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Heterogeneity of Astrocytic Form and Function

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Abstract

Astrocytes participate in all essential CNS functions, including blood flow regulation, energy metabolism, ion and water homeostasis, immune defence, neurotransmission, and adult neurogenesis. It is thus not surprising that astrocytic morphology and function differ between regions, and that different subclasses of astrocytes exist within the same brain region. Recent lines of work also show that the complexity of protoplasmic astrocytes increases during evolution. Human astrocytes are structurally more complex, larger, and propagate calcium signals significantly faster than rodent astrocytes. In this chapter, we review the diversity of astrocytic form and function, while considering the markedly expanded roles of astrocytes with phylogenetic evolution. We also define major challenges for the future, which include determining how astrocytic functions are locally specified, defining the molecular controls upon astrocytic fate and physiology and establishing how evolutionary changes in astrocytes contribute to higher cognitive functions.

Keywords

Astrocyte; NG2 cell; Glia; Glia progenitor; Potassium buffering; Epilepsy; Calcium signaling; Purinergic receptors

1. Introduction

Rudolf Virchow first proposed that neuroglia comprised the connective tissue of the brain and was composed of cellular elements in 1858 (1) . Just over a decade later, Camillo Golgi visualized astrocytes within the nervous system, and further advanced the concept that these cells comprised the “glue” of the brain (2) . Yet the term “astrocyte,” which referred to the stellate morphology of these cells, was first used only in 1893, by Michael von Lenhossek (3) . These cells were soon subdivided into fibrous and protoplasmic astrocytes by Kolliker and Andriezen (4, 5). Yet not until Ramón y Cajal, whose drawings first revealed the extraordinary pleomorphism of astrocytes, was their diversity first appreciated (6) (Fig. 1).

Based on his histological studies, Cajal and others postulated several roles for this diverse class of cells, including maintaining brain architecture, homeostasis, and nutrition (7) . Since then, numerous studies have further revealed the morphologic and functional diversity of astrocytes. In addition, more recent studies have revealed inter-species differences in astrocytic form and function, which together highlight the potential importance of astrocytic function in complex brain processing (8, 9).

2. Astrocytes Are Both Heterogeneous and Pleomorphic

In the nineteenth century, two classes of central astrocytes were first described using a nomenclature that largely survives today: fibrous astrocytes of the white matter, and protoplasmic astrocytes of the grey matter (4, 5). Their distinct morphological differences were first appreciated by Golgi staining, which revealed that protoplasmic astrocytes are complex cells with numerous fine processes, while fibrous astrocytes are less complex, with

fewer branching processes. Whereas protoplasmic astrocytes appear distributed relatively uniformly within cortical gray matter, fibrous astrocytes are organized along white matter tracts, within which they are oriented longitudinally in the plane of the fibre bundles. In addition to these two classes of astrocytes, specialized astrocytes within different areas of the brain were also defined in the late nineteenth and early twentieth centuries; these included the Bergmann glia of the cerebellum, and the Muller glia of the retina (7). It was not until 1919 that oligodendrocytes and microglia were first recognized as separate cell types (7) , observations that led to our current conception of central glia, as comprised of three major cellular classes that include microglia, oligodendrocytes, and astrocytes. More recently, a number of groups have pointed out that parenchymal glial progenitor cells, typically noted as either oligodendrocyte progenitor cells or as NG2 cells based upon their expression of the NG2 chondroitin sulfate proteoglycan, may comprise a fourth category of central glia (10).

Astrocytes have not yet been associated with a canonical molecular signature that specifically and selectively defines their phenotype; their morphological features and relationships with both neurons and capillaries define their phenotype more so than any single molecular marker. Nonetheless, glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed in astrocytes, is typically used to distinguish and identify astrocytes within the central nervous system (11). Yet, even though this marker has been used for over 30 years as a standard for the definition of an astrocyte, it has become clear that not all astrocytes express GFAP and not all cells in the CNS that express GFAP are astrocytes (12, 13). For instance, neural stem cells of the subventricular zone express GFAP (14), but do not otherwise meet the criteria for phenotypic assignment as astrocytes (15). Indeed, although a number of proteins have been reported as selectively expressed by astrocytes, none have proven to be entirely specific for, ubiquitously expressed by, and absolutely restricted to, astrocytes. Rather, studies of astrocytic biology have revealed the great diversity of these cells, in such features as their developmental lineage, mitotic control, ion channel expression, receptor expression, gap junction connectivity, electrophysiological and calcium signaling properties (16). These studies have revealed a remarkable heterogeneity among astrocytes, the elucidation of which is ongoing.

One group recently attempted to define classes of astrocytes within the rodent CNS using a combination of GFAP-driven GFP expression, GFAP protein expression, and S100ß immunostaining. Using this combinatorial approach to empiric classification, Emsley and Macklis defined nine different classes of astrocytes, that included Bergmann glia, ependymal glia, fibrous astrocytes, marginal glia, perivascular glia, protoplasmic astrocytes, radial glia, tanycytes, and velate glia (17). These authors reported differences in astrocytic density among different brain regions, as well as in the morphologies thereof, and confirmed that astrocytic phenotype is in part a function of both local cytoarchitecture and region-specified functional demands.

3. Protoplasmic Astrocytes Exhibit a Domain Organization

Although astrocytes are thought of as star-like based upon both Golgi staining and GFAP immunolabeling, it has become clear that astrocytes are much larger than their silver stain or GFAP-defined profiles might suggest, as they have numerous fine processes that are GFAP-negative. In fact, it has been estimated that GFAP immunostaining reveals at best 15% of the total astrocytic volume in rodents, in which protoplasmic astrocytes reveal manifestly spongi-form morphologies (18). In addition, the conception of protoplasmic astrocytes as geometrically ovoid was challenged by dye injection studies, which revealed a variety of fusiform morphologies that allowed astrocytes to penetrate otherwise dense areas of neuropil (18, 19). Furthermore, the longstanding concept that astrocytic processes interdigitate to

create a scaffold for neuronal organization was also challenged, following dye injection studies that revealed minimal overlap – less than 5% of total astrocytic volume – between neighboring hippocampal astrocytes (18, 19). Instead, these studies and others revealed that hippocampal astrocytes are organized in distinct, nonoverlapping domains, with little interaction between adjacent cells. Since then, other groups have revealed that this domain organization is also found in the rodent cortex (20–22).

