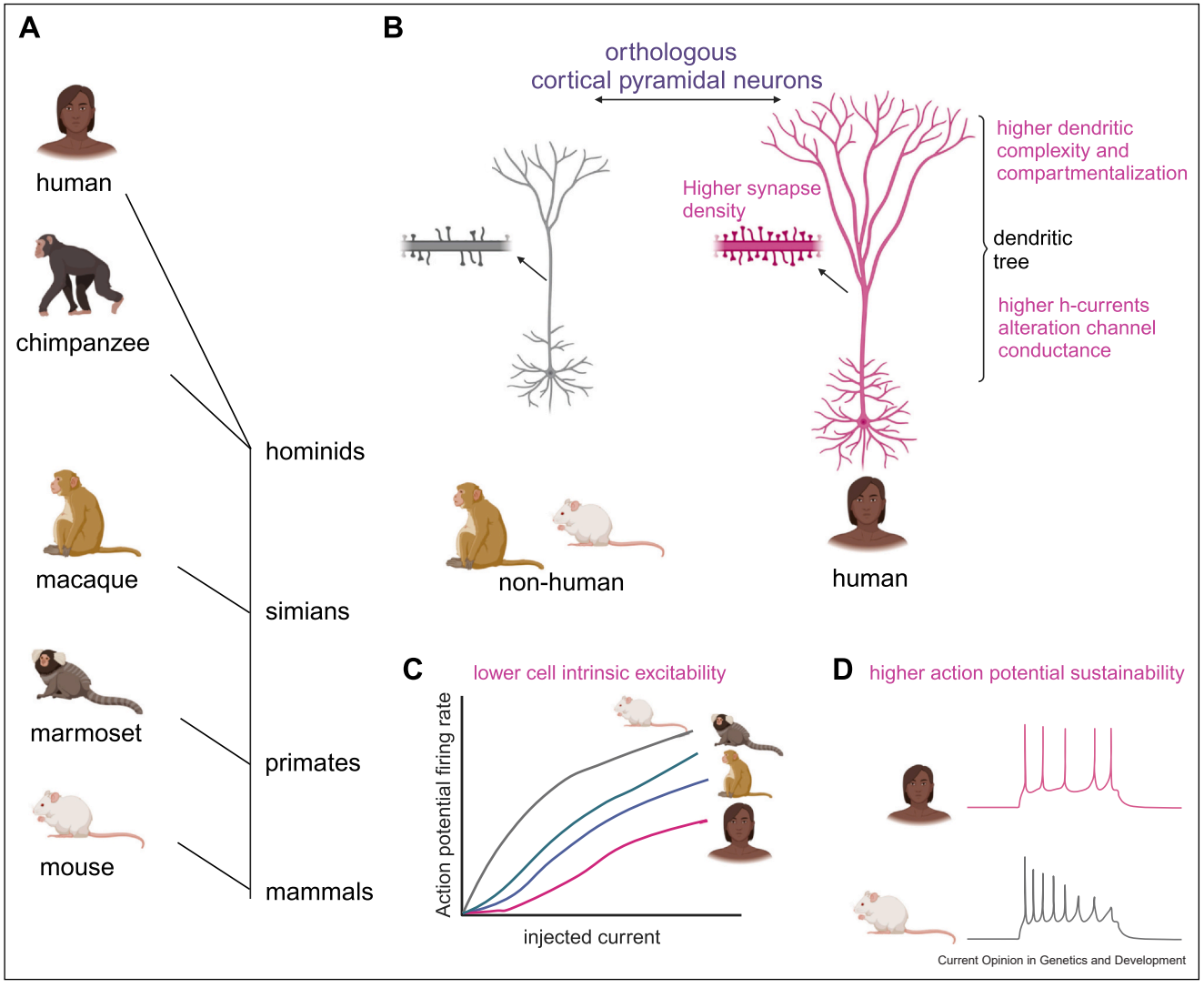
But this is not all. Recent comparative transcriptomic analyses have revealed that hominid (human and great apes), simian, primate, and mouse adult cortex display a

