Functional diversity of astrocytes in neural circuit regulation

Lucile Ben Haim[1] *and David H. Rowitch*[1,2]

Abstract | Although it is well established that all brain regions contain various neuronal subtypes with different functions, astrocytes have traditionally been thought to be homogenous. However, recent evidence has shown that astrocytes in the mammalian CNS display distinct inter- and intra-regional features, as well as functional diversity. In the CNS, astrocyte processes fill the local environment in non-overlapping domains. Therefore, a potential advantage of region-specified astrocytes might be their capacity to regulate local development or optimize local neural circuit function. An overview of the regional heterogeneity of neuron–astrocyte interactions indicates novel ways in which they could regulate normal neurological function and shows how they might become dysregulated in disease.

Astrocytes comprise the largest class of glial cells in the mammalian CNS. They have key roles in maintaining the blood–brain barrier[1], regulating regional blood flow, providing trophic, antioxidant and metabolic support to neurons[2], neurotransmitter recycling[3], and regulating synaptogenesis and synaptic transmission[4–6]. Astrocytes have traditionally been considered to be a homogenous cellular population, and it was assumed that astrocytes from different CNS regions were functionally interchangeable; however, glial biologists now have an appreciation of the important ways in which astrocytes are functionally diverse, a concept that fundamentally alters the way that we consider the regulation of the local neuron–glial cell unit and CNS organization.

The variety in the morphological and physiological properties of astrocytes led to early speculation about their functional diversity[7–10] (FIG. 1). However, defining the precise ways in which they differ — in terms of expression of individual genes and functions — has proven difficult. Importantly, there was a lack of technical approaches for the interrogation of the intrinsic properties of astrocytes to determine how they might differ. Unlike neurons, astrocytes are electrically silent, meaning that researchers had to monitor neuronal activity as a proxy for astrocyte function and neuron–astrocyte interactions[11]. In addition, isolating putatively diverse regional astrocyte subsets is challenging because — unlike neurons, which can display distinct morphology, projection structures or regional localization according to the subtype — astrocytes occupy the microenvironment of CNS in a relatively homogenous manner. However, recent advances in imaging and reporter methods, genetic tools and gene profiling have permitted an unravelling of key functional differences among astrocytes[12,13] (BOX 1).

In this Review, we summarize the emerging evidence for functional and molecular heterogeneity of astrocytes in the normal CNS and in disease (see also REF. 11). We focus on the mature mammalian CNS and on astrocyte-mediated neural circuit regulation because the developmental diversification of astrocytes has been recently covered elsewhere[14–16]. We also discuss experimental strategies that could be used to further decipher astrocyte heterogeneity in future research (BOX 1).

Astrocyte heterogeneity in the CNS

The morphological heterogeneity of astrocytes has been recognized since the time of Ramón y Cajal and is apparent when the human or rodent brain is stained to reveal the expression of glial fibrillary acidic protein (GFAP)[17] (FIG. 1). But how might this morphological heterogeneity translate into heterogeneity of function? This topic has only recently begun to gain traction in the research community and is the subject of several recent reviews[10,11,14,18]. Here, we provide representative examples of astrocyte heterogeneity.

Early studies based on morphology and expression of astrocyte-enriched proteins suggested that there are as many as nine putative 'types' of astrocytes in the murine CNS[19]: these include grey matter (protoplasmic) astrocytes, white matter (fibrous) astrocytes and specialized astroglia, such as Bergmann glia in the cerebellum or Müller cells of the retina (FIG. 1). Human and non-human primates display several morphological subtypes of cortical astrocytes[20,21], and stem cells with astrocyte features are found in the rodent subventricular zone and dentate gyrus[14].

Some of the morphological differences among these astrocyte types are associated with distinct patterns of protein expression, which vary according to their position

[1]*Department of Pediatrics, Neurological Surgery and Edythe Broad Institute for Stem Cell Research and Regeneration Medicine, University of California San Francisco, San Francisco, California 94143, USA.*
[2]*Department of Paediatrics and Cambridge Stem Cell Institute, University of Cambridge, Cambridge, CB2 OQQ, UK.*

Correspondence to D.R.
dhr25@medschl.cam.ac.uk

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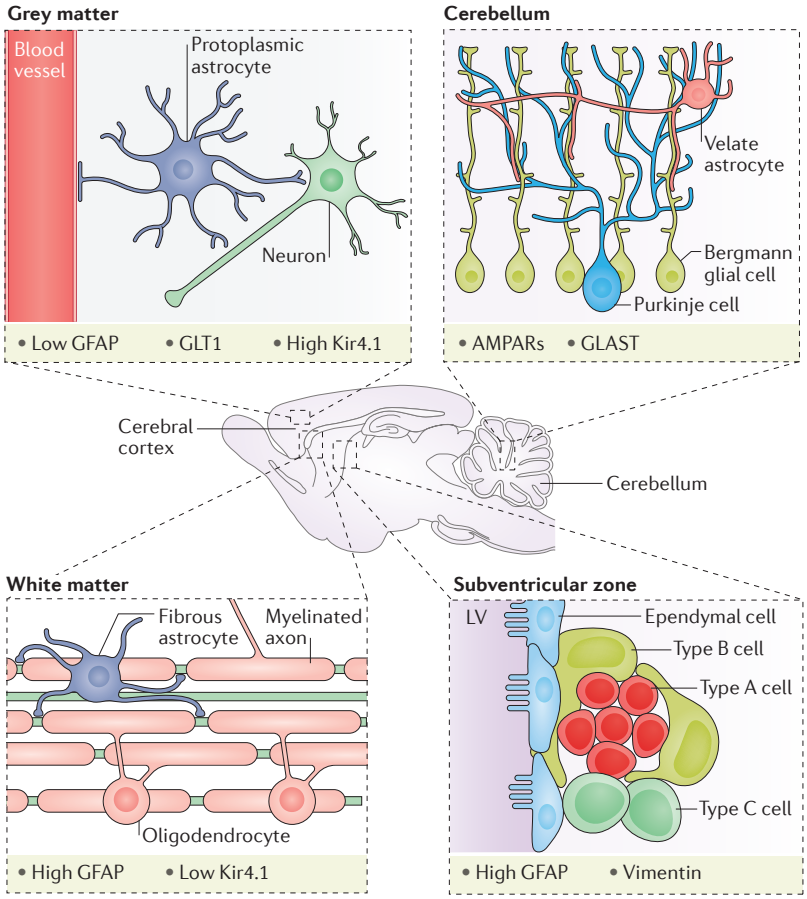


