REVIEW

Morphological and physiological features of human cerebral cortical astrocytes in regenerative medicine: a narrative review

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Abstract

Astrocytes are essential for various functions in the brain, including regulating synaptic transmission, maintaining ion homeostasis, and supporting neuronal network activity. Despite over a century of recognition that human astrocytes differ morphologically from those of other mammals, detailed functional studies have been limited due to challenges in obtaining live human brain tissue. This narrative review revisits recent findings on the morphological and physiological properties of human astrocytes, particularly those focusing on cortical astrocytes. I summarize the main anatomical and functional features of the different types of human astrocytes and briefly compare human astrocytes with what is known about astrocytes in mice and/or primates. I greatly summarize the physiological properties of human astrocytes, with emphasis on their intrinsic properties, potassium ion uptake and syncytium connectivity, emphasizing recent advances that have enhanced our understanding of astrocytic functionality in the human brain and their possible role in regenerative medicine.

Key words: astrocytes; biomedical engineering; fibrous astrocytes; human brain; interlaminar astrocytes; neocortex; protoplasmic astrocytes; regenerative medicine; regenerative therapy; synaptic transmission; varicose projection astrocytes

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Introduction

Astrocytes play an active role in regulating synaptic transmission, shaping many functions highlighted in recent review papers, including providing metabolic and trophic support,[1] regulating synaptogenesis,[2, 3] maintaining ion homeostasis,[4] facilitating neurotransmitter uptake,[5] preserving the integrity of the blood–brain barrier,[6] and shaping neuronal network activity[7, 8] and behavior.[9, 10] While the role of astrocytes in synaptic transmission is a significant and rapidly growing area of research, it is not the primary focus of this review. Readers are encouraged to consult the referenced review papers for a more comprehensive analysis.

Recent advancements have underscored the importance of astrocytes in therapeutic research.[11] Although studies using animal models, primarily rodents, have provided valuable insights into the underlying mechanisms, it is crucial to explore the distinct characteristics of human astrocytes and consider how these findings can be effectively translated into human biology. This is especially important given that the astrocyte-to-neuron ratio increases with evolution — from 1:3 in the mouse cortex to 1:1.4 in the human cortex.[12, 13]

The idea that astrocytes might function differently in the human brain from in animals emerged over a century ago when an early Golgi staining study revealed that human astrocytes with long, cable-like protrusions up to 1 mm in length, never observed in rodents (**Figure 1**).[14] Recent studies have detailed the morphological differences between human astrocytes and other mammals and have identified at least four distinct types of $GFAP^+$ astrocytes in the adult human brain (**Figure 2**).[15-18] Astrocytes with stellate-like morphology include protoplasmic astrocytes in layers 2–6 and fibrous astrocytes in the white matter. They have the classical astrocytic morphology of oval soma and multiple radial protrusions. Additionally, two distinct types of astrocytes are found only in apes and humans: interlaminar astrocytes (ILAs) in layer 1 and varicose projection astrocytes (VPAs) in layers 5–6. One of the

main characteristics of these cell types is the ability of several long protrusions to travel through cortical layers. Novel insights into the specific morphology of human astrocytes have prompted a reconsideration of their role in synaptic transmission. Although research on the functional diversity of human astrocytes has historically progressed slowly — mainly owing to limited access to live human brain tissue — recent studies have overcome this challenge by advancing the usage of fetal samples and nonpathological brain tissue resected during surgeries to perform direct measurements of astrocytic activity, thereby shedding light on human astrocyte functionality. This narrative review revisits recent findings on the morphological and physiological properties specific to human cortical astrocytes, discussing how these insights may enhance our understanding of astrocytic functionality in the human brain.

Search Strategy

The literature search targeted studies using *ex vivo* human brain tissue. Key research on human-specific astrocyte functions and relevant reviews from recent and foundational studies were included.