surprisingly similar repertoire of neuronal types [8–10]. This echoes recent findings that cortical cell types are conserved between most vertebrates despite considerable changes in their relative proportions and location [11,12]. In contrast, recent work focusing on completely different neuronal features, that is, electrophysiological recordings from human and non-human cortical tissue, also converged to the same conclusion that individual cortical neurons display species-specific intrinsic functional properties [13]. Thus, human brain evolution could also rely on divergent properties in 'orthologous' (same cell types across different species) cell types found in the mammalian cortex, leading to species-specific features at the individual neuron level, as described in other species [3,14]. Here, we will present an overview of recent work that identifies species-specific molecular and physiological properties among human cortical neurons and then discuss how they have started to be linked more mechanistically, opening novel opportunities to study the impact of neuronal divergence on human brain function.

Human cortical neurons display specialized functional properties

Most human cortical neurons display species-specific morphological features compared with their rodent or non-human primate counterparts. For instance, human CPNs are overall much larger, displaying larger dendritic arbor length [15] and increased numbers of synapses per neuron compared to all other species examined, including chimpanzee [16] (Figure 1a,b). How about functionality? Recent work, comparing *ex vivo* human and nonhuman cortical tissue

Figure 1



Evolution of human CPNs. **(a)** Phylogenic tree with human species and other hominid (chimpanzee), simian (macaque), primate (marmoset), and mammalian (mouse) species. **(b–d)** Human CPNs display species-specific morphological and physiological cell-intrinsic properties (in purple).

using electrophysiological single-cell recordings, has revealed that human CPNs display a high number of specialized functional features (Figure 1b–d). These include stronger synapses [17] and higher levels of synaptic plasticity [18], which could contribute to the distinct functionality of cortical circuits. Even more surprisingly, human CPNs were found to display specialized biophysical and physiological properties, including at the level of basic features such as action potential (AP) generation [19], likely reflecting distinct properties or subcellular distribution of ion channels and/or their regulators [20–25], as discussed below.

One such critical determinant of neuronal function that displays human specificities is intrinsic excitability: recent work shows that human CPNs do not follow allometric rules between neuronal size and excitability, resulting in lower excitability of human CPNs compared with counterparts in other species [20,24] (Figure 1c). Neuronal excitability, or *input–output relationship*, is a critical property by which CPNs produce different patterns of AP trains (e.g. AP frequency) (=*output*) in response to synaptic stimuli (=*input*) [26]. These electrophysiological differences characterizing similar neuronal subtypes between species could have significant consequences at the level of cortical circuits, for instance, by modifying their coding properties or plasticity [27], but they could also reflect scaling processes to maintain circuit function of neural circuits composed of more and/or larger CPNs and synapses.

Cellular and molecular mechanisms proposed so far to explain the decreased neuronal excitability of human CPNs focused on the dendritic compartment, including enhanced compartmentalization of human CPNs compared to rodents and changes in the conductance of specific ion channels (voltage-gated potassium and Hyperpolarization-activated Cyclic Nucleotide–gated) [20,21,25]. However, recent studies also highlighted molecular differences at the level of the axon initial segment (AIS), the site of AP generation in CPNs [19,28], as discussed below, as well as in GABAergic interneurons [29].

Finally, recent findings in the human cerebellum indicate that Purkinje cells are more complex in their dendritic tree arborization and also intrinsically less excitable than mouse counterparts [30,31], suggesting that such divergent properties might be shared with other human cell types in other brain areas. Evolutionary tinkering has thus led to functional divergence in human neuron function, including in basic properties such as excitability, as previously reported in invertebrate and lower vertebrate species [3].