Figure 1 | **Astrocytes have a range of morphologies and molecular profiles.** The morphological diversity of astrocytes has been recognized since Ramón y Cajal's drawings of astrocytes in various brain regions[104]. The schematics illustrate the location and characteristics of several different types of astrocytes in the rodent brain. Protoplasmic astrocytes in the grey matter have a radial morphology and contact neuronal synapses and blood vessels (upper left panel). By contrast, fibrous astrocytes in the white matter have an elongated morphology and are in close contact with oligodendrocytes and myelinated axon tracts (bottom left panel). In the cerebellum, Bergmann glia extend long processes into the molecular layer and enwrap Purkinje cell dendrites (upper right panel). Velate astrocytes are protoplasmic astrocytes found in the cerebellar molecular layer. In the rodent subventricular zone, astrocyte-like type B cells line the lateral ventricles (LVs). These different types of astrocytes differentially express generic astrocyte markers. These include intermediate filament proteins (glial fibrillary acidic protein (GFAP) and vimentin), glutamate transporter 1 (GLT1) and glutamate aspartate transporter (GLAST), the inward rectifying potassium channel Kir4.1 and AMPA receptors (AMPARs). In addition to these different types, protoplasmic astrocytes can also display inter- and intra-regional molecular differences.

in specific CNS areas or circuits (FIG. 1). Indeed, astrocytes display both inter- and intra-regional differences in their protein expression profiles[22]. For instance, the ATP-dependent inwardly rectifying potassium channel Kir4.1 is selectively enriched in astrocytes in the ventral horn of the mouse spinal cord, and its expression is differentially regulated throughout postnatal development in various brain regions[22]. Intra-regional differences in morphology and mRNA expression profile are also observed[13,23] (FIG. 1). Differential protein expression is also observed in astrocytes in particular anatomical circuits, such as the mouse somatosensory cortex[24] or olfactory bulb[25]. For example, the astroglial gap-junction proteins connexin 30 and connexin 43 are enriched in the barrels of the somatosensory cortex when compared with areas outside the barrels[24].

At the functional level, several studies have provided evidence of diversity. Cell culture experiments have demonstrated differences in the ability of astrocytes that are derived from different regions of the mouse brain to support the development of midbrain neurons[26,27]. A classic study showed that adult rat hippocampal neural stem cells differentiated into neurons when co-cultured with hippocampal astrocytes but not when co-cultured with astrocytes derived from the spinal cord[28]. Differences in electrophysiological properties, calcium signalling and astroglial coupling have also been reported in specific brain regions. For instance, the transient increase in intracellular Ca^{2+} levels that is elicited during running in mice displays different dynamics in Bergmann glia and neocortical astrocytes[29]. In the rat cortex, the dynamics of spontaneous oscillations in Ca^{2+} levels show layer-specific patterns[30]. The electrophysiological features of rat hippocampal astrocytes also vary according to their localization in different subregions of the hippocampus[31]. Finally, astroglial coupling also shows subregional differences. For example, astrocytes in CA1 and CA3 of the rat hippocampus display different levels of coupling[31]. Astrocyte–astrocyte communication is also enhanced in particular functional circuits, such as the barrels of the somatosensory cortex[24] and the olfactory glomeruli of the olfactory bulb in mice[25].

These and other studies show that astrocytes display regional differences and/or specificities in terms of their morphology, protein expression patterns and intrinsic functional features. However, numerous questions remain: what are the functional consequences of these differences for the neurons or local circuits with which the astrocytes interact? Can we identify astrocyte functional subtypes and how might this definition be achieved? Is astrocyte heterogeneity primarily determined by intrinsic factors or is it induced by the microenvironment (for example, by neuron-derived cues)? As outlined below, recent studies have advanced our understanding of the molecular and functional heterogeneity of astrocytes, which ranges from broad regional differences in gene expression profiles to synapse-specific neuron–glia interactions.

Regional neuron–astrocyte interactions

Specificity of interactions

The brain is regionally organized into functional centres and local circuits in which astrocytes play various parts. Astrocytes are known to respond to numerous stimuli by generating a range of calcium signals[11]. These responses display regional and/or subregional specificities that might enable astrocytes to support and modulate particular neuronal networks in different ways (FIG. 2). In the mouse somatosensory cortex, for example, astrocytes specifically respond to the stimulation of layer 4 neurons that project to layer 2/3 (REF. 32). This selective response suggests that astrocytes discriminate between neuronal inputs within an anatomical circuit.

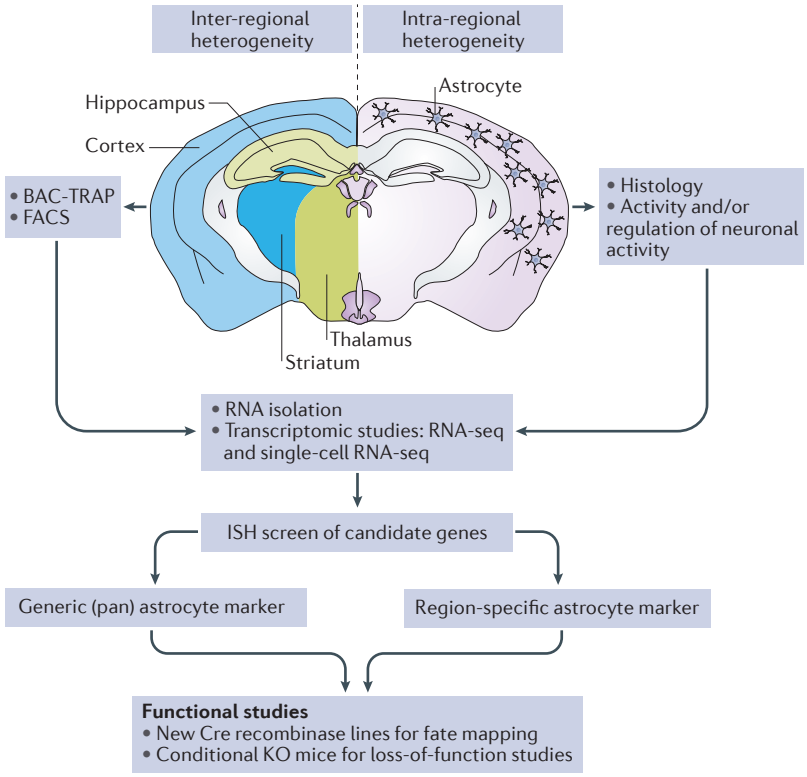
Inwardly rectifying potassium channel
A member of a family of K^+ channels enabling the entry of K^+ into the cell. These channels have many physiological functions in astrocytes and are necessary for neuronal repolarization after an action potential.