Interlaminar Astrocytes

Human ILAs are $GFAP^{+}$ astrocytes found in the cortex with cell bodies in layer 1 and processes extending into deeper cortical layers.[18-20] Recently, ILAs have been further subdivided based on the location of their cell bodies and occupied anatomical domains. The upper zone of layer 1 contains astrocytes with oval-shaped cell bodies, often called rudimentary ILAs.[16] These astrocytes have lower cell density and less branching of protrusions. Their soma are usually in contact with the pia surface, whereas short processes typically stay within layer 1 and form the glia limitans.[21]

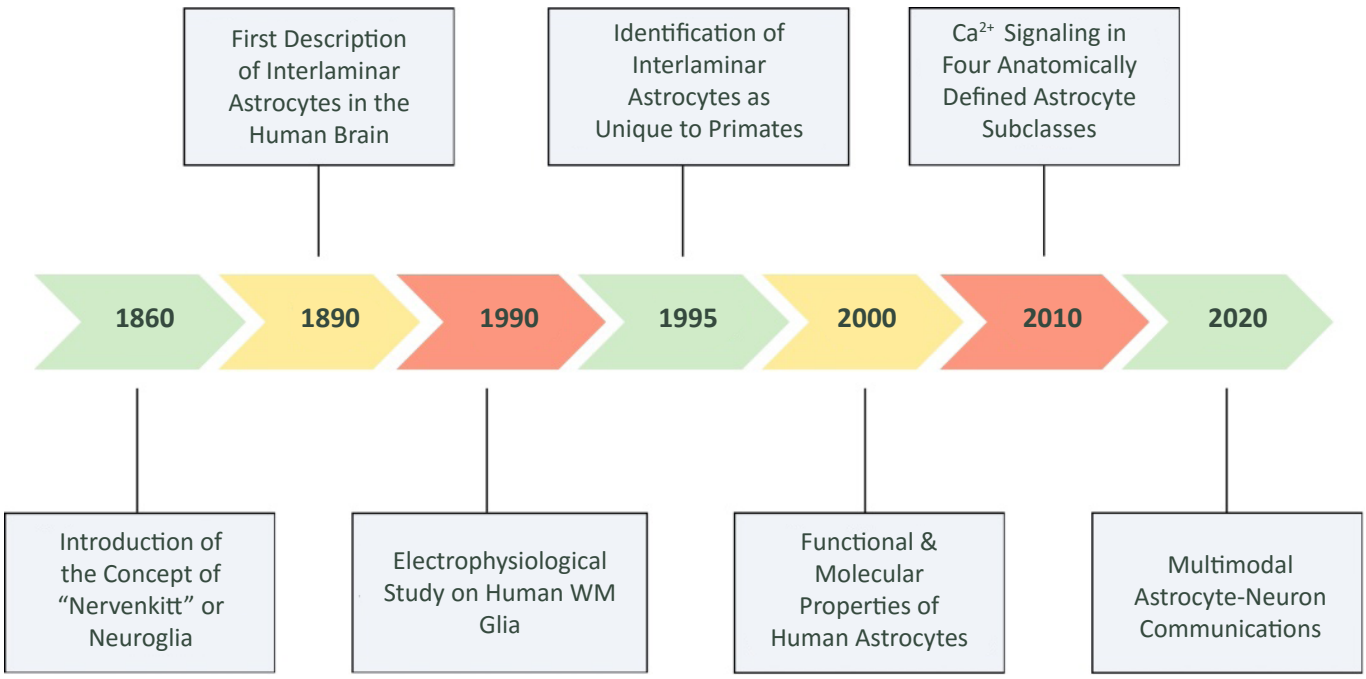

Figure 1: Timeline of research progress in human astrocytes.
Note: This timeline illustrates key milestones in the study of human astrocytes, beginning with the introduction of the concept of "Nervenkitt" or neuroglia by Rudolf Virchow in 1858, which laid the foundation for identifying glial cells in the brain.[47] In the 1890s, the first description of interlaminar astrocytes in the human brain was provided by Andriezen and Retzius, significantly advancing the understanding of astrocyte morphology.[14, 47] By the 1990s, electrophysiological studies on human white matter glia further revealed ion channel expression in astrocytes, marking a pivotal moment in astrocyte research.[48] In 1995, Colombo and colleagues described interlaminar astrocytes as unique to primates, emphasizing species-specific differences in astrocyte structure and function.[19, 49] In the 2000s, significant progress was made in understanding the functional and molecular properties of human astrocytes through the combination of various techniques, including patch–clamp recording, fluorescent imaging, and 3D reconstruction, contributing to a more comprehensive view of astrocyte roles in the cortex.[22, 24, 25, 28, 35, 36] In 2009, research described four distinct subclasses of astrocytes with Ca^{2+} signaling properties, emphasizing their functional diversity in the human brain.[18] Studies have also shown that human astrocytes enhance neuronal survival, promote synapse formation, engulf synaptosomes, and respond robustly to glutamate with increased Ca^{2+} levels.[29] In the 2020s, advanced molecular and imaging techniques continued to unveil the complex interactions between astrocytes and neurons.[15, 16, 21, 27, 30, 50-52] Created with Canva.com.