The significance of the domain organization is unclear. The many fine processes of protoplasmic astrocytes penetrate all areas of the local neuropil, encompassing synapses and the microvasculature alike. It has been estimated that within the domain of a single hippocampal astrocyte, there are approximately 140,000 synapses (18). Thus, single astrocytes contact and may control large sets of contiguous synapses as well as the vascular bed regulating blood flow to those synapses. This architecture places the astrocyte in a prime position to coordinate synaptic activity and blood flow, potentially independent of neuronal metabolic activity.

The domain organization may also play a role in pathology. Studies examining gliosis have shown that the domain organization is lost in reactive astrocytes in several experimental models of epilepsy, but maintained in reactive astrocytes in a mouse model of Alzheimer disease (Fig. 2) (22). In all models of epilepsy studied, including acute and chronic cortical iron injection, kainate injection, and genetic epileptic mice (SWXL mice), cortical astrocytes manifested severe reactive changes. Concurrently with an increase in cellular diameter, process hypertrophy, and upregulated GFAP, the reactive astrocytes of these epileptic models lost their domain organization and displayed on average a >15-fold increase in process overlap between neighboring cells. Reactive astrogliosis and loss of domain organization in the epileptic brains were paralleled by changes in neuronal structure, including a reduction in spine density and dendritic morphology. Interestingly, astrocytic domain organization was in part preserved if the frequency of seizures was reduced by valproate (22). Moreover, astrocytes in a transgenic model of Alzheimer's disease exhibited an increase in GFAP, but maintained the domain organization at an age of 12–14 months (22, 23), suggesting that reactive astrocytosis per se was insufficient to abrogate domain architecture. Thus, while the significance of domain organization is not well understood, it seems likely that the preservation of this astrocytic architecture may be critical to normal brain physiology and function.

4. Astrocytes Are Diverse in Physiology as Well as in Form

Traditionally, astrocytes were considered as contributing primarily to the structural organization of the brain, since they are not electrically excitable; they do not conduct action potentials like their neuronal counterparts. Yet astrocytes sustain a very low resting potential, typically −85 to −90 mV, by virtue of their dense expression of potassium channels (24). Most are also highly coupled by gap junctions, composed primarily of connexin 43, which confers a low input resistance upon cells within the astrocytic synctium (25). When depolarized, astrocytes respond with a linear current–voltage relationship and are thus not electrically excitable (26, 27). Yet the more detailed electrophysiological characteristics of astrocytes are not all the same: recent studies have determined that astrocytes within different brain regions can express different levels and types of ion channels and may thus have subtle differences in electrophysiological properties, including in their resting membrane potentials. For instance, astrocytes may vary substantially with respect to their expression of inwardly rectifying potassium channels (K_{ir}) (28). This large family of channels is expressed by protoplasmic astrocytes, fibrous astrocytes, hippocampal astrocytes, and both Muller and Bergmann glia and is also differentially expressed during development (28–32). Yet despite its ubiquitous expression as a class, the levels and specific

subtypes of K_{ir} channels can vary among astrocytic populations as a function of region and cellular relationships. For instance, in the spinal cord, astrocytes in the ventral horn express high levels of K_{ir} 4.1, while those in the apex of the dorsal horn express low levels, resulting in intraseg-mental gradients in the rate of potassium buffering, and hence in local thresholds for synaptic transmission (32). Additionally, expression of K_{ir} 4.1 changes during development: In the hippocampus, K_{ir} 4.1 is down regulated within 10 days after birth, concurrently with a fourfold decrease in astrocytic inward current density (29). Bergmann glia also exhibit developmental changes in K^+ channel expression; delayed outward and inward rectifying K^+ currents predominate during the first post-natal week, while mature Bergmann glial cells display both voltage and time independence currents (33).

5. NG2-Expressing Cells Comprise a Glial Phenotype Distinct from Astrocytes

Regional differences notwithstanding, the electrophysiological properties of different subclasses of astrocytes are largely similar, including across regions (14). As a group, they are readily distinguished from the only other electrically polarized glial phenotype, the NG2 cell, also referred to as the oligodendrocyte or glial progenitor cell (34, 35), or polydendrocyte (36). NG2 cells may be viewed as a separate class of glial cells and are characterized by a lack of gap junction coupling, high input resistance, and voltage-dependent sodium and potassium conductances (36). NG2 glial progenitor cells are themselves a heterogenous group (37, 38) and have been found to express AMPA, NMDA, and GABA receptors in different brain regions, and form synapses with neurons in both grey and white matter, even participating in forms of LTP (39–43). Furthermore, NG2 cells have differing expression of glutamine synthetase in the hippocampus and have been shown to have differing morphologies and electrophysiological properties based on brain region (44, 45). Although still under intense study, the fate of NG2 cells also appears to be varied. In culture, $NG2^+$ glial progenitor cells are readily bipotential for astrocytes and oligodendrocytes (35, 46–48), and under serum-free culture, conditions can generate neurons as well as glia, with a fraction revealing neural stem cell potential (37). In vivo though, fate mapping studies have revealed a more restricted phenotypic potential, by which endogenous NG2 cells can generate oligodendrocytes in both brain and spinal cord, and protoplasmic astrocytes in the gray matter of the ventral forebrain and spinal cord (49–52). Yet in these studies, no white matter fibrous astrocytes were derived from NG2 cells. Other studies using similar cell fate mapping strategy based upon the expression of PDGFα identified derived oligodendrocytes and additional NG2 cells, as well as small numbers of pyriform neurons, yet failed to see astrocytes in grey or white matter (53). Therefore, while it seems likely that the oligodendrocyte lineage is derived from NG2 cells, the generation of astrocytes from these cells–both in normal physiology and in reactive states–remains controversial.