Box 1 | **Approaches to uncover astrocyte diversity**

Various approaches are taken by many laboratories to discover novel astrocyte-specific genes and functions. Even the invertebrates are involved in the action, and there has been progress in the identification of glial cell types with regulatory functions in *Caenorhabditis elegans* and *Drosophila melanogaster*[71,102].

Most evidence suggests that there is astrocyte heterogeneity both between brain regions (inter-regional heterogeneity) and within brain regions (intra-regional heterogeneity) (see the figure). For example, adjacent astrocytes in a given brain region exhibit diversity in their expression of specific proteins and in their functional ability to regulate the activity of neuronal subtypes[33,37,67]. New techniques using single-cell suspensions to interrogate the transcriptome of individual astrocytes will unravel the molecular diversity of these cells with an unprecedented depth. Indeed, gene expression profiling studies are a crucial point of entry for insights into astrocyte diversity. The comparison of the transcriptome of astrocytes from various regions and subregions will help us to uncover the molecular diversity of astrocytes throughout the CNS. This can be done through fluorescence-activated cell sorting (FACS)-based purification using cells from mice expressing a reporter with pan-astrocytic expression (such as aldehyde dehydrogenase family 1 member L1 (ALDH1L1)–green fluorescent protein (GFP) mice)[13], bacterial artificial chromosome (BAC)-translating ribosome affinity purification (TRAP)[23] to isolate translated mRNA from different CNS regions or single-cell RNA sequencing (RNA-seq) to study individual cells[103] (see the figure). Candidate genes can be validated for regional and cell type-specific expression by immunohistochemistry and *in situ* hybridization screens (ISH) in the developing and adult CNS.

Profiling of astrocytes from various brain regions also identifies 'generic' genes that are present in astrocytes across brain regions, which might be useful to generate new reporters and tools for conditional knockout (KO) of specific genes in astrocytes, such as those specifically targeting mature astrocytes. Such new tools would address the significant caveats to the use of standard human glial fibrillary acidic protein (GFAP) enhancer sequences, which are expressed during neurogenic phases and are not restricted to astrocyte progenitors during development[50]. Candidate enhancers with compelling cell-type, temporal and spatial specificity would be used to generate new mature astrocyte Cre lines and cell type-specific viral vectors (such as adeno-associated viral vectors) for both loss- and gain-of-function experiments. There are examples of studies suggesting that the manipulation of single genes in subsets of astrocytes influences neuronal circuits[13,44,46]. Therefore, genetic manipulation of regional and subregional astrocyte genes, in combination with functional studies of neuron–glia interactions in specific networks, will bring insights into the diversified functions of astrocytes.



A recent study showed that subsets of astrocytes in the mouse dorsal striatum specifically respond to the stimulation of either striatonigral or striatopallidal medium-sized spiny neurons[33]. Indeed, following local activation of dopamine receptor D1 (DRD1)-expressing or DRD2-expressing medium-sized spiny neurons, different subsets of striatal astrocytes exhibited increased intracellular Ca^{2+} levels, which were elicited by the release of endocannabinoids (FIG. 2a). Calcium uncaging in DRD1- or DRD2-responding astrocytes additionally evoked a transient synaptic potentiation only in homotypic pairs of synaptically connected neurons (that is, pairs of neurons expressing either DRD1 or DRD2) (FIG. 2a). Therefore, subsets of striatal astrocytes seem to be specialized to respond and modulate synaptic transmission in a neuron subtype- and synapse-specific manner[33]. Interestingly, such interactions do not rely on spatial segregation because DRD1- and DRD2-expressing neurons are intermingled in the rostral part of the dorsal striatum[34].

Another example of the specificity of astrocyte–neuron interactions is found in the primary visual cortex. In this area, neurons receive sensory information from the retina and display unique functional features such as orientation selectivity and ocular dominance[35]. In the ferret primary visual cortex, both astrocytes and neurons respond to visual stimulation; astrocytes display orientation and spatial-frequency tuning, as well as orientation-preference maps that match those of their neighbouring neurons[36]. This suggests that astrocytes display network-specific features that enable them to interact with subsets of neurons in the visual cortex. However, how astrocytes responses influence synaptic transmission is not clear. A recent study showed that optogenetically activated astrocytes modulate synaptic activity in the mouse primary visual cortex *in vivo*[37]. Interestingly, astrocytes displayed a dual role in controlling neuronal activity according to neuronal subtype: astrocyte photoactivation increased the spontaneous firing frequency of inhibitory parvalbumin-expressing

Astroglial coupling
The communication of neighbouring astrocytes through gap junctions that provide ionic and metabolic connections in the astrocyte network.

Calcium uncaging
An approach that is used to control the local intracellular concentration of Ca^{2+} and Ca^{2+}-induced intracellular signalling events. Cells are loaded with high-affinity Ca^{2+} chelator derivatives that decrease their affinity upon photostimulation, therefore releasing bound Ca^{2+}.

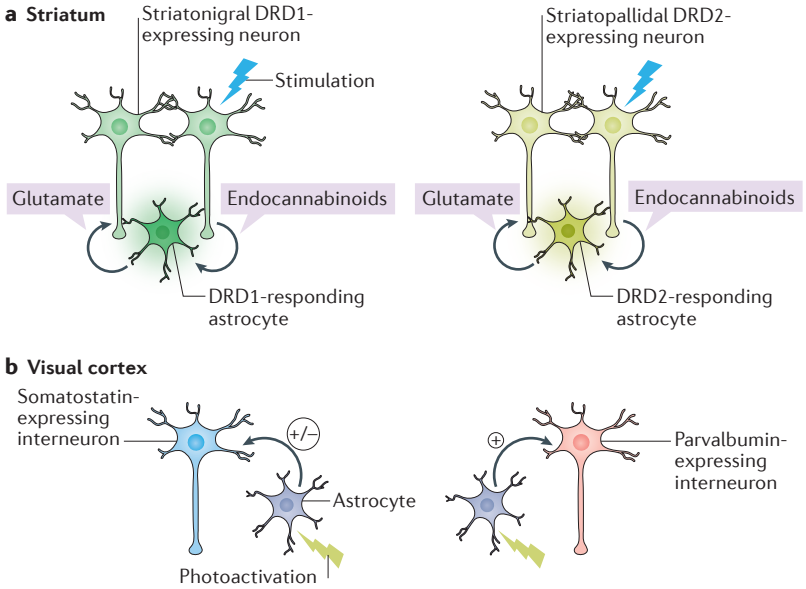


Figure 2 | **Synapse-specific neuron–astrocyte interactions.** Astrocytes are capable of discriminating between neuron subtypes within a brain region and differentially modulating their synaptic activity. **a** | In the mouse striatum, astrocytes selectively respond (by generating calcium signals) to the stimulation of distinct striatal neuron subtypes (specifically, those expressing either dopamine receptor D1 (DRD1) or DRD2)[33]. Stimulation of DRD1- or DRD2-expressing neurons results in the release of endocannabinoids that activate specific populations of astrocytes. In turn, astrocytes modulate synaptic activity selectively in homotypic pairs of neurons (expressing the same dopamine receptor) by releasing glutamate. **b** | In the mouse visual cortex, photoactivation of astrocytes increases (in the figure, indicated by '+') the spontaneous firing frequency of parvalbumin-expressing interneurons, whereas it either increases or decreases (in the figure, indicated by '+/–') somatostatin-expressing interneuron activity[37]. Whether astrocytes mediating these differential effects on interneuron subtypes are distinct subsets of astrocytes or are interchangeable is not known.