Species-specific transcriptional regulation in human cortical neurons

What could be at the origin of the species-specific functional properties of human cortical neurons? While morphological and hodological features are likely the consequence of divergent gene regulatory programs taking place during brain development [5], the specialized functional properties more likely reflect the molecular properties of human cortical neurons at the adult stage. Several recent studies of comparative single-cell transcriptomics of hominid, simian, primate, and mouse adult cortex have pointed to strong patterns of molecular divergence of cortical neurons, despite a strong conservation of cellular composition. In a nutshell, these analyses have revealed that although a higher diversity of cell subclasses may be observed in humans compared to other species [7,8], all examined mammalian species display a remarkably similar repertoire of cortical neuronal types [8–10]. And while most of these conserved types share patterns of gene expression defined using single-cell transcriptomics, they also display species-specific gene expression. This divergence seems to be particularly high for human CPNs, as chimpanzee CPNs appear to be closer to gorilla CPNs than to human counterparts [8], which is unexpected given the equivalent phylogenetic distances between humans and chimpanzees to gorillas. Hundreds of genes are thus expressed in a human-specific fashion in cortical neurons, some of which are specific to certain neuronal subtypes, while others are expressed throughout cortical cell types. Interestingly, the divergent genes displaying human-specific expression are enriched in genes involved in neuronal and/or synaptic function, thus representing potential modifiers of neuronal function [8].

What are the origins of these species-specific differences in gene expression patterns? Comparative genomics has provided many insights on the changes at the genomic level that could lead to the evolution of gene regulatory networks (GRNs) in the human brain (reviewed in Ref. [32]). Recent high-scale cross-species genomic, transcriptomic, and epigenomic comparisons have revealed various classes of human-specific putative cis-regulatory elements, including so-called human accelerated regions (HARs), human-specific deletions in non-coding genomic regions (hCONDELs), human gain/lost enhancers, and human ancestor quickly evolved regions (HAQERs) [33–37]. These genomic human novelties are likely linked to human-specific neuronal gene expression, as for instance HARs, HAQERS and hCONDELs are enriched next to genes displaying human-specific expression in cortical neurons [8,36]. Moreover, many of them are associated to neurological diseases [36,38–40], suggesting human-specific sensitivities to some of these disorders.

Overall, these data strongly suggest that these divergent genomic regions are directly linked to human neuronal evolution. This remains to be tested in a more systematic way by exploring the physiological consequences of such differences in cortical neurons. It will also be important to determine the conservation of the structure and function of human-specific elements

within the human population. Finally, it will be fascinating to explore the evolution of genomic features in non-primate species (like cetaceans or elephants) that also display large neuronal size and extended life span, searching for convergent evolution of similar GRN features. The lack of high quality genomic resources in many closely related species in those lineages (especially transcriptomic resources) makes this a challenging endeavor but could also be partially addressed with pluripotent stem cell modeling from these species [41].

On the other hand, post-transcriptional and translation regulation will be important to explore in the context of human neuron evolution. For instance, proteomic analyses on synaptosomes isolated from the cortex of human and nonhuman mammalian species have uncovered human-specific differences that are not always related to transcriptional differences [42].

While the bulk of evolutionary changes are linked to changes in gene regulation, other genomic changes contribute to evolution. These include divergence in coding regions, although the sequences of brain-associated genes tend to be strongly conserved in primates compared to genes associated with other organs [38]. Interestingly, a recent mass spectrometry analysis from cerebral cortex samples reported >200 human-specific (compared to mouse and macaque) open reading frames coding for small peptides (<15 amino acids) [43], for which the ligands and the function remain to be explored.

Last but not least, another important driver of evolution is the emergence of novel genes through segmental duplication. Gene duplication is a major mechanism of gene diversification, where an *ancestral* gene is duplicated one or several times into several related *paralogous* genes. While many of these duplicates are expected to turn into pseudogenes, some constitute a rich substrate for evolutionary changes. In this context, hominid-specific or even human-specific segmental gene duplications have received a lot of recent attention in relation with human brain evolution. Indeed, several of them have been linked to human speciesspecific features of cortical development, from neurogenesis to synaptic development and connectivity. As these were extensively reviewed recently [5], we focus here on one of these, called LRRC37B, which was recently implicated in the evolution of human adult cortical neuronal physiology [28], thus providing a surprisingly direct molecular mechanism between genomic and neuronal evolution, as discussed further in the next section.

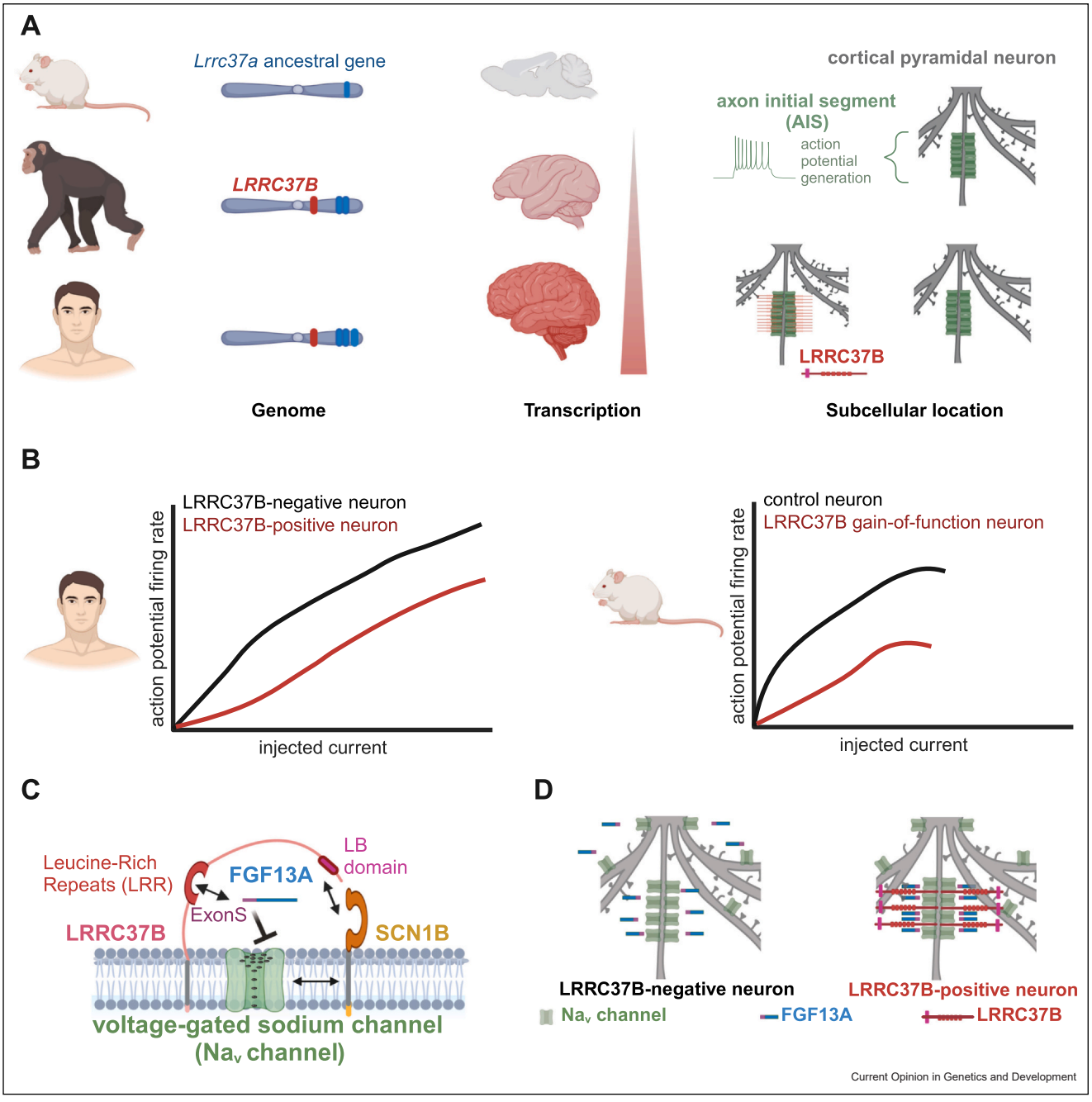
LRRC37: a hominid-specific gene family linking human species-specific molecular and functional properties of cortical neurons