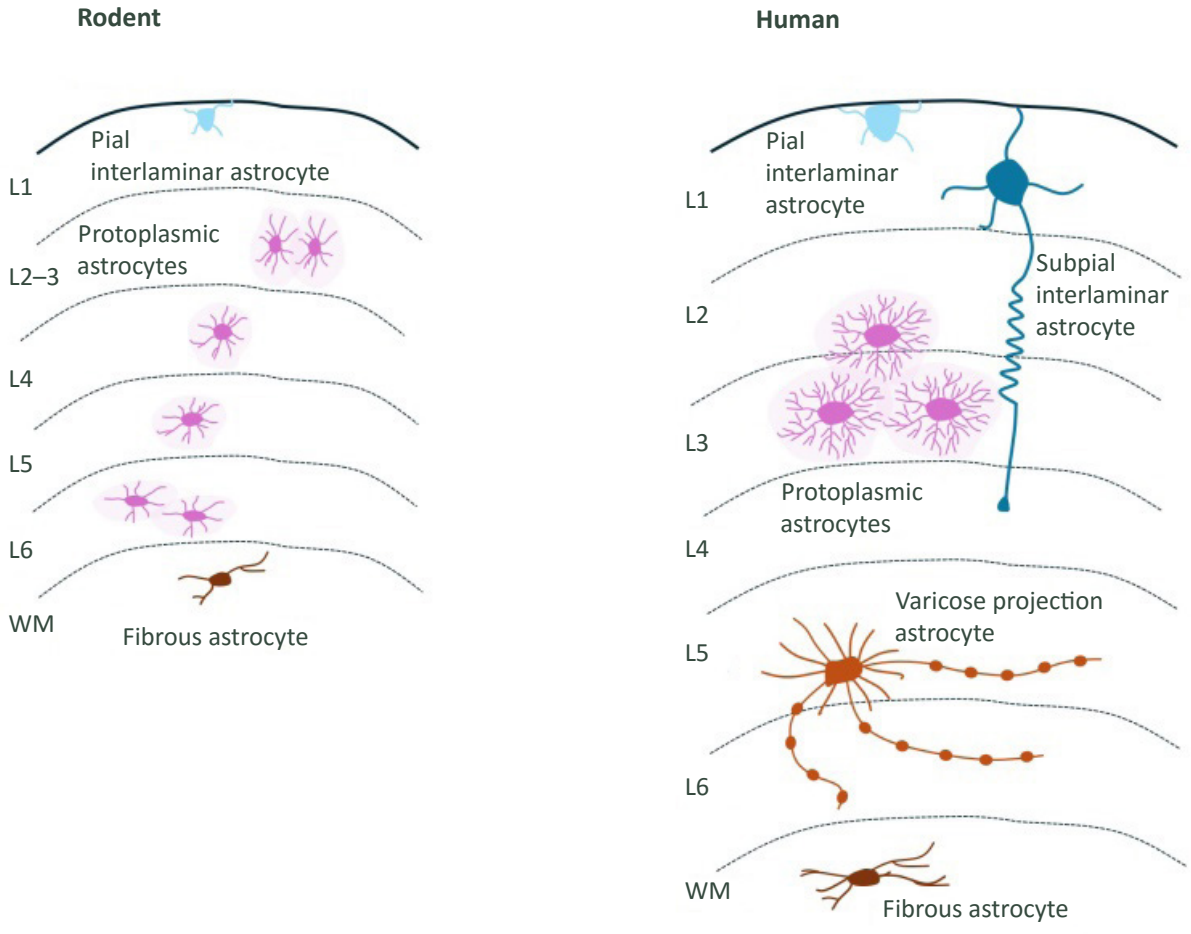

Figure 2: Schematic representation comparing astrocyte distribution and morphological properties across cortical layers in rodents and humans.
Note: (A) Three types of astrocytes have been identified in rodents: pial interlaminar astrocytes (L1), protoplasmic astrocytes (L2–6), and fibrous astrocytes (WM). Compared with those in layer 6, astrocytes in layers 2/3 exhibit greater radial elongation, process arborization, and synaptic cleft ensheathment, indicating morphological and synaptic interaction differences across layers.[53] (B) In humans, astrocytes display greater morphological complexity and are distributed as follows: pial and subpial interlaminar astrocytes (L1), protoplasmic astrocytes (L2–6), varicose projection astrocytes (L5–6), and fibrous astrocytes (WM). WM: White matter.

Rudimentary ILAs are found in the ventral and dorsal cortices of rodents and other mammals.[20] The lower zone contains subpial types of astrocytes called typical ILAs. Their soma does not directly contact the pia but connects with it via short processes and sends long processes to the deeper layers of the cortex.[20, 21] Typical ILAs are found only in the dorsal cortex of primates.[20] This cell type originates prenatally from radial glial cells and proliferates during gestation.[16] Throughout postnatal development, ILAs increase in density and process complexity and express markers characteristic of stem cells and astrocytes.[16] Typical ILAs have two primary types of processes: multiple radially oriented protrusions within the same layer and 1–2 long, cable-like protrusions that extend orthogonally to the pial surface and the gray matter. These protrusions have a tortuous shape, reaching lengths of up to 400–600 µm at 2 months after birth,[22] and terminate in club-like masses within layers 3–4.[18] Electron microscopy studies have shown that the endings of these interlaminar processes possess a multilamellar structure and contain mitochondria.[19] Furthermore, most interlaminar processes end on blood vessels.[23]