6. Astrocytic Glutamate Transport and Modulation of Transmission Varies by Region

One of the major functions of astrocytes within the CNS is glutamate uptake, which influences excitatory neurotransmission and prevents excitotoxicity. Astrocytes accomplish this through expression of glutamate transporter proteins, predominantly GLAST and GLT-1 (EAAT2) (54). It is now known through transgenic studies in which the fluorescent protein DsRed was placed under the control of the GLAST promoter, and GFP under the GLT-1 promoter, that there is heterogenous expression of these important proteins in different areas of the CNS as well as during development (Fig. 3) (55). GLAST is expressed primarily in radial glia as well as cortical astrocytes during development, but does persist in the adult

brain in the Bergmann glial cells of the cerebellum, fibrous astrocytes of the ventral white matter tracts of the spinal cord, as well as several niches in the forebrain such as the progenitor cells of the subgranular layer of the dentate gyrus. GLT-1 is the predominant gluta-mate transporter expressed in the adult brain and is highly active in both protoplasmic and fibrous astrocytes accounting for 90% of glutamate uptake in the CNS (56, 57). However, in the spinal cord, there is tenfold less expression of GLT-1 compared to brain, which is correlated with decreased glutamate uptake (55). Additionally, a splice variant of the type 2 excitatory amino acid transporter, exon 9 skipping EAAT2/GLT1, is highly expressed in fibrous astrocytes of the white matter and only expressed weakly in subsets of protoplasmic astrocytes and radial glia (58). Protoplasmic and fibrous astrocytes may thus differ substantially in their glutamate uptake capabilities and capacity.

7. Astrocytic Neurotransmitter Receptor Expression and Calcium Response

Unlike their neuronal counterparts, astrocytes are not electrically excitable; rather they are a chemically excitable system. It was first observed in the early 1990's that cultured astrocytes could respond to stimuli such as glutamate by increasing intracellular calcium and initiate calcium wave propagation between neighboring cultured astrocytes (59). Recently, it was observed that astrocytes can increase their intracellular calcium in small volume compartments, near membranes in the fine astrocytic processes as well as in the cell somata (60). It is now recognized that astrocytes express numerous metabotropic receptors coupled to second messenger systems; in slice preparations, these have been shown to increase intracellular calcium in a phospholipase C (PLC) and inositol (1, 4, 5)-trisphosphate-dependent fashion, in response to neurotransmitters that include glutamate, ATP, GABA, adenosine, and norepinephrine, acetylcholine, prostaglandins, and endocannabinoids (59, 61–67). Additionally, it has been shown that astrocytes have the capability to increase intracellular calcium intrinsically, without the influence of neuronal activity (68–71). Interestingly, it has been shown that astrocytes within different areas of the central nervous system respond to different collections of neurotransmitters. Because the in vitro environment can artifactually alter astrocytic receptor expression, the work highlighted here derives from in vivo and in situ studies. In the cortex, astrocytes respond to glutamate and norepinephrine with increases in calcium (72–74), while hippocampal astrocytes exhibit calcium responses to ATP, GABA, glutamate, acetylcholine, prostaglandins and endocannabinoids (64, 75–80). Studies of brain slices from the cerebellum show that astrocytes in this region respond to ATP, norepinephrine, glutamate, and nitric oxide (81–84). Astrocytes in the olfactory bulb have also been shown in brain slice preparations to respond to ATP and glutamate and in the retina to ATP (85, 86). The physiologic responses in most cases have been correlated with neurotransmitter receptor expression, highlighting the heterogeneity of astrocytes within different brain regions.

Astrocytes also vary in their calcium responses. There are two major types of whole-cell calcium signals in astrocytes that include intrinsic calcium oscillations within single cells and calcium waves propagated from one cell to others. Both forms of calcium signaling can occur both independent of neuronal activity, as well as in response to neurotransmitters as described above (68–71). Spontaneous calcium oscillations differ in different layers of the somatosensory cortex. In live anesthetized rats, astrocytes in layer 1 display mostly asynchronous calcium oscillations that are more than twice as frequent as those of astrocytes in layers 2/3, which display more synchronized calcium responses (87). In addition, the downstream effects of astrocytic Ca^{2+} signalling are context and phenotype-dependent, so that activation of different receptors can mediate fundamentally different responses. For example, activation of either $P2Y_1$ or PAR-1 receptors can increase cytosolic Ca^{2+} in hippocampal astrocytes, yet only PAR-1 receptor activation triggered astrocytic glutamate

release, as detected by NMDA receptor-mediated slow inward currents in nearby neurons (88).

Calcium waves also differ by astrocytic class and location. Calcium waves in gray matter protoplasmic astrocytes rely on gap junction coupling to propagate. In slice preparation of connexin 43-deficient mice, the principal gap junction protein expressed in astrocytes, there was no calcium wave propagation (89). In contrast, fibrous astrocytes of the white matter in the corpus callosum can propagate calcium waves without gap junction coupling and are instead dependent on ATP release. This was demonstrated by calcium wave propagation in the connexin 43 knockout mice, as well as by the sensitivity of calcium wave transmission in the corpus callosum to purinergic receptor blockers, but not to gap junction blockers (89, 90). Thus, different classes of astrocytes may utilize both different moieties and modalities of communication within the glial synctium.

8. Astrocytes Can Communicate via Gliotransmitters

Since astrocytes exhibit diverse responses to a variety of neurotransmitters, it follows that astrocytes may tap a diverse collection of gliotransmitters by which to communicate with their neighbours. Increases in astrocytic intracellular calcium have been shown to trigger the release of several gliotransmitters, including glutamate, ATP, adenosine, d-serine, TNF-α, and eicosanoids, which then can modulate the activity of surrounding cells including other astrocytes, neurons, microglia, and the vasculature (91–95). Astrocytes in the cortex and hippocampus have been shown to release ATP and glutamate leading both to excitation and inhibition of neuronal activity (69, 79). Numerous studies on hippocampal astrocytes demonstrate the astrocytic release of glutamate, ATP/adenosine, D-serine and TNF alpha with effects ranging from increased excitatory as well as inhibitory post-synaptic currents to modulation of LTP and synaptic scaling (64, 78, 96–102). In the cerebellum, astrocytes have been shown to release ATP and adenosine, resulting in depression of spontaneous excitatory post-synaptic currents (103). Additionally, Bergmann glia have also been shown to release GABA through bestrophin 1 channels, as a mechanism of tonic synaptic inhibition (104). Astrocytes within the olfactory bulb can release both glutamate and GABA modulation slow inward and outward currents, respectively, and those in the retina release glutamate, ATP/adenosine, and d-serine leading to modulation of light-evoked neuronal activity (105–107).

It is important to note that it remains unclear whether gliotransmitter release is a normally occurring physiological event. Gliotransmitter release has only reliably been demonstrated in vitro and has been shown to play a role in synaptic plasticity in situ only under nonphysiological conditions. One characteristic shared by all gliotransmitters is that they are present within the cytosol of astrocytes in mM concentrations. Since gliotransmitter release has been studied in slice preparations using manipulations (e.g., high frequency stimulation, UV photolysis) that potentially can lead to the opening of channels (volume-sensitive channels or Cx-hemichannels), with an inner pore diameter large enough to allow efflux of glutamate, ATP, or D-serine, it is possible that gliotransmitter release is fundamentally nonphysiological. In other words, the experimental manipulations may activate signaling pathways that are not operational under normal conditions. In support of this concept, several recent reports have documented that agonist-induced Ca^{2+} signaling in astrocytes is not linked to gliotransmitter release (108–110)

9. Astrocytes Can Coordinate Syncytial Communication Using Gap Junction Coupling

Traditionally, astrocytes are thought to be highly coupled cells through the expression of connexins (Cx), mainly Cx43 and Cx30 (111). However, different astrocyte classes have

different degrees of coupling. Additionally, depending on anatomic location, the extent and organization of coupling can differ. Finally, studies have shown that age may also play a part in the level of astrocytic coupling.

Protoplasmic astrocytes of the cortex are highly coupled cells. After a single cell injection of biocytin, a gap junction-permeable dye, an average of 94 cells spanning a radius of approximately 400 μm can be visualized and hence appear networked through gap junctions (89). In contrast, it is now thought that fibrous astrocytes within the major white matter tracts are not highly coupled. Using the same technique as in cortex, Haas et al. were only able to visualize 1–2 cells labeled with biocytin and never a network as observed in protoplasmic astrocytes (89). These data suggest that protoplasmic and fibrous astrocytes have very different degrees of coupling, which has significant implications in regard to their respective calcium wave signals, resting membrane potentials, potassium buffering, and glutamate metabolism, as well as to their respective abilities to exchange second messengers, metabolites and other signal intermediates between cells.

As a higher-order level complexity, the organization of gap junction coupling can be dependent on anatomic localization within the cortex. Protoplasmic astrocytes in both the cortex and hippocampus are highly coupled. However, it is now thought that not all astrocyte networks are circular in nature. In the cortex, in layers 1 and 2/3, gap junction networks have been shown to be in parallel with the surface of the cortex (112). In the deeper layers, 4 and 5, astrocytic networks through gap junctions were shown to be more circular. This was also the case in the hippocampus where astrocytes near the pyramidal cell layer have networks that remain in parallel to this anatomic structure, yet astrocytes in the stratum radiatum have circular networks (112). In more specialized areas of the cortex, such as the barrel cortex of rodents, astrocyte networks were shown to be more oval in shape within a barrel field compared to circular networks in areas outside the barrel fields in layer IV (113). Additionally, gap junction communication is restricted to within a barrel due to little gap junction coupling of astrocytes within the septa between the barrel fields (113). In the Bergmann glia of the cerebellum, which are also highly coupled by Cx 43, the shape of the network is perpendicular to the parallel fibres, forming long strings of coupled cells unlike the circular or oval networks seen in the cortex and hippocampus (114). Therefore, anatomic localization as well as cellular class plays a role in the shape of the networks in which astrocytes are coupled.

Age may also play a role in the degree of astrocytic coupling. When astrocytes in the hippocampus are injection with the gap junction-permeable dye biocytin, there was a much smaller network of coupled cells observed compared to early postnatal rodents (16). Therefore, age, cell type and anatomic localization all play a part in the determinate of gap junction coupling of astrocytes which has implications for variations of cellular properties and functions. Despite the implied importance of gap junction coupling in astrocyte function, knockout mice of Cx43 as well as double knockout mice of Cx43 and Cx30 have been generated (115–117). Surprisingly, other than some changes in potassium homeostasis, there is little phenotype in both of these knockout mice, suggesting that either there is compensatory upregulation of other connexins or pannexin molecules in these animals, or perhaps coupling may not be as integral as once thought to astrocyte function (117).

In addition to gap junction coupling, the expression of Cx43 is also thought to have an important role in formation of hemichannels, or an unopposed half of a gap junction. Hemichannels can open during a variety of both physiologic and pathologic conditions and can lead to release of several gliotransmitters including ATP and glutamate (111, 118–120). At this point, it is unclear whether any heterogeneity exists with regard to the number of functional Cx43 hemichannels within the different classes of astrocytes.

10. The Ontogenetic Basis of Astrocytic Heterogeneity

The heterogeneity of astrocytes could arise due to separate astrocyte lineages, plasticity of differentiated cells, or a combination of both phenomena (121). It is well-known that mature astrocytes can exhibit forms of plasticity, most notably after injury when astrocytes become reactive, upregulate GFAP and other intermediate filament proteins, become larger, and in some pathologies loss of the domain organization of the protoplasmic astrocytes (22, 122, 123). Another example of plasticity of the mature astrocyte is astrocyte motility. Time lapse studies of astrocytes in acute slice and slice culture have shown that astrocyte processes act much like dendritic spines; they are frequently motile and contact active synapses (124, 125). One role of this motility may be in synaptic remodeling, in that direct contact of astrocytic processes has been shown to be necessary for dendritic spine maturation (126). Additionally, this plasticity may be involved in regulating synaptic strength (127). In the hypothalamo-neurohypophysial system, lactation determines the amount of astrocytic coverage of synapses. During lactation, astrocytic processes retract from active synapses, distancing glutamate transporters and therefore increasing the glutamate concentration within the synaptic cleft (127). This in turn activates inhibitory interneurons leading to homo and heterosynaptic depression of neurotransmitter release and is thought to be important for the regulation of lactation (127, 128). Therefore, mature astrocyte plasticity may be critical for modulating neuronal activity and important for the development of astrocyte heterogeneity.

The diversity of astrocytes may reflect the underlying diversity of glial progenitor cells. Gliogenesis occurs perinatally in the germinal niches of the CNS, the ventricular and subventricular zones (129). There are several distinct pools of progenitors within the VZ/SVZ that may give rise to astrocytes, which include both radial cells of the ventricular zone and glial progenitor cells of the subventricular zone. Initial lineage tracing studies in birds revealed that radial cells are multipotential (130, 131), and later studies confirmed the multilineage competence of radial cells in mammals as well. Yet other studies have pointed out that some radial cells may directly give rise to a subset of cortical astrocytes (132), and that such radial cell astrocytic progenitors persist postnatally (133). In addition, some cortical and white matter astrocytes are derived from distal-less homeobox 2 (Dlx2) migratory progenitors from the dorsolateral subventricular zone, which are distinct from radial glia (134). In addition, some astrocytes may be generated locally from glial progenitor cells after their migration into the marginal zone (135). Furthermore, protoplasmic astrocytes may also be generated postnatally from $NG2^+$ glial progenitor cells arising from the SVZ of the ganglionic eminences and later ventral striatum (51). Importantly, $NG2^+$ glial progenitors may not contribute significantly to either fibrous astrocytes of the white matter or protoplasmic astrocytes in the dorsal telencephalon, in which locally generated, dorsally derived Dlx-2+ progenitors may give rise to mature astrocytes. In this regard, cell fate studies of Olig2+ glial progenitors have shown that these cells in the SVZ/VZ may give rise to astrocytes of the dorsal pallium (136). Thus, astrocytes may derive from different cells of origin, suggesting an ontogenetic basis for their mature heterogeneity.

This also seems to be the case in the development of astrocytes within the spinal cord. Cell lineage tracing studies have found that astrocytes (in addition to motor neurons and oligodendrocytes) at the ventral surface of the spinal cord are produced from Olig2+ progenitors in a subset of the ventral ventricular zone of the spinal cord named the pMN domain (137–139). Astrocytes in the spinal cord are also derived from cells outside the pMN domain in the ventral ventricular zone in a position-dependant manner. Recent studies have demonstrated three distinct domains of the ventral ventricular zone, which give rise to distinct white matter astrocyte subpopulations in the spinal cord (140). These subtypes of fibrous astrocytes can be distinguished through the combinatorial expression of reelin and

slit1, while their positional identities may be defined by the expression of the homeodomain transcription factors Pax6 and Nkx6.1. Thus, in the spinal cord as well as in the forebrain, considerable heterogeneity may be observed in astrocytic lineage and phenotype.

11. Human and Hominid Astrocytes Are More Complex than Those of Infraprimates

In addition to the functional heterogeneity of astrocytes within the rodent cortex, it is now clear that significant inter-species heterogeneity exists among glial cells. In particular, human and primate cortical astrocytes are substantially larger and more complex than their rodent counterparts (9). Furthermore, there are more subtypes of cortical astrocytes found in primates and humans compared to other mammals (Fig. 4). A recent study made a direct comparison between cortical astrocyte found in human, primate, and rodent brains (8).

Compared to the rodent cortex, primates harbor two novel astrocyte subclasses: interlaminar astrocytes and varicose projection astrocytes (5, 8, 141–143) (Fig. 5). Varicose projection astrocytes which have hitherto been observed only in humans and chimpanzees are $GFAP^+$ cells that reside in layers 5–6 (8). They are characterized by the shorter straighter main processes compared to protoplasmic astrocytes and the striking extension of one to five long processes of up to 1 mm in length. These long processes are notable for evenly spaced varicosities approximately every 10 μm. The long processes terminate in the neuropil or along the vasculature. Human varicose projection astrocytes are more complicated and larger than those observed in the chimpanzee brain. The function of these cells specific to higher-order primates remains to be determined.

Interlaminar astrocytes abundantly populate cortical layer 1 in both humans and primates. In humans, they are characterized by spheroid cell bodies close to the pial surface and extend several short processes that contribute to the pial glial limitans, creating a thick network of GFAP fibres (8, 141–143). Additionally, they extend one to two processes from layer 1, terminating in layers 2–4 of the cortex, resulting in numerous millimetre-long processes radiating through the outer cortical layers in a columnar manner. Human interlaminar astrocytes are distinct in that primate inter-laminar astrocytes have oblong cell bodies directly opposed to the glial limitans and are less numerous than seen in the human cortex. Both in humans and other primates, the millimetre-long processes of interlaminar astrocytes are tortuous and terminate in the neuropil or on the vasculature. Their function remains unknown, but the long processes have been shown to be able to support calcium wave propagation in humans (8).

Protoplasmic astrocytes in humans are manifestly distinct from those of rodents (Fig. 6). They are 2.6-fold greater in diameter, with >10-fold more abundant GFAP-defined processes (8). Like their counterparts in rodents, human astrocytes are also organized into domains, but with significantly more overlap in proportion to their increased diameter. In the rodent, one astrocytic domain may encompass 20,000–120,000 synapses (8). Yet in accord with the increased size of protoplasmic astrocytes in humans, and the high synaptic density of the human cortex, the domain of one human protoplasmic astrocyte may encompass 270,000 to 2 million synapses (8, 9). Furthermore, protoplasmic astrocytes from human brain are able to propagate calcium waves far more rapidly than their rodent counterparts, with a speed of 36 μm/s, approximately four to tenfold that seen in rodents (8). Similarly, fibrous astrocytes of the white matter in humans are similarly larger and more complex than those of rodents (8). Overall then, human astrocytes are both morphologically and functionally distinct from those of infraprimate mammals, exhibiting larger size, far greater architectural complexity and pleomorphism, and more rapid syncytial calcium signalling than their murine counterparts (8, 9, 144). As such, the unique aspects of astrocytes in

humans may provide a cellular substrate for many of the distinct neurological capabilities and increased functional competencies of the human brain. Indeed, better understanding of how the evolution of astrocytes might contribute to human neural processing, and hence the species-specific capabilities intrinsic to human cognition, is a key question for the future.

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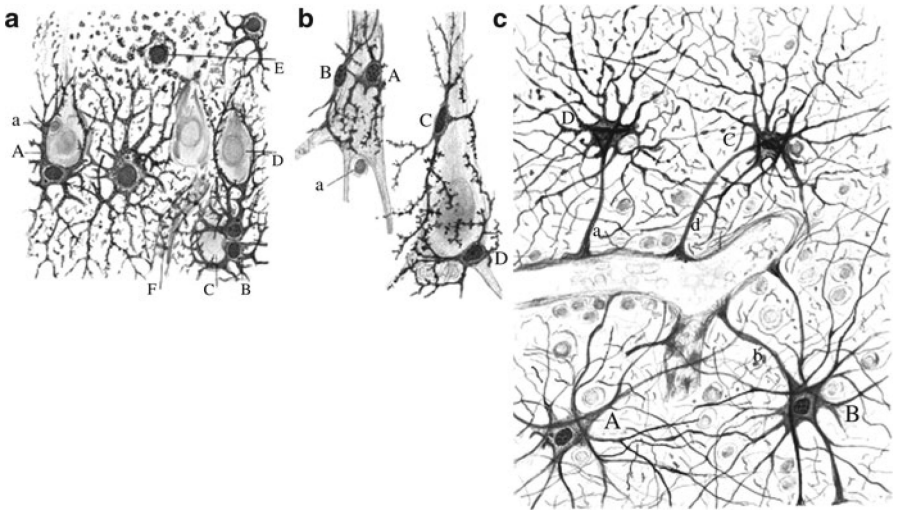

Fig. 1.

Prototypical astrocytic morphologies. (**a**) Cajal's drawing of astrocytes (indicated by “A”) in the pyramidal layer of the human hippocampus (indicated by “D”), twin astrocytes (indicated by “B”) and a satellite cell called the “third element” by Cajal (indicated by “a”). Sublimated gold chloride method. (**b**) Different astrocytes (indicated by “A,” “B,” “C” and “D”) surrounding neuronal somas in the pyramidal layer of the human hippocampus. (**c**) Cajal's drawing of fi brous astrocytes of human cerebral cortex surrounding a blood vessel. Reproduced from (145).

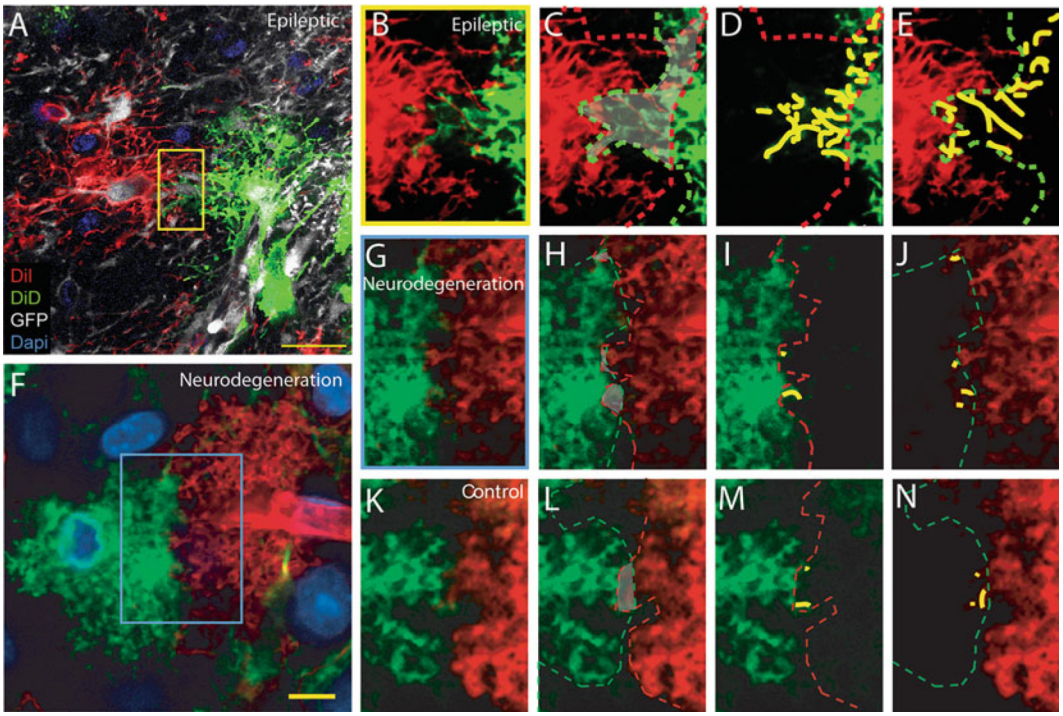

Fig. 2.

Astrocytic domain organization varies with pathology. The domain organization of protoplasmic astrocytes is lost in epileptic brains, but maintained in neurodegeneration. (**a**) Reactive astrocytes 1 week post-iron injection lose the domain organization. Diolistic labelling of the cortex of a GFAP-GFP mouse 1 week post-iron injection near injection site. Two adjacent GFP positive astrocytes are labeled with DiI and DiD. DAPI, *blue*, GFP, *green*, DiI, *red*, DiD, *white*. (**b**–**e**) High power of yellow box in (**a**). area of overlap delineated in *grey*, *red line* is border of the domain of the *red* cell, *green line* is the border of the domain of the *white* cell. (**g**–**h**) *Yellow lines* indicate the processes of the cell that pass into the domain of the adjacent cell's domain represented by the dotted line. (**f**) Cortical astrocytes in an Alzheimer disease model Tg2576 become reactive, but do not lose the domain organization. Diolistic labelling of cortical astrocytes in Tg2576 mouse. (**g**–**j**) High power of *blue box* in (**f**) showing limited overlap between adjacent cells. (**k**–**n**) Adjacent control astrocytes demonstrating the domain organization. Scale: (**a**) 20 μ m; (**g**–**h**) 10 μ m. From (22).