interneurons, whereas it either increased or decreased excitatory and somatostatin-expressing neuron activity[37] (FIG. 2b). Through this differential effect on cortical interneurons, astrocytes could modulate various aspects of the visual cortex network and shape sensory information processing[37]. It is still not known whether distinct subtypes of photoactivated astrocytes are involved in this differential modulation or whether astrocytes are interchangeable and regulate neuronal activity according to the adjacent neuronal subtype.

Influence on homeostasis and survival

As described above, astrocytes can modulate neuronal activity in a subtype- and synapse-specific manner. But how might region-specific astrocyte functions influence neuron subpopulations? Several studies have addressed this question through loss-of-function approaches that aimed to determine the role of region-specific astrocyte genes on neuronal homeostasis.

In the mouse cerebellum, Bergmann glia express functional Ca^{2+}-permeable AMPA receptors (AMPARs) on their processes, which enwrap Purkinje cell synapses[38]. Selective and temporally controlled deletion of Bergmann glia AMPARs during cerebellar development causes a retraction of their processes, alters Purkinje cell electrophysiological activity and delays the formation of glutamatergic synapses[39]. In adult mice, deletion of Bergmann glia AMPARs was associated with altered fine motor control at the behavioural level. Thus, the molecular and functional features of Bergmann glial cells influence synaptic transmission in the cerebellum.

Central pattern generators are unique neural networks that generate rhythmic neuronal activity and are involved in various behaviours such as walking or breathing[40]. Astrocytes might have regional roles in such processes, as they have been shown to modulate central pattern-generator networks. In *Drosophila melanogaster*, astrocytes regulate the activity of the adult pacemaker neurons that are involved in the generation of circadian rhythms[41]. Indeed, glia-specific alterations of vesicular transport or calcium signalling caused abnormal circadian behaviour in flies[41]. In the rat brain, astrocytes modulate the inspiratory rhythm in response to a change in pH in the ventral brainstem respiratory area[42]. Furthermore, astrocytes of the dorsal trigeminal main sensory nucleus, which has rhythmic activity, respond to the stimulation of sensory fibres[43]. Using electrophysiological recordings and calcium imaging, a recent study showed that, upon stimulation, astrocytes release the Ca^{2+}-binding protein S100β, which triggers neuronal rhythmic burst firing by decreasing local extracellular Ca^{2+} concentration[43]. These studies indicate that astrocytes are able to sense and interact with neurons in a network-specific manner.

In the hypothalamic arcuate nucleus, astrocytes are specialized to express leptin receptors (LEPRs). Conditional knockout of *Lepr* in mouse astrocytes led to a reduction in the astrocytes' synaptic coverage and the subsequent alteration of synaptic transmission in feeding control neurons expressing either the orexigenic agouti-related peptide (AgRP) or the anorexigenic pro-opiomelanocortin (POMC)[44] (FIG. 3a). As a result, the disruption of LEPR signalling in astrocytes led to a leptin resistance phenotype and to increased fasting- or ghrelin-induced hyperphagia. In another study, a designer receptor exclusively activated by designer drug (DREADD) approach was used to modulate Ca^{2+} activity *in vivo* in mouse astrocytes[45] (FIG. 3a). Astrocyte activation was shown to increase leptin-induced anorexia and to decrease ghrelin-evoked hyperphagia through adenosine-mediated inactivation of AgRP neurons. Thus, regionally specified astrocytes participate in the control of neural circuits that regulate feeding behaviour.

An elegant study, published recently, demonstrated that insulin signalling in hypothalamic astrocytes is required for brain glucose sensing and systemic glucose homeostasis[46]. The authors used both a conditional knockout and a viral approach to delete insulin receptors in astrocytes of the mouse hypothalamus. Mice lacking insulin signalling in astrocytes showed increased fasting-induced hyperphagia and impaired regulation of systemic glucose levels in response to hyperglycaemia. At the cellular level, absence of insulin signalling in astrocytes altered glucose-induced activation of POMC-expressing neurons. This study provides further evidence that astrocytes carry out functions in normal physiological homeostasis and that they comprise important regulators of metabolic control circuits in the hypothalamus.

Designer receptor exclusively activated by designer drug (DREADD). A chemogenetic tool that modulates G protein-coupled receptor signalling to control cellular activity. It uses mutated muscarinic receptors that can only be activated by an exogenous ligand, clozapine-*N*-oxide.