Although the specific functions of ILs are not fully understood, several hypotheses offer insights into their potential roles. Unlike human protoplasmic astrocytes, ILAs show weaker immunostaining for glutamate excitatory amino acid transporters (EAAT1 and EAAT2) and glutamine synthetase, suggesting a reduced involvement in local glutamate regulation.[23] Instead, ILAs could be key players in coordinating long-distance communication owing to their extensive coverage of greater distances within the cortex and passing through multiple domains of protoplasmic astrocytes.[18, 23] They

connect blood vessels in a deeper cortical layer to the pial surface, suggesting a critical role in regulating blood flow and supporting long-distance metabolic functions. The high expression of aquaporin-4 in their end feet further supports its involvement in water regulation and interregional metabolic processes.[23, 24] The disruption and loss of long astrocytic interlaminar processes are described in Alzheimer's disease and Down syndrome. In Down syndrome, these disruptions start early in life and progressively worsen, particularly in the dorsolateral prefrontal cortex. These patchy disruptions lead to a notable reduction in protrusion depth, causing deterioration by adulthood, especially in patients with Alzheimer's disease.[22] In Alzheimer's disease, particularly in advanced stages, these processes are often severely altered or absent, accompanied by prominent gliosis, thickened terminal segments, and enlarged terminal masses that may appear disconnected from the rest of the astrocytes.[25]

ILAs respond to purinergic and glutamatergic stimuli by increasing intracellular calcium (Ca^{2+}) levels, which further propagate as Ca^{2+} waves.[18, 26] Given that ILAs have passive electrical membrane properties, which limit the propagation of electrical signals,[23] Ca^{2+} signaling may be predominant for communication within the astrocytic network. Moreover, ILA connectivity to neighboring protoplasmic astrocytes through gap-junctions may enhance the integration of these cells into a broader astrocytic network, thereby improving the speed and volume of astrocytic communication.[18, 23]