Previous studies identified over 30 genes that arose through segmental duplications, specifically in hominids, and that are dynamically expressed during human corticogenesis [44]. Among these, the *leucine-rich-repeat containing 37 (LRRC37)* genes encode a novel class of membrane receptors that are selectively amplified in primate genomes and expressed in cortical neurons. *Lrrc37* genes can be found in all amniotes, but they expanded during primate evolution to reach more than a dozen of genes in the human genome [45]. One of these, *LRRC37B*, stands out as it is present only in the human and great ape genomes and expressed at highest levels in the human cerebral cortex at adult stages [28] (Figure 2a). Most strikingly, the examination of LRRC37B protein distribution in the human cerebral cortex revealed that it is expressed selectively at the level of the axon initial segment AIS of human CPNs but not of nonhuman primate or mouse CPNs (Figure 2a). The AIS is a key specialized segment of the proximal part of the axon, highly enriched for voltage-gated sodium channels (Na_v). The AIS is the major site of AP generation and thereby of regulation of neuronal excitability [46] (Figure 2a). Intriguingly, *LRRC37B* expression was found to be heterogeneous among CPNs: it is expressed in about half of them, independently of their subtype identity (Figure 2a).

What could be the neuronal function of LRRC37B? Overexpression of LRRC37B in the mouse cortex revealed a similar distribution of the receptor at the AIS level. Electrophysiological recordings of control and LRRC37B-expressing mouse CPNs then indicated that the forced expression of LRRC37B leads to a decrease in their intrinsic excitability, selectively reducing the ability of neurons to trigger APs at the level of the AIS. Using *ex vivo* electrophysiological recordings of human cortical tissue obtained from neurosurgery resections, it was found that the CPNs that display LRRC37B at their AIS are less excitable compared with those whithout (Figure 2). Thus, LRRC37B is a human-specific component of the AIS of cortical neurons that regulates their excitability [28] (Figure 2b).

Each *LRRC37* gene encodes a putative membrane protein with an extracellular domain containing a leucine-rich repeat (LRR) and a short intracellular domain, thus suggestive of a receptor function. The ligands of LRRC37B were discovered using *in vitro* binding screens and *ex vivo* immunoprecipitation experiments from human brain. LRRC37B was found to bind with high affinity to FGF13A, an extracellular ligand of the FGF family. Also, it was found to bind to the Na_v subunit 1 (SCN1B), a transmembrane protein localized at the AIS and a regulator of Na_v (Figure 2c). Further biochemical and cellular investigations revealed that FGF13A also acts as a negative regulator of neuronal excitability by binding directly to $Na_V1.6$, which is selectively present at the AIS. By binding to FGF13A, LRRC37B promotes its targeting to the AIS and thereby

Figure 2



A novel evolutionary molecular pathway based on LRRC37-FHF-SCNb protein interaction in human neurons modulates their physiological properties. **(a)** LRRC37B displays several evolutionary levels: 1) emergence of the gene in hominid species, 2) higher transcript abundance in humans compared to chimpanzees, 3) LRRC37B protein location at the axonal initial segment (AIS) selectively of human CPNs. Of note, it is a subset of human CPNs that display LRRC37B at their AIS. **(b)** LRRC37B protein location at the AIS is correlated with a lower neuronal excitability in humans, and it leads to lower neuronal excitability when expressed in mouse CPNs. **(c)** LRRC37B binds to secreted FGF13A and transmembrane SCN1B that both are modulators of voltage-gated sodium channels (Na_v channels). **(d)** LRRC37B acts by concentrating the FGF13A ligand at the AIS where it inhibits Na_v channels.

enhances its inhibitory effect on neuronal excitability, selectively at the level of the AIS (Figure 2d).