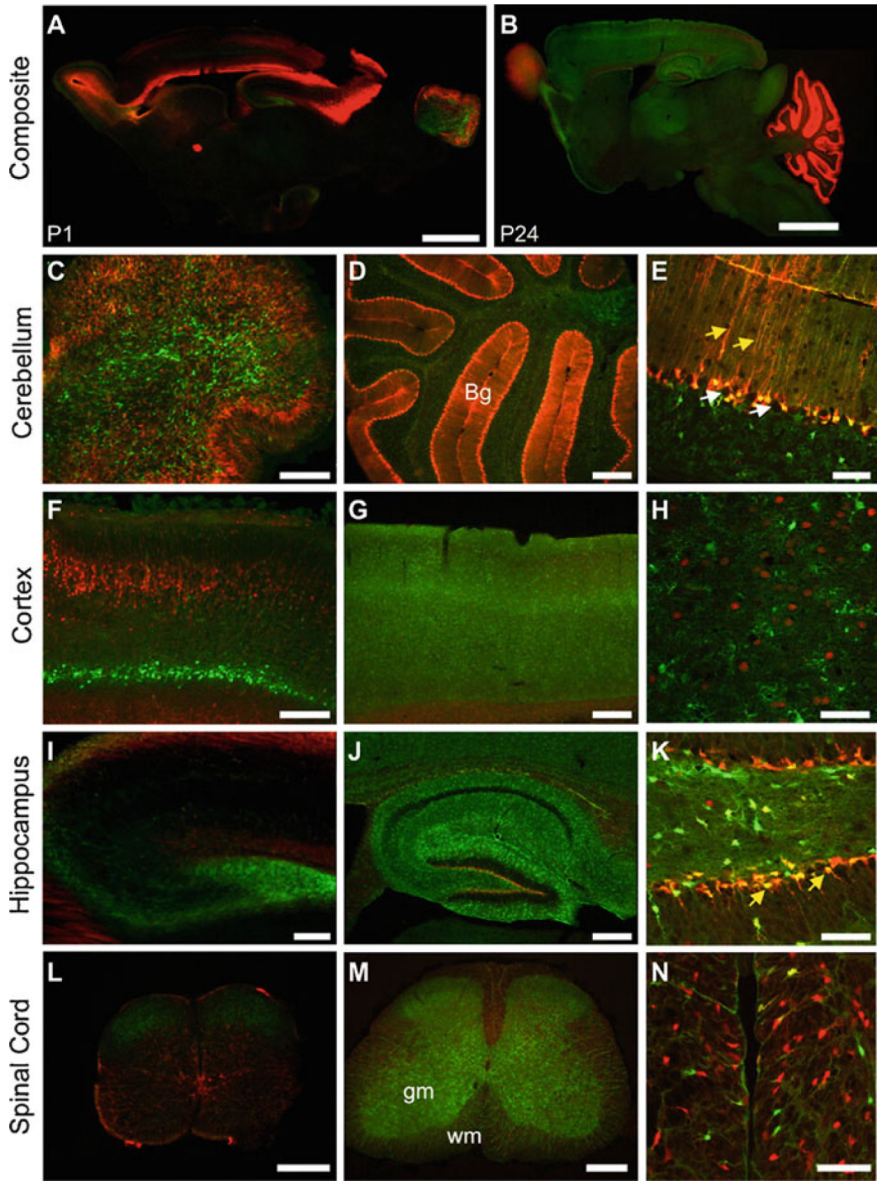

Fig. 3.

Astrocytic expression of glutamate transporters varies in different areas of the CNS. Double-transgenic mice expressing fl orescent proteins under the GLAST and GLT-1 promoters were used to study the expression of GLAST and GLT-1 during development of the CNS. All images are sagittal sections from GLAST–DsRed/GLT-1–eGFP double-transgenic mice. (**a**) Composite fluorescent image showing the expression of DsRed (GLAST) (*red*) and eGFP (GLT-1) (*green*) in the brain at P1. (**b**) Composite image showing the expression of DsRed (GLAST) and eGFP (GLT-1) in the P24 brain. (**c**–**e**) Fluorescent images of the cerebellum at P1 (**c**) and P24 (**d**, **e**). Both GLAST and GLT-1 promoters were active in Bergmann glia (Bg) (*yellow arrows* in (**e**)), although neither promoter was active in Purkinje neurons (*white arrows* in (**e**)). (**f**–**h**) Fluorescent images of the cortex from P1 (**f**) and P24 (**g**, **h**). Layer 4 is shown at higher magnification in (**h**). (**i**–**k**) Fluorescent images of the hippocampus from a P1 (**i**) or P24 (**j**, **k**). Both DsRed (GLAST) and eGFP (GLT-1) were expressed by radial glia in the dentate gyrus (*yellow arrows* in (**k**)). (**l**–**n**) Fluorescent images of spinal cord from a P1 (**l**) or P24 (**m**, **n**). *gm* gray matter; *wm* white matter. A region of the

ventral white matter is shown at a higher magnification in (**n**). Scale: (**a**–**b**) 2 mm; (**c**, **d**) 300 μ m; (**e**) 50 μ m; (**f**, **g**) 300 μ m; (**h**) 50 μ m; (**i**, **j**), 300 μ m; (**k**) 50 μ m; (**l**, **m**) 300 μ m; (**n**) 50 μ m. From (55).

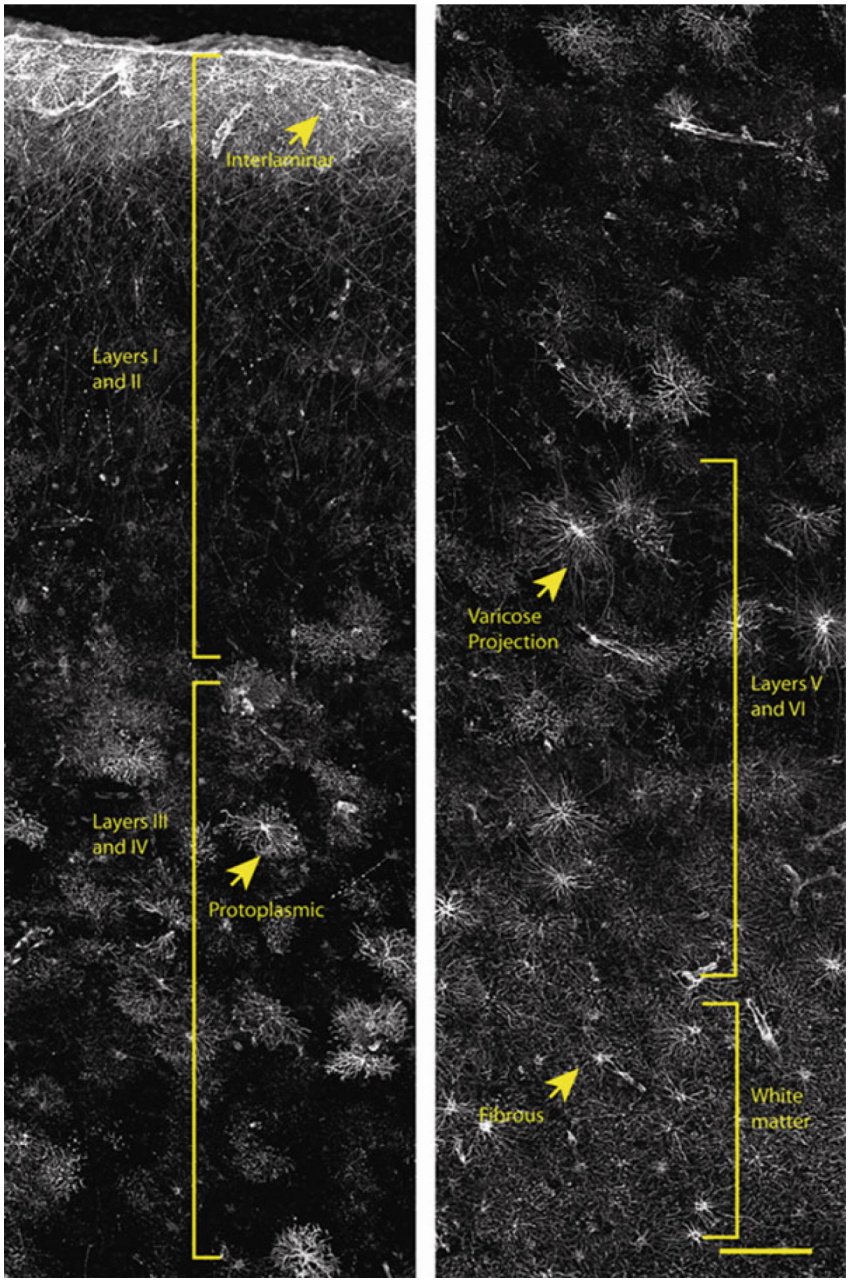

Fig. 4.

Four major classes of GFAP$^+$ cells coreside within the human neocortex. Human brains were immunolabeled with GFAP and analyzed throughout all layers of the cortex to determine subclasses of human astrocytes. Layer 1 is composed of the cell bodies of interlaminar astrocytes, whose processes extend over millimetre lengths through layers 2–4 and are characterized by their tortuous morphology. Protoplasmic astrocytes, the most common, reside in layers 2–6. Polarized astrocytes are found only in humans and are seen sparsely in layers 5–6. They extend millimetre-long processes that are characterized by varicosities. Fibrous astrocytes are found in the white matter and contain numerous overlapping processes. *Yellow lines* indicate areas in which the different classes of astrocytes reside. Scale = 150 μm. Reproduced from (8).

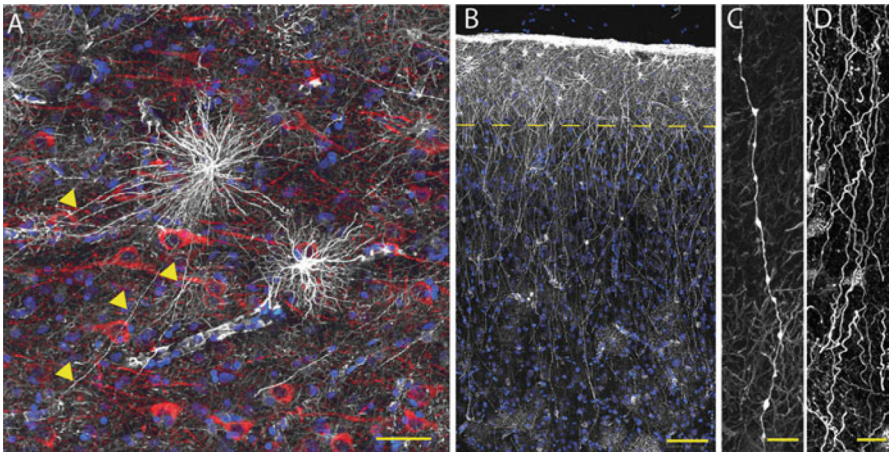

Fig. 5.

Hominid-specific astrocytic phenotypes pervade the human brain. (**a**) Varicose projection astrocytes reside in layers 5–6 and extend long processes characterized by evenly spaced varicosities. GFAP, *white*, MAP2, *red*, DAPI, *blue*. *Yellow arrowheads* indicate numerous long processes. (**b**) Pial surface and layers 1–2 of human cortex. GFAP, *white*, DAPI, *blue*. *Yellow line* indicates border between layers I and II. (**c**) Process from a varicose projection astrocyte. GFAP, *white*. (**d**) Interlaminar astrocyte processes characterized by their tortuousity. GFAP, *white*. Scale: (**a**,**b**) 100 μm; (**c**,**d**) = 10 μm. Reproduced from (8).

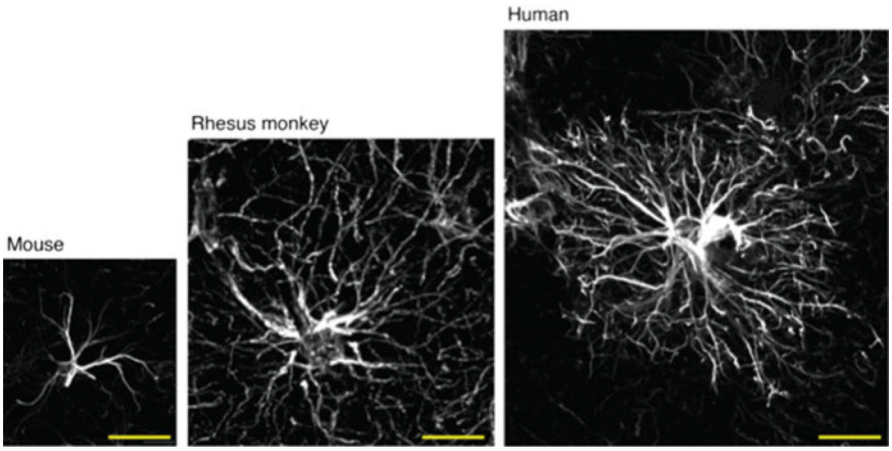

Fig. 6.

Human astrocytes are larger and more complex than rodent and other primates. Mouse, Rhesus Monkey, and Human astrocytes are compared by GFAP staining (*white*). Scale = 20 μm.