Protoplasmic Astrocytes

Compared with rodent astrocytes, cortical protoplasmic astrocytes are located mainly in layers 2–6.[18, 27] Compared with rodent astrocytes, human protoplasmic astrocytes exhibit 10 times more primary processes and possess more complex and thicker $GFAP^+$ processes throughout their domains. Each astrocytic domain in humans spans from 100 to 400 µm in diameter, making them, on average, 2.55 times larger than those in rodents.[18, 28, 29] This allows them to cover a much larger volume and contact more synaptic terminals (up to 2 million synapses[18]). Refined by the connectomic imaging approach, diolistic labeling and the loading of individual cells with fluorescent dye revealed that protoplasmic astrocytes in the human cortex occupy distinct domains, similar to those observed in rodents, but with a slightly greater overlap of border processes. Specifically, the overlap in human astrocytes is 204.7 ± 44.1 µm², whereas it is 11.8 ± 2.2 µm² in rodents.[18, 27, 30]

Protoplasmic astrocytes in the cortex and hippocampus have electrically passive membrane properties, with a resting membrane potential close to the reversal potential for potassium.[30, 31] These human astrocytes have a significantly larger capacitance, most likely due to a larger soma and an increased number of processes. Moreover, astrocytes filled with fluorescent dye display functional gap–junction connections.[23, 30] The coupling between astrocytes in the human hippocampus is achieved mainly by connexin 43, which is present in approximately 10 times greater abundance than connexin 30 in both humans and mice.[32]

One of the crucial functions of astrocytes in rodents is to rapidly regulate extracellular potassium (K^+) levels, modulating neuronal excitability.[33] Studies using acute human slices and K^+-sensitive microelectrodes have provided insights into similar mechanisms in humans. Recordings of the extracellular K^+ concentration in slices from temporal lobe epilepsy (TLE) patients revealed that the application of extracellular barium, which primarily blocks K^+ inward rectifying (Kir4.1) channels at lower concentrations, led to an increased accumulation of extracellular K^+ in the nonsclerotic hippocampus.[34, 35] This effect was minimal in the sclerotic hippocampus, where astrocytes demonstrated significantly reduced K^+ channel current density.[36] Further studies have reported astrocytic Kir4.1 channel dysfunctions in patients with epilepsy-associated lesions[36] and those suffering from severe disabling seizures caused by loss-of-function mutations in the Kir4.1 and SLACK channels,[37] further highlighting the important function of this channel subtype in the human brain.

The uptake of K^+ by astrocytes is closely associated with the activity of the excitatory amino acid transporter EAATs, which are responsible for removing glutamate from the synaptic cleft and preventing glutamate spillover.[38] Protoplasmic astrocytes in the human adult brain demonstrate immunostaining for EAAT1 and EAAT2 through development.[39] During recordings from human hippocampal astrocytes, the application of glutamate to outside-out membrane patches triggers inward currents. These responses were blocked by the glutamate transporter blocker TBOA and unaffected by the AMPA/kainate receptor antagonist NBQX, indicating that they resulted from glutamate uptake through transporters.[17]

Another important characteristic of protoplasmic astrocytes in rodents is that they are known to respond to ATP and glutamate application with increased intracellular Ca^{2+}, which further modulates synaptic

transmission and plasticity.[40] The human fetal astrocytic Ca^{2+} response does not change when exposed to glutamate.[29] However, adult astrocytes in both the CA1 and CA3 regions of the hippocampus and in layers 2–5 of the cortex respond to glutamate application through mGluRs-mediated increases in intracellular Ca^{2+} in cell somas and processes.[18, 26, 29] Both fetal and adult human astrocytes respond to ATP application with increased intracellular Ca^{2+}.[18, 26] These events propagate concentrically as waves at ~30–40 µm/s in most recordings and are unaffected by TTX but abolished by thapsigargin. Additionally, astrocytes respond to direct stimulation of neurons with elevated Ca^{2+} levels, indicating a direct interaction between neuronal activity and astrocytic Ca^{2+} levels. Propagating Ca^{2+} waves can travel through rodent astrocytes and their syncytia, triggering gliotransmitter release in some cases, which further influences neuronal activity.[41, 42] Although the exact mechanisms underlying gliotransmitter release are not fully understood and have long been debated,[43] recent studies using single-cell RNA sequencing have identified nine different types of astrocytes in the mouse hippocampus, including one type that selectively expresses glutamate-releasing machinery.[44] Similarly, a specific group of glutamate-releasing astrocytes has been identified in the human brain, particularly in regions CA1, CA2, CA3, and the dentate gyrus, highlighting the presence of distinct astrocyte subtypes with specialized roles in brain function. Moreover, direct recordings from human neurons revealed bidirectional communication with astrocytes, which respond to ATP by increasing Ca^{2+} levels and enhancing NMDA-mediated slow inward current frequency in neurons, suggesting that astrocytes can release glutamate, thereby influencing neuronal excitability.[26]