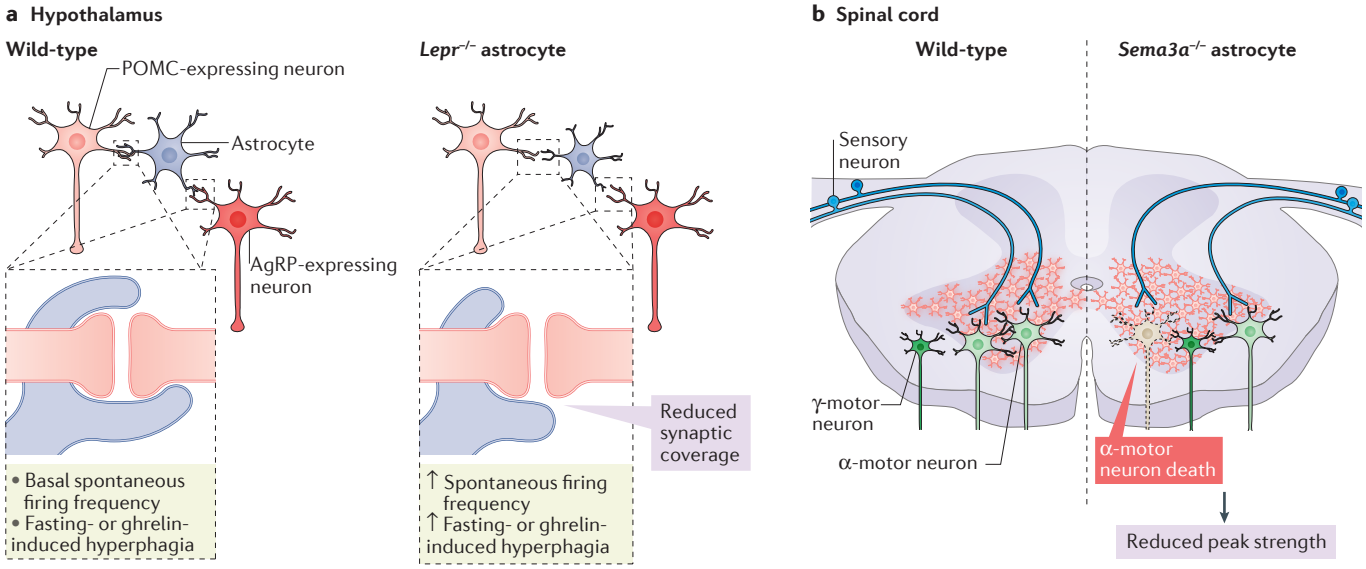


Figure 3 | **Functions of regionally specialized astrocytes.** Examples of region- and circuit-specific astrocyte functions that are necessary for neuronal network homeostasis and survival are shown. **a** | In the mouse hypothalamic arcuate nucleus, astrocytes modulate synaptic transmission in pro-opiomelanocortin (POMC)-expressing and agouti-related peptide (AgRP)-expressing neurons[45]. Conditional deletion of the leptin receptor in astrocytes (*Lepr*$^{-/-}$ astrocytes) results in a reduction of synaptic coverage by astrocytic processes and subsequent changes in the frequency and amplitude of spontaneous activity in POMC- and AgRP- expressing neurons. These cellular changes are associated with leptin resistance and increased fasting- or ghrelin-induced hyperphagia[44]. **b** | In the spinal cord, sensory and motor circuits are spatially segregated in the dorsal and ventral horns, respectively. Astrocytes from the dorsal and ventral part of the spinal cord display distinct protein expression patterns[13]. In mice, ventral astrocytes selectively express the axon guidance molecule semaphorin 3A (SEMA3A). Conditional deletion of *Sema3a* in astrocytes (*Sema3a*$^{-/-}$ astrocytes) causes several local ventral horn abnormalities, including a loss of α-motor (but not γ-motor) neurons. This specific loss of α-motor neurons would be predicted to result in reduced peak strength at the behavioural level.

The spinal cord sensorimotor circuit provides a system to investigate the precise interactions between astrocytes and particular subtypes of neurons (FIG. 3b). It has been shown that astrocytes that are purified from the dorsal and ventral mouse spinal cord display distinct gene expression profiles[13]. Interestingly, only about 40 genes in total could distinguish dorsal versus ventral astrocytes at the transcriptomic level, raising the question of whether these subtle differences could be functionally important. The most highly upregulated ventral astrocyte gene was semaphorin 3A (*Sema3a*), which encodes an axon guidance molecule that binds to the neuropilin 1 receptor on motor neurons. Conditional deletion of *Sema3a* in astrocytes caused several local ventral horn abnormalities, including changes in synaptogenesis and axon orientation. However, the most striking phenotype was the loss of about half of the α-motor neurons throughout the spinal cord. By contrast, γ-motor neurons and interneuron populations did not show losses (FIG. 3b). Because α-motor neurons in mice are necessary for peak strength[47] — corresponding to the maximal force that is developed to grip an object — it is predicted that this parameter would be lost in mice lacking astrocytic *Sema3a* (FIG. 3b). This study demonstrates that astrocyte heterogeneity can be defined at the single-gene and/or functional level, and that ventral horn astrocytes are specialized to support α-motor neuron homeostasis.

Together, these examples show that subtypes of astrocytes have specific roles in the modulation of local and sometimes distant neuronal networks. Astrocytes can regulate complex synaptic activity and the associated behavioural outputs in defined CNS regions. In this context, astrocyte diversity is particularly important because it enables the creation of highly specialized neuron–glia units.

The basis of astrocyte heterogeneity

Although the examples that were described above serve to illustrate that astrocytes can function in diverse ways with respect to their neuronal neighbours and/or regions, it remains unclear whether such differences are intrinsically encoded and/or determined by inductive cues from neurons. Several reviews that were published recently describe developmental patterning and astrocyte heterogeneity[14,16,48]. Therefore, we summarize the key points of patterning during astrocyte development but focus on the neuronal and activity-dependent signals that could influence astrocyte subtype elaboration in the adult CNS.

Evidence for developmental patterning

During CNS development, spatiotemporal patterning programs are known to diversify neuronal subtypes, and similar mechanisms have been proposed for gliogenic progenitors[48–50]. Embryonic patterning of the mammalian CNS is determined by dorsoventral gradients of

secreted organizing signals (such as bone morphogenetic proteins and Sonic hedgehog (SHH))[51] (FIG. 4a). In the spinal cord, astrocyte and oligodendrocyte progenitors are regionally specified by this dorsoventral template and the downstream functions of basic helix–loop–helix and homeodomain proteins[52,53]. The homeodomain code that specifies ventral white matter astrocyte subtypes (VA1, VA2 and VA3) in the spinal cord mirrors the progenitors domains (p1, p2 and p3) in the neural tube from which these astrocytes derive[53], suggesting that astrocytes residing in the three different domains derive from positionally distinct progenitors. Therefore, it is possible that a spatiotemporal regulation of gene expression that spans developmental stages from glial progenitors to mature astrocytes influences astrocyte diversification (FIG. 4a). Indeed, it has been shown that this regional specification mechanism is tightly linked to the ultimate astrocyte spatial position in the adult CNS[54]. Furthermore, astrocytes retain their regional adherence even after injury, suggesting that they might express domain-specific positional cues that enable a range of functions.

Supporting the possibility that astrocyte diversity is programmed independently of neurons, it has been shown that cultures of human embryonic stem cells can be patterned to adopt anterior or posterior identity and that these cells give rise to astrocytes with corresponding regional identities in the absence of inductive cues from neurons[55]. This suggests that specific aspects of intrinsic patterning programs are maintained *in vitro*. In further support of this notion, we have shown that astrocytes that

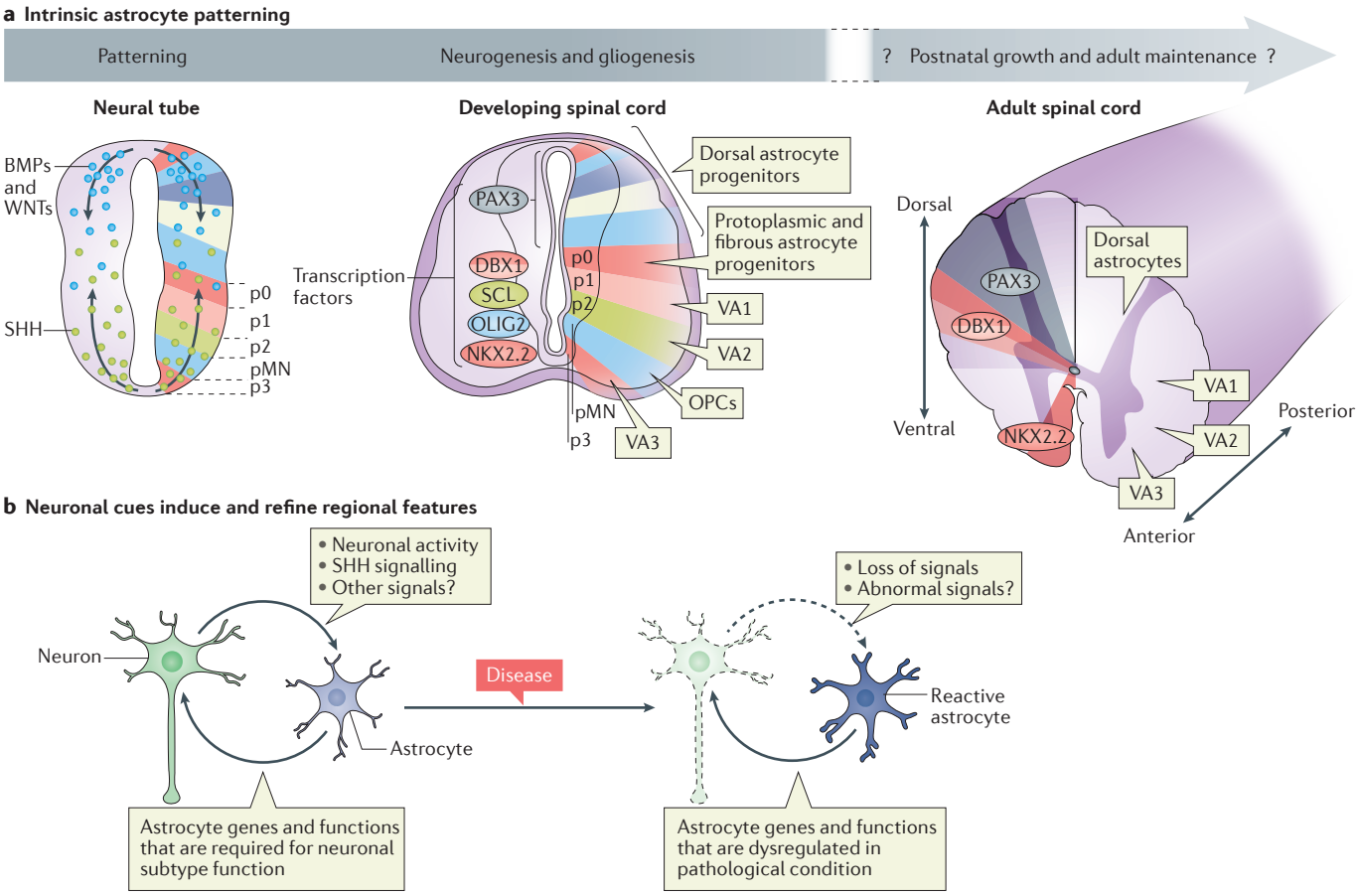


Figure 4 | **Establishment and refinement of astrocyte heterogeneity.** **a** | Embryonic patterning of progenitors is established by dorsoventral gradients of secreted organizing signals (including bone morphogenetic proteins (BMPs), wingless (WNT) proteins and Sonic Hedgehog (SHH)) in the neural tube (left panel). These gradients control the expression of homeodomain proteins and transcription factors that determine region-restricted ventral progenitor domains (p0, p1, p2, p3 and pMN) (left and middle panels). Neuronal and oligodendrocyte progenitors are found in these regionally restricted domains: interneurons (not shown) derive from progenitors in domains p0, p1, p2 and p3, whereas motor neurons (not shown) and oligodendrocytes are generated from progenitors located in the pMN domain. After neurogenesis, SHH signalling regulates the expression of transcription factors — including OLIG2 and SCL — that determine the segmental template according to which astrocyte progenitors are generated[50]. Regionally restricted progenitors that are located in domains p1, p2 and p3 give rise to three white matter astrocyte subtypes (VA1, VA2 and VA3) that can be distinguished by the combinatorial expression of SLIT1 and reelin[53]. Progenitors in the intermediate spinal cord (p0) expressing DBX1 give rise to both protoplasmic and fibrous astrocytes[54]. In the dorsal spinal cord, PAX3-expressing progenitors give rise to all dorsal astrocytes. These regionally restricted astrocyte domains are maintained into adulthood (right panel). **b** | In the adult CNS, neuronal signals (activity or secreted molecules) refine the regional features of astrocytes to generate highly specialized neuron–glia units in specific neuronal networks (left panel). Such region-specific astrocyte functions are required for the maintenance of CNS homeostasis and neuronal survival. In disease, such region-specific astrocyte genes and functions can be dysregulated and affect neuronal networks (right panel). Part **a** is adapted with permission from REF. 48, Cold Spring Harbor Laboratory Press.

are derived from ventral and dorsal parts of the spinal cord maintain key regionally distinct features in culture in the absence of neurons[13]. Thus, patterning of multipotent progenitors might be sufficient to diversify astrocytes.

Indeed, in an elegant series of papers, it was shown that type B astrocytes of the subventricular zone can be parsed into dorsoventral domains that give rise to distinct neuronal progeny[56,57]. In this case, the subventricular zone seems to be instructed by classic organizing cues, such as SHH signalling, to impose identity to distinctive subtypes of astroglial stem cells. This strongly supports the idea that diversified astrocyte identity is generated at embryonic stages and may be irreversible, as even culture manipulation and transplantation did not alter the progeny of position-restricted type B stem cells[56]. Indeed, the finding that regional identity is 'hard wired' at an early stage is supported by cell culture studies showing that astrocytes maintain region-associated expression and functions[13,28]. However, it is important to note that studying astrocytes *in vitro*, especially in primary cultures, has several caveats. These include significant changes in the gene expression profile and in morphological and physiological properties of astrocytes that are maintained *in vitro* as compared with their *in vivo* counterparts[58].

Despite these caveats, this series of observations indicate that astrocytes are patterned to produce heterogeneity. This is an elegant mechanism in principle and takes advantage of the tremendous amount of positional information that is intrinsic to the vertebrate embryonic CNS[49]. In summary, astrocytes show regional differences in gene expression that are maintained *in vitro*, do not require neuronal inductive cues and are reflected by the astrocytes' different functional effects on neighbouring neurons. Although these findings collectively show that patterning and cell intrinsic (transcriptional) mechanisms are sufficient to specify and allocate diverse astrocyte subtypes in various regional domains, other lines of evidence suggest important roles for neuronal activity-dependent and inductive cues. Indeed, one possibility is that embryonic patterning programs astrocyte responsiveness for their later maturation in locales containing particular neuronal subtypes.

Evidence for extrinsic cues

Extracellular signals can trigger a wide range of intracellular changes in astrocytes[48], including the regulation of the expression of different sets of ion channels, transporters and receptors[22,23,59]. Through these signals, the local microenvironment can thus influence and maintain astrocyte heterogeneity in the adult CNS.

Early *in vitro* studies suggested that astrocyte mRNA and protein levels are regulated by neuronal activity: for example, when cultured with neurons, astrocytes exhibited changes in their expression of glutamate transporters[60], connexins[61], glutamine synthetase[62,63] and neuropeptide receptors[64]. More recently, it was shown *in vivo* that whisker stimulation increases the levels of glutamate aspartate transporter (GLAST; also known as excitatory amino acid transporter 1 (EAAT1)) and glutamate transporter 1 (GLT1; also known as excitatory amino acid transporter 2 (EAAT2)) in astrocytes of the somatosensory cortex[65]. In an interesting study, it was shown that neuronal activity induces GLT1 expression through the induction of astrocytic heterogeneous nuclear ribonucleoprotein K (hnRNP K)[66]. Therefore, it is possible that differential synaptic activity, which varies according to the brain region, might modulate astrocyte molecular features.

Might there be other specific inductive cues for astrocyte identity and function? A recent study showed that SHH released from Purkinje neurons regulated the molecular signature that distinguished between Bergmann glia and velate protoplasmic astrocytes in the cerebellum[67]. Abrogating SHH signalling in mouse Bergmann glia through conditional knockout of the gene encoding the SHH receptor smoothened decreased the expression of several Bergmann glia markers, including AMPAR subunits (glutamate receptor 1 (GluR1; also known as GRIA1) and GluR4 (also known as GRIA4)), GLAST and Kir4.1. Conversely, overexpression of a constitutively active form of smoothened in cerebellar velate astrocytes resulted in a shift towards a Bergmann glia-like expression profile[67]. Thus, neuronal SHH signalling determines two distinct astrocyte subtypes in the cerebellum. In other brain regions such as the hippocampus or the cortex, constitutive activation of smoothened in astrocytes increased Kir4.1 expression but did not affect other astrocyte protein levels, suggesting that, in these regions, SHH signalling modulates a specific function rather than astrocyte identity[67]. Because SHH is not expressed in all regions of the adult CNS and because Bergmann glia represent a peculiar type of astroglia, other putative neuronal signals must be invoked to explain forebrain astrocyte diversity[23]; however, the example of SHH induction of cerebellar astrocytes makes the important point that neuron-derived cues are crucial in some instances to impose astrocyte regional identity and function.

The activation of different signalling cascades (such as mitogen-activated protein kinases (MAPKs), Janus kinase (JAK)–signal transducer and activator of transcription (STAT) or nuclear factor-κB (NF-κB)) regulates gene expression in astrocytes and reactive astrocytes[1,68]. Therefore, it is possible that — according to the cellular microenvironment — various signalling cascades are triggered and differentially regulate gene expression in astrocytes, contributing to the generation and maintenance of astrocyte diversity (FIG. 4). Numerous factors might influence astrocyte gene transcription in a given CNS region or subregion, including neuronal subtypes and properties (such as firing frequency or neurotransmitter type), metabolic requirements, synaptic inputs, the density of different components (including other cell types, myelinated axon tracts and blood vessels) and the proximity to specific structures such as neurogenic niches.

Human astrocytes display marked regional and subregional differences in their microRNA expression patterns[69]. Interestingly, it has been shown that *in vitro* GLT1 translation in astrocytes is regulated by the release of neuron-derived exosomes containing microRNAs[70]. This

type of regulation could be involved in setting regional differences in astrocyte protein expression profiles and could be determined by intrinsic or extrinsic cues.

In addition to neurons, other glial cell types could potentially influence the development or maintenance of astrocyte diversity in various CNS regions. It is becoming increasingly recognized that cells of the oligodendrocyte lineage are diverse[71–73]. In addition, it was recently reported that microglia also display marked differences in their expression profile in different regions of the CNS[74]. For example, in the hippocampus, microglia express a greater number of genes in common with activated macrophages, suggesting that their vigilance state depends on the brain region[74]. Therefore, other glial cell types might be as diverse as astrocytes and establish region-specific interactions that shape astrocyte heterogeneity during development and in a mature CNS (FIG. 4).

Astrocyte heterogeneity in disease

Given that the contribution of astrocyte heterogeneity to normal CNS function has yet to be fully established, its role in human diseases is unclear. However, some recent studies suggest that astrocytes respond differentially to various types of injury or disease in animal models. Astrocytes become reactive in response to a range of pathological conditions in the CNS, from acute traumatic injury to chronic neurodegenerative diseases. Reactive astrocytes are generally identified by morphological changes (hypertrophy) and upregulation of intermediate filament proteins such as GFAP and vimentin[1]. However, many outstanding questions remain. Do all — or just a subset of — astrocytes respond to injury and disease? What is the role (or roles) of reactive astrocytes in disease and is this role beneficial or toxic? Do astrocytes that become reactive retain their spatial identity and circuit-specific features? Recent studies are beginning to shed light on these issues[68].

Reactive astrocyte heterogeneity

Differential astrocyte reactivity has been observed between brain regions at different time points after injury[75]. In chronic diseases, as for example neurodegenerative disorders, that primarily target a specific CNS region, astrocytes display a reactive phenotype in the vulnerable area[76–78]. This suggests that astrocytes selectively respond to a region-specific pathological stimulus and can react to the presence of abnormal, or the loss of, local physiological signals.

In certain pathological conditions, reactive astrocytes aggregate at the site of the injury, forming a 'glial scar'. Morphological distinctions can be made between glial scar-forming reactive astrocytes (which are the elongated reactive astrocytes at the lesion centre) and hypertrophied reactive astrocytes (which lie further from the lesion core)[18,79]. The molecular changes elicited in these different subsets of reactive astrocytes have been well described in acute injury models such as ischaemia[80] or spinal cord injury (SCI)[81]. In addition, a transcriptomic analysis that compared reactive astrocytes' expression profiles after ischaemia or neuroinflammation in mice revealed that reactive astrocytes display disease-specific changes in gene expression[12].

In addition to diverse molecular changes, reactive astrocytes display marked differences in their dynamic reaction to injury. Using *in vivo* two-photon imaging, it was shown that only subsets of astrocytes react to stab wound injury in the mouse cortex either by polarization towards the lesion or by proliferating (juxtavascular astrocytes)[82]. The latter 'type' of reactive astrocytes is also observed after SCI[83].

Although these results emphasize the diversity of molecular and dynamic changes elicited during reactivity — which may putatively correspond to different types of reactive astrocytes — the functional roles of such subsets are less understood. Several recent studies have shed light on disease-specific changes in reactive astrocytes' gene expression or function and their impact on disease phenotype.

Functional differences

Recent evidence suggests that reactive astrocytes display heterogeneous functional changes in particular pathological conditions. For example, it was demonstrated that the K^+-buffering capacity of astrocytes was altered in the striatum of mice carrying the mutation that causes Huntington disease (HD mice) and that this alteration contributed to the synaptic dysfunction of striatal neurons[84]. Kir4.1 is highly expressed in astrocytes, in which it acts to buffer K^+ from synapses. Kir4.1 levels are decreased in HD mice, resulting in increased extracellular K^+ *in vivo* and altered electrophysiological properties of striatal neurons. Kir4.1 overexpression in astrocytes improved some neurological features in HD mice, suggesting that altered astrocyte K^+ buffering contributed to the disease phenotype[84]. It is important to note that, in this disease model and in this cerebral region, astrocytes do not show hypertrophy or GFAP upregulation, the classical hallmarks of reactivity, highlighting the regional diversity of reactive astrocyte phenotypes.

In Alzheimer disease (AD), reactive astrocytes are found around amyloid plaques in patients and in mouse models of AD[85,86], and take up amyloid-β peptides[85–87]. Several alterations of physiological astrocyte function have been reported in the context of AD, including hyperactive Ca^{2+} transients[88,89] and abnormal release of GABA (which was shown to contribute to cognitive impairment) in mouse models of AD[90,91].

Recently, an interesting study suggested that reactive astrocytes could transfer functional mitochondria to neurons to support neurons in a model of stroke[92]. The authors identified CD38 signalling in astrocytes as being responsible for mitochondria transfer to neurons: blocking astrocytic CD38 expression in mice decreased mitochondria transfer after focal ischaemia and worsened disease outcome. Whereas neurons can transfer mitochondria to astrocytes for recycling purposes[93], this study suggests that reactive astrocytes can support neuronal function and influence disease phenotype by a similar mechanism. Further investigations are needed to determine whether such mechanisms are also involved in other neurological disease models.

Last, a recent study showed that scar-forming reactive astrocytes not only have a protective function but also promote axonal regrowth after SCI, challenging a

long-held dogma[94]. Using two different transgenic mice to either prevent or inhibit glial scar formation, the study showed that there is a failure in axonal regrowth following removal of reactive astrocytes in both acute and chronic glial scars. In addition, reactive astrocytes were shown to increase their expression of more axon-growth permissive factors, such as laminins, than the expression of inhibitory molecules, such as specific chondroitin sulphate proteoglycans. More importantly, the presence of scar-forming reactive astrocytes, when combined with neurotrophins, supported axonal regrowth[94]. Overall, this suggested that scar-forming reactive astrocytes are necessary for maintaining tissue integrity after SCI and are even involved in endogenous repair mechanisms.

Gene expression patterns in reactive astrocytes can give us insights into their functions and potential involvement in pathology. For example, it was shown that reactive astrocytes display injury-specific changes in gene expression that might correlate with types of reactive astrocytes[12]. Under neuroinflammatory conditions, reactive astrocytes might have potentially detrimental consequences on neighbouring cells, including neurons and other glial cells. Deciphering the molecules that are responsible for the induction of this type of reactivity will provide insight into the functions of reactive astrocytes and, potentially, therapeutical interventions. In addition, identifying putative disease-specific or regionally-enriched reactive astrocyte subtypes might be particularly relevant to understand the selective vulnerability of neuronal populations in neurodegenerative disorders.

However, astrocyte reactivity is not a 'black and white' process[79], and it is possible that, in most pathological conditions, these different types of reactive astrocytes are intermingled and thus influence disease outcome in a complex manner, involving regional, temporal and injury- or disease-specific components.

How does reactivity affect diversity?

Although disease-specific astrocyte alterations have been reported[95], it is not clear how well astrocyte functional diversity is maintained under pathological conditions (FIG. 4b). Indeed, it is possible that, under pathological conditions involving neuronal dysfunction, astrocytes lose the extrinsic cues that specify them in a given network, thus altering local neuron–glia interactions (FIG. 4b). Interestingly, several lines of evidence support the idea that a loss of extrinsic signals (potentially from neurons) is sufficient to elicit astrocyte reactivity in the absence of pathological stimulus[96,97]. For example, mice in which the SHH receptor, smoothened, is conditionally deleted, develop astrocyte reactivity in subsets of cortical astrocytes that express the transcription factor GLI1, but not in the striatum, where this protein is not expressed[98]. Similarly, manipulating fibroblast growth factor signalling in astrocytes triggers global astrocyte reactivity in the mouse forebrain[99]. However, whether this type of astrocyte reactivity is associated with functional changes (such as changes in K^+ buffering) is not known.

Very few studies have compared astrocyte reactivity in different CNS regions after the same insult or compared disease models in which multiple brain regions are targeted. Such approaches would be useful to characterize the regional heterogeneity of reactive astrocytes and identify putative region-specific molecular and functional changes. A better understanding of such regional diversity is particularly relevant for neurodegenerative diseases, in which specific neuronal populations are vulnerable.

In conclusion, the heterogeneity of reactive astrocytes at both regional and subregional levels needs to be investigated more carefully using cell type-specific approaches and dynamic techniques to monitor astrocyte reactivity in disease.

Conclusions and perspectives

Although the concept of a functional heterogeneity of astrocytes is no longer novel[7,8,100], it remains challenging to find abundant experimental proofs of such diversity at the molecular and functional level. As a result, this Review has focused on only a few examples. The past 15 years have seen excellent progress in our understanding of astrocyte function and of how these cells regulate neuronal networks[101]. Using a combination of new tools and experimental systems, future studies will be able to characterize region- and network-specific astrocyte features. Gaining further insights into the molecular and functional features of astrocytes and reactive astrocytes throughout CNS regions will contribute to our full understanding of the roles of these ubiquitous cells in brain functions in normal and pathological conditions.

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Competing interests statement
The authors declare no competing interests.