Thus, LRRC37B acts as a human species-specific modifier of neuronal excitability and, as such, constitutes a striking example of how molecular tinkering can influence species-specific properties of the brain. What could be the impact of LRRC37B at the level of cortical circuits and brain function? One can only speculate at this point, considering its negative effect on excitability of some but not all human cortical neurons. LRRC37B could affect how neurons process and encode signals, adapting the neuronal responses to synaptic inputs, and influence synaptic transfer of information through gain modulation, which depends on output modulation at the level of the AIS [26,47]. This could then influence attention, learning and/or multisensory integration, or neuronal plasticity, whether Hebbian or homeostatic. Thus, through its ability to modulate input/output relationships in the CPNs in which it is expressed, LRRC37B could have a critical impact on key properties of cortical circuits, including some that display human species-specific features, such as CPN synaptic plasticity and information transfer efficiency [13]. Alternatively or in addition, LRRC37B expression in the human cortex could be an adaptive feature for the increased synaptic connectivity of human CPNs. Future work should examine these possibilities in more depth, for instance, using mouse models in which LRRC37B expression is 'humanized', an approach that was previously successful to reveal the functional impact of another human-specific gene, *SRGAP2C*, on cortical circuits [48].

Human genetics also points to a potentially important role for normal human brain function. On the one hand, *LRRC37B* copy numbers are fixed in the human population compared to other paralogs [28], pointing to a strong selection on the dosage of the gene, thus suggesting important functional relevance. On the other hand, while no pathogenic mutations of *LRRC37B* have been found (so far) in patients presenting alterations of neuronal excitability, it is striking to note that its three identified partners, *FGF13A*, *SCN1B*, and *SCN8A* ($Na_v1.6$), were all previously shown to be mutated in severe forms of epilepsy [49–51]. Thus, through its ability to modulate the function of FGF13A and Na_v channels, LRRC37B could act as a human-specific modifier of brain disorders including forms of epilepsy and autism spectrum disorders.

Changes in voltage-gated channel biophysical properties enable sustained fast action potential signaling in human cortical pyramidal neurons

Human CPNs display a decreased excitability but yet can produce a fast AP firing rate when performing cognitive tasks [19]. Recent work showed that during such high trains of spiking, the shape of individual APs remains stable in human CPNs compared to mouse CPNs [19] (Figure 1c). This work linked this sustainability of AP to the biophysical properties of the Na_v channels (AP initiation) and K_v channels (AP termination) [19]. Human CPNs displayed a larger fraction of Na_v channels that can be activated compared to mouse CPNs [19]. As described above, Na_v channels not only activated more slowly but also inactivated more slowly and yet recovered more quickly than mouse CPNs [19]. In addition, K_v channels activated faster in human CPNs compared to mouse CPNs [19]. This highlights the fact that lower excitability (channels activated more slowly) can be a companion of easier AP generation (more channels available) in long trains of AP (for which the soma vs. AIS contribution remains to be determined), which can be key for high cognitive tasks in the human species. Further work would decipher whether these properties are shared with other primates and how channel biophysical properties are changed between species.

Concluding remarks and perspectives

Today, we would draw a *human* neuron as an orthologous CPN, shared between primates and mammals, that yet depicts divergent structural and functional cell-intrinsic properties compared to other hominids, explained by species-specific gene expression patterns, hominid-specific duplications, and changes in the biophysical properties of voltage-gated channels. Taken together, these genetic changes relate to most of the genomic changes in the human lineage after the separation from our common ancestor with great apes, and these could account for species-specific sensitivities to brain disorders. Intriguingly, a recent study also suggested the possibility of species-specific, sex-related differences in gene expression, which needs to be explored further [52]. Last but not least, little is known about brain differences between modern humans and archaic hominin species, notably because of the absence of brain primary tissues, although they display a number of genomic differences that might be potentially relevant to neuronal evolution [53].

"The air pilot [drew] 3 pictures of a sheep, that the child refused one after the other […] the air pilot drew a box with holes, asserting that the ideal sheep was inside. This time, the child sounds happy with his sheep". Like the sheep in the *Little Prince*, our knowledge of human species-specific features of neurons is still buried in a box with holes of unknowns, but the recent convergence of cross-species comparative -omics and neurobiology constitutes an exciting venue to reveal the singularity of human neural cells.

CRediT authorship contribution statement

BL-P: Conceptualization, Writing – original draft, Funding acquisition. **PV:** Conceptualization, Writing –

review & editing, Funding acquisition. **FP:** Writing – review & editing, Funding acquisition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

BL-P and PV are inventors of a PCT application related to the work on LRRC37B.

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