Varicose Projection Astrocytes

VPAs are found only in apes and humans. Somas of these cells are located in layers 5–6, and like ILAs, these cells have two types of protrusions: multiple relatively short and spiny protrusions and up to five long protrusions containing varicose protrusions every 10–15 µm.[15, 18] VPAs are notably absent in other mammals with large brain sizes, such as elephants, giraffes, and whales, despite initial considerations for their presence as adaptations for larger brains.[15] This suggests that VPAs are not solely linked to brain size but may result from specific neurobiological mechanisms evolved in a common hominoid ancestor. Limited data exist on the functionality of VPAs, but their location, long processes, and irregular occurrence across species provide insights into their potential roles. Oberheim and colleagues suggested that VPAs may be involved in facilitating long-range signaling between different cortical regions or across the gray and white matter. Additionally, the termination of their processes on blood vessels hints at a potential role in regulating blood flow over more extensive areas than those typically influenced by protoplasmic astrocytes.[18] Interestingly, VPAs were observed in some but not all brain slices, with their presence being independent of factors such as the time required for slice preparation (in some cases >24 hours), the age of the subjects, or the cause of death. In slices where varicose projections were found, ILAs also displayed varicosities along their long processes. Since ILAs do not consistently exhibit these varicosities, their presence may be induced by specific conditions. VPAs, which share close proximity and elongated process morphology characteristic of fibrous astrocytes, are considered to be modified forms of these cells. Falcon et al.[15] suggested that VPAs undergo morphological changes in response to stress, aging, and certain pathological conditions.

Fibrous Astrocytes

Fibrous astrocytes are located in the white matter. They are known to be twice as large as their rodent counterparts, approximately 180 µm in the diameter of the $GFAP^+$ defined domain, and have straighter, fewer, and less elaborate processes.[18] Unlike protoplasmic astrocytes, fibrous astrocytes do not exhibit domain organization but are equally distant from one another, likely owing to their role in supporting axon tracts. These astrocytes are crucial for metabolic support, as they frequently contact blood vessels. Additionally, they play a role in ion regulation at the nodes of Ranvier, contributing to the maintenance of the ion balance in these regions.[45]

Limitations

This review prioritizes studies using *ex vivo* human brain tissue, which, while valuable, does not incorporate insights from iPSC-derived astrocytes, organoids, and chimeric animal models that may not fully capture the complexities of astrocytic architecture. This review highlights studies that predominantly use the $GFAP^+$ marker for astrocyte labeling, which tends to focus on astrocytes in superficial cortical layers. While $GFAP^+$ has been invaluable in astrocyte research, incorporating alternative markers such as ALDH1L1, which labels a

broader population, and Cx43, which is present in all cortical layers, could increase our understanding of astrocyte diversity. Additionally, markers such as AQP4 and GLT-1, which label distal processes and endfeet, offer further insights into the complexity of astrocyte architecture throughout the cortex.[46]

Conclusion

In the last two decades, astrocytes have been recognized as active components of synapses and potential therapeutic targets for various neurological disorders. However, most of our understanding of astrocyte functionality stems from animal model studies. Translating these findings to humans necessitates an in-depth understanding of both similarities and differences. Compared with their rodent counterparts, human astrocytes are larger, with more elaborate protrusions, suggesting extended functionality. Two types of astrocytes unique to humans, ILAs and VPAs, are particularly intriguing. The roles of their long, cable-like protrusions and the physiological significance of their varicosities remain largely unknown. Like rodents, human astrocytes maintain gap-junction connections, yet they exhibit faster Ca^{2+} waves, indicating more rapid signaling. Recent studies in both rodents and humans have revealed significant variability among astrocyte subtypes, implying that astrocytic functionality is likely subtype specific.

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The author declares no conflicts of interest.
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