Functional roles of reactive astrocytes in neuroinflammation and neurodegeneration

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Abstract | Despite advances in uncovering the mechanisms that underlie neuroinflammation and neurodegenerative disease, therapies that prevent neuronal loss remain elusive. Targeting of disease-defining markers in conditions such as Alzheimer disease (amyloid-β and tau) or Parkinson disease (α-synuclein) has met with limited success, suggesting that these proteins do not act in isolation but form part of a pathological network. This network could involve phenotypic alteration of multiple cell types in the CNS, including astrocytes, which have a major neurosupportive, homeostatic role in the healthy CNS but adopt reactive states under acute or chronic adverse conditions. Transcriptomic studies in human patients and disease models have revealed the co-existence of many putative reactive substates of reactive astrocytes. Inter-disease and even intra-disease heterogeneity of reactive astrocytic substates is well established, but the extent to which specific substates are shared across different diseases is unclear. In this Review, we highlight how single-cell and single-nuclei RNA-seq and other 'omics' technologies can enable the functional characterization of defined reactive astrocyte states in various pathological scenarios. We provide an integrated perspective, advocating

cross-modal validation of key findings to define functionally important substates of astrocytes and their triggers as tractable therapeutic targets with cross-disease relevance.

Introduction

Astrocytes were first described by neuroanatomists in the mid-19th century, contemporaneously with neurons[1]. Despite this temporal coincidence in discovery, however, our understanding of astrocyte biology has failed to keep pace with our understanding of their neuronal counterparts. To some degree, this discrepancy can be attributed to a lack of investigative tools, leading to an initial low-resolution assessment and the claim that astrocytes were largely uniform and lacked the interesting electrophysiological properties of neurons. Subsequent developments in technology have permitted higher-resolution studies, which unambiguously establish the fundamental roles of astrocytes in CNS development, physiology and pathophysiology.

Astrocytes are now widely accepted as being essential for neuronal survival and function in the CNS (Fig. 1). Once fully mature, they occupy distinct three-dimensional domains along the neuraxis and are responsible for a wide range of functions, such as pH, ion and redox buffering, and regulating blood flow to neurons. However, under pathological conditions, astrocytes can undergo morphological and molecular changes to adopt so-called ‘reactive’ states, which may alter their function and alter the balance between their neurosupportive and neurotoxic. The underlying mechanisms that determine whether astrocytes have a net neurosupportive or neurotoxic effect is likely to depend on the state of multiple, possibly overlapping pathways and is not well understood. Moreover, a single astrocyte might be neurosupportive or neurotoxic depending on the nature of the insult or homeostatic challenge with which it is faced. How the diverse and increasingly well-defined reactive astrocyte states are spatiotemporally regulated and affected in adverse contexts of neurodegeneration and acute insult is a key issue to address.

Here, we review the evidence that astrocytes are key drivers of the neuroinflammatory cascade across a range of neurodegenerative disorders. We describe how single-cell and single-nuclei RNA sequencing (scRNA-seq and snRNA-seq, respectively) and other ‘omics’ technologies are enabling the functional characterization of different reactive astrocyte states in various pathological scenarios and discuss the therapeutic implications of this research.

Inflammation and neurodegenerative disease

Broadly speaking, inflammation is the protective response of the body to or infection with non-self invading pathogens such as bacteria and viruses. In addition, inflammation is widely

accepted to be a standard cellular response to many endogenously produced disease-associated molecules, including amyloid-β (Aβ), tau, α-synuclein and mutant huntingtin. In the periphery, the inflammatory response is mounted by specialized peripheral blood and tissue-resident immune cells such as leukocytes, along with platelets and other key cellular components such as cytokines and chemokines. The resulting rapid migration of these cells to sites of infection or injury results in swelling, production of heat and release of cytokines, which often leads to recruitment of additional immune cells. Ultimately, this process leads to destruction of any invading pathogens, preservation of tissue, and initiation of recovery and regeneration.

In the CNS, the inflammatory response is mediated largely by microglia[2,3] (the resident macrophages of the CNS), as well as infiltrating peripheral immune cells including T cells[4]. However, macroglia, in particular astrocytes, are key downstream effectors[2,5-7]. Understanding the spatial and temporal interactions between astrocytes and microglia is crucial for determining how astrocytes contribute to the neuroinflammatory cascade across health and disease, leading to a diverse range of functional outcomes for the neural circuit and organism. The possibility of manipulating this interaction is of great interest for developing new therapeutic strategies in neurodegenerative disease[8-10].

Neurodegenerative diseases such as Alzheimer disease (AD), Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS) represent other drivers of inflammation, although the underlying mechanisms have yet to be fully resolved. Convincing data suggest that in some neurodegenerative diseases, the initial pro-inflammatory stimulus may come from neurons, triggering a secondary inflammatory response resulting from intercellular interactions between astrocytes and microglia to drive disease progression[11-13] (BOX 1). However, the effects of disease-causing gene mutations on neurons might be insufficient to drive pathology, as has been shown in ALS, and glial cells likely represent a primary source of neurotoxicity[11,12] Astrocyte reactivity is known to be triggered by altered microglial states in some instances[8], which might themselves be triggered by various stimuli, including other CNS-resident cells (Fig. 2) and the gut microbiome — specifically, tryptophan metabolites via the aryl hydrocarbon receptor[14].

Effective therapies for neurodegeneration are likely to be combinatorial, targeting multiple CNS cells simultaneously to disrupt the complex intercellular events that drive pathology. For example, a therapy that decreases the susceptibility of neurons to synapse loss, axonal damage and/or cell death, combined with therapies that block adverse microglia–astrocyte signalling and reactive astrocyte induction, and interfere with the production or release of reactive-astrocyte

derived neurotoxic compounds, could be an effective approach for slowing disease progression. Astrocyte-centred therapies require a knowledge of the integration and synthesis of local and remote triggers for inducing astrocyte reactivity in all its forms. This is an intensely researched subject that will illuminate key mechanisms of interaction between astrocytes and inflammatory signals, as well as other disease-causing agents (such as misfolded proteins), leading to molecularly characterized, therapeutically targetable processes.

Studying astrocyte function and pathology

The rise of transcriptomics

In an effort to standardize investigations across groups and through time, many researchers have sought to characterize and name different substates of reactive astrocytes. Initial attempts focused on the identification of gross morphological differences between different astrocyte populations (for example, white versus grey matter astrocytes[15,16]). Although these descriptions were helpful for characterizing the physiological location of cells, they offered few functional insights

Following the development of antibodies to the cytoskeletal protein glial fibrillary acidic protein (GFAP), the field moved towards identification of ‘reactivity’ on the basis of this single marker, as increased GFAP levels were assumed to be attributable to a phenotypic change from physiological to reactive astrocytes[17,18]. Although this is true in some — and perhaps most — cases, GFAP levels show regional heterogeneity in the healthy CNS and can be differentially altered depending on the stimulus involved[19-23]. Subsequently, the use of RT-PCR to investigate changes in small numbers of ‘reactive’ genes was an important step forward in defining astrocyte reactivity, but again lacked the fidelity to identify functionally heterogeneous populations. Similar problems arose with the switch to cDNA array[24], microarray[25-27] and then next-generation RNA sequencing[28-31]. Although new methods enabled increasing numbers of genes to be probed, they all still relied on bulk analysis of astrocytes. These astrocytes were purified using astrocyte-specific reporter mice (for example, *Aldh1l1*-EGFP[32]), astrocyte-specific expression of tagged ribosomes (as in the *Aldh1l1*-EGFP/Rpl10a mouse line, also known as *Aldh1l1* bacTRAP)[33,34]) or fluorescence-activated cell sorting using cell-surface markers such as IGTB5[35], HEPACAM[29], CD49f[36], or other commercial systems using ACSA1 (GLAST)[37] or ACSA2 (ATP1B2[38,39].

The use of these techniques in animal models of various conditions, including frontotemporal dementia, AD[40-42], ALS[30,43-45], demyelination[46], Huntington disease (HD)[47], ischaemic stroke[27,48,49], acute neuroinflammation[27,30,50], spinal cord injury[51], in addition to normal

ageing[52,53], has proved powerful for detailing specific transcriptomic changes in astrocytes in response to inflammation, ischaemia and other neurological disease processes. In addition, baseline gene expression profiling of astrocytes in different brain regions, including the cortical layers[28,29,31,38,54,55], has provided a basis for multiple follow-up studies. Importantly, combined investigations using RNA-seq and proteomics in the striatum, cortex and hippocampus has added extra layers of information about subtle differences in baseline gene expression between these brain regions[31]. Subsequent investigations took advantage of automated fluorescent in situ hybridization methods to identify gene expression differences in astrocytes from different cortical layers[23], which were further validated by integration of single-cell, single-nuclei and spatial transcriptomic data sets in both rodents and humans[56]. The interpretation of these datasets however, has mainly assumed a uniform response of astrocytes to an external insult, and the studies have lacked the power to probe the existence of substates of astrocytes with the potential to respond to an initial acute stimulus with different transcriptomic and functional changes, or to respond over different timeframes to prolonged stimuli.

We have seen an explosion in the use of scRNA-seq and snRNA-seq to investigate the baseline and reactive responses of astrocytes in different brain regions and across multiple species, including rodents[23,27,28,30,31,38,47,50,51,57-61], humans[29,36,40,46,62-64]), non-human primates[49,65], ants[66], *Drosophila*[67] and zebrafish[58,68]. In addition to validating the existence of cortical layer-specific astrocytes[56], these studies have identified many transcriptomically defined physiological subtypes and reactive substates of astrocytes Providing a list of current astrocyte-specific scRNA-seq and snRNA-seq studies would be a futile exercise given the surge in uptake of this technology, which would quickly render such a list outdated. The field would benefit from a well-curated repository of such resource datasets to expedite ongoing investigations and increase data accessibility. Such efforts are beginning to emerge, for example, the FAIR principles[69,70], which are intended to provide guidelines to improve the findability, accessibility, interoperability and reuse of digital assets.

Both historical and modern sequencing datasets have provided important seeds for ongoing research, but many questions still remain. For example, how does astrocyte heterogeneity change during development and ageing, or with respect to sex? Also, what is the extent of heterogeneity in reactive astrocyte populations within a particular disease, and how does it alter with disease progression? Possibly most pertinent of all, at what level will we have finally defined the extent of biologically relevant baseline astrocyte heterogeneity? These questions will only be addressed by careful generation of properly powered scRNA-seq and snRNA-seq datasets, which has so far proved difficult for the investigation of astrocytes[50,71].

Many of the published scRNA-seq and snRNA-seq datasets capture large numbers of individual cells but have been underpowered for astrocytes, often with only a few dozen of these cells being collected from each individual animal. Combined with the low sequence depth afforded by these method, this has led to a number of attempts at gene clustering that have been unable to define differentially expressed genes that are representative of specific astrocyte populations in vivo. This artifact of clustering driven by low numbers of astrocytes has also caused several reactive astrocyte substates to be missed owing to low abundance[50]. This is an important limitation, as even some well-accepted reactive astrocyte states, such as scar-forming astrocytes, represent less than 5% of all astrocytes in the CNS. Interpretation of weakly powered scRNA-seq and snRNA-seq datasets has often led to the erroneous conclusion that transcriptional changes associated with astrocyte reactivity are purely disease specific[72], in contrast to the homogeneity of some substates of microglia[73] and oligodendrocytes[56,74] across diseases. These high-powered studies[56,73,] show that while both microglia and oligodendrocytes have considerable transcriptomic heterogeneity within a disease or brain region, some of these reactive substates are common across disease-states. Several of these microglia and oligodendrocyte states are also reported following inflammation (a common response to nearly all neurodegenerative diseases, infections, and traumas). In addition, these gene expression profiles have some overlap with proliferative scar-forming reactive astrocytes, or astrocytes that are associated with plaque/plaque-associated gene signatures[75,]. Further studies focusing on integration of multiple datasets from different disease models and/or patient cohorts are likely to further validate the presence of substates of reactive astrocytes across different diseases, as each additional dataset included in such meta-analyses will further increase astrocyte numbers and, hence, the power of downstream interpretation.

Experimental challenges

Interspecies differences

Although animal models have provided crucial insights into astrocytes, increasingly recognized interspecies differences highlight the need to integrate animal studies with human data. Compared with their rodent counterparts, human astrocytes occupy an almost 30-fold larger volume, extend 10-fold more processes and have a considerably more complex structure[77,78]. Beyond these morphological differences, some transcriptomic divergence has also been observed, with expression of several hundred genes showing enrichment in human but not mouse astrocytes[29]. Key differentially expressed genes indicate divergent properties between

human and mouse astrocytes, such as variations in calcium handling, defence responses and metabolic pathways (Box 2)[29,79-81].

Despite such differences, animal models remain an indispensable tool to understand astrocyte biology in response to disease or trauma in vivo, owing to the capacity to induce adverse conditions, analyse spatiotemporal responses, and probe disease mechanism through genetic or pharmacological intervention. By contrast, experimental studies aimed at understanding the function of human astrocytes in health and disease have largely been confined to in vitro systems, and have historically been hampered by limited accessibility to enriched cultures. Despite some elegant work using immunopanning approaches in human post-mortem tissue[29,80], the functional viability of these astrocytes is impaired, or at least variable[82].

Human induced pluripotent stem cells (hiPSCs) offer an promising model system to interrogate the role of astrocytes in neurodegeneration and ageing. By virtue of the ability to bypass the need to artificially overexpress or knock down disease-associated genes, hiPSCs could provide an ideal preclinical platform to elucidate pathogenic mechanisms and enhance drug discovery. Of note, both glia and neurons derived from hiPSCs are approximately fetal in their maturational status[83-85] and lack the dynamic environment of intact neural circuits and complex intercellular interactions, reinforcing the importance of cross-modal validation using animal models and human post-mortem tissue. Protocols are being developed to generate more mature ‘adult-like’ astrocytes (at least at the transcriptomic level) in culture. This maturation process can take hundreds of days[86], although more efficient methods of achieving aged phenotypes from hiPSC-deriived astrocytes are emerging[85]

How best to approach the study of astrocytes is a complex issue. Although human cells would be expected to provide the most useful biological insights, the caveat of possible culture artifacts should not be understated. Newer approaches using organoids or multicellular organ-on-a-chip approaches are partially overcoming these limitations, but no approach can fully recapitulate the complex CNS milieu afforded by in vivo experimentation in the mature brain, especially when investigating complex disease trajectories and validating astrocyte-specific drug targets. Many researchers have found a happy medium by focusing their research on evolutionarily conserved functions that are likely to be replicable in human patients, rather than investigating species-specific astrocyte functions or responses to disease pathology.

Translating transcriptomics to function

Despite the increasing availability and accessibility of sequencing technologies to define reactive astrocyte responses to inflammatory insults and during neurodegenerative disease, our understanding of the impact of these changes on astrocytic function is still rudimentary. Measuring these functions in vivo and recapitulating the in vivo characteristics of astrocyte biology in vitro have proved to be technically challenging. As early as the 1970s, when the McCarthy and DeVellis method was first developed to purify astrocytes from postnatal rodents[87], it was apparent that these astrocytes were not fully representative of astrocytes in the intact CNS. One reason for these differences is that astrocytes need a constant set of molecules secreted by neurons and endothelial cells[35], as well as neuronal contact[88-91], to maintain a transcriptomic state resembling that seen in vivo. Removal of astrocytes from their in vivo environment deregulates dozens of genes[35], which becomes hundreds of altered transcripts with the addition of serum to culture media[26,87].

Co-culture of astrocytes with neurons restores a more complex stellate morphology[92], and studies have shown that through a mechanism involving juxtacrine notch signalling, neurons induce the expression of glutamate transporters[93] and partly rescue the transcriptional perturbations that occur when astrocytes are removed from their in vivo environment and grown in the presence of serum[91]. Therefore, functional validation of changes to astrocytes in neurodegenerative disease should ideally be performed in vivo or in acute slices, although several caveats require consideration. For instance, slices are inherently damaged tissue owing to the severance of neuronal axons, damage to blood vessels, and induction of microglial and astrocytic inflammation and trauma responses. In vivo models are the gold standard for studying cellular gene expression and function but can lack the fidelity to disentangle individual cell–cell interactions and to identify in an unbiased way the specific functions attributed to individual substates of reactive astrocytes. In vitro models enable the investigation of homogenous populations of astrocytes, including defined reactive substates[8,50]. The addition inclusion of microglia to astrocyte–neuron co-cultures enables some aspects of astrocyte–microglia cross-talk to be investigated under basal and inflammatory conditions[90,91,94].

To date, the most successful functional substate modelling has been accomplished through the addition of small molecules or cytokines to cultures of pure, transcriptomically and physiologically ‘normal’ astrocytes — either primary astrocytes from rodents[8,30,50,95], or astrocytes derived from hiPSCs[36,96,97] — grown in defined culture media devoid of serum components[35]. These studies used transcriptomic modelling at the bulk and single-cell level to define a cocktail of factors that, when added to the culture medium, recapitulated the transcriptomic signature seen in astrocytes during inflammation induced by systemic injection of

lipopolysaccharide in vivo. The astrocytes that were exposed to these factors could then undergo further functional characterization — for example, with regard to synaptogenesis, neurotrophic and neurotoxic effects, phagocytosis, and glutamate reuptake (Fig. 1), and in vivo validation (in the case of rodent studies). For example, if astrocyte functions such as glutamate uptake were perturbed, leading to excitotoxicity[98], a modest inflammatory response could have profound effects on neuronal health and function[8,95]

Other 'omics' analyses can identify protein, lipid and other molecular signatures to add to the characterization of reactive astrocyte substates. For example, using bulk RNA-seq datasets[27], we discovered that tumour necrosis factor, IL-1α, and complement component 1q secreted by microglia were drivers of the gene expression profiles of neurotoxic reactive astrocytes[8,36], and subsequent unbiased protein, lipid and metabolomics analyses uncovered long-chain free fatty acids as astrocyte-derived toxic molecules[95]. To date, only two inflammation-induced reactive astrocyte substates — neurotoxic[8,36,95] and interferon-responsive [50] — have been recapitulated using these methods. These substates have been identified in several disease models and human post-mortem tissues at the transcriptomic and protein level[8,9,30,50,99].

A particularly exciting new advance has been use of microfluidic-based multicellular culture methods to study the inflammatory response of astrocytes to Aβ[100,101]. These 'brain-on-a-chip' methods have key advantages over existing methods: first, they take advantage of multicellular interactions, which are important for understanding the complex inflammatory responses in diseases such as AD; and second, they use human cells, thereby raising the prospect of patient-specific drug screening. These high-throughput, reproducible, patient-specific screening platforms are likely to prove integral to furthering our understanding of complex diseases such as AD, and to enable translational researchers to better model therapeutic effectiveness in patients with diverse genetic backgrounds.

Astrocyte dyshomeostasis versus neurotoxicity

Functional changes to astrocytes that can lead to disease progression seem to fall into two broad categories, 'dyshomeostasis' and 'cytotoxicity', which include many substates with different functional consequences. Dyshomeostasis refers to astrocytes failing to carry out their normal neurosupportive and other homeostatic roles, such as neurotransmitter uptake, energy metabolism, ionic balance and immunomodulation (Fig. 1). These processes are thought to decline with ageing and in disease and are to a certain extent interdependent. Although gene expression changes and indirect measures such as PET and microdialysis strongly point to

these processes being impaired, functional interrogation is needed to provide full confirmation. For example, glutamate uptake capacity can be measured by whole-cell patch-clamp recordings to validate the functional consequences of altered transporter mRNA expression[91]. A similar electrophysiological approach can be used to probe alterations in potassium buffering capacity, and genetically encoded probes for various metabolites can interrogate astrocytic glycolytic flux, a process that is regulated by neuronal activity[91]. Cytotoxicity refers to astrocytes not merely neglecting their supportive roles but actively driving pathological progression, for example, through the release of toxic factors such as inflammatory cytokines and neurotoxic lipids[95,102]. Although dyshomeostasis and cytotoxicity in astrocytes have been found to coexist under adverse conditions, the precise nature of the relationship between these two broad functional changes has yet to be established.

Several important astrocyte functions are reported to be lost or severely downregulated in the context of acute inflammation and chronic neurodegenerative diseases. During development, astrocytes secrete several synaptogenic molecules, including SPARCL1[103], TSP1/2[104] and GYP4/6[105]. These molecules are downregulated at the transcriptomic level in both rodent and human neurotoxic reactive astrocytes, and these cells showed a reduced capacity to support synapse formation when co-cultured with neurons [8,36]. The pruning of excess synapses, which is typically associated with specialized phagocytes such as microglia, can also be functionally attributed to astrocytes. Synapse engulfment requires the combined function of MERTK and MEGF10 receptors[106], which are downregulated in several reactive astrocyte substates[8,30,36,50]. From a functional standpoint, the loss of astrocyte phagocytic capacity could lead to problems during development (for example, defects in synaptic pruning and neuronal network development), as well as during disease (for example, loss of debris clearance following trauma, or lack of removal of toxic pathogenic protein aggregates such as Aβ and α-synuclein). Synapse density loss in the early prodromal stages of AD has been attributed, at least in part, to increased microglia- pruning[107]: however, it could be exacerbated by a lack of compensatory synaptogenesis owing to local astrocytes losing their synaptogenic potential.

In addition to synapse-interacting functions, reactive astrocytes show profound alterations in glutamate reuptake and recycling, which can lead to excitotoxic synapse loss or neuronal death owing to a build-up of ambient glutamate. This phenomenon has been studied extensively in mouse and in vitro models of ALS, and also in other neurodegenerative diseases such as AD, PD, HD and epilepsy[2,108]. It is largely driven by decreases in the transporters EAAT1/GLAST

(encoded by *SLC1A3*) and EAAT2 (encoded by *SLC1A2*), which is a common occurrence in many disease contexts[40,56,62,109,110] and animal models[8,30,40,47,50,57,76,111].

Astrocytes also display intracellular calcium signalling dynamics that are reported to underlie important physiological functions including modulation of neural circuit function and alterations in blood flow[112,113]. Whether these functions are directly mediated by calcium signalling remains to be seen, and methods to target astrocytes and visualize such signalling in vivo in real time should help to resolve this issue[114]. Astrocytic calcium signalling has been shown to increase or decrease in response to a wide array of extracellular stimuli, including inflammation[115], disease[116-120] and ischaemia[121,122], and in response to pain[123] and changing metabolic demands[80,81]. Normalization of these calcium transients has been reported to rescue cognitive deficits in a β-amyloidopathy model[124]. However, no consensus exists on precisely how disturbed astrocytic calcium signals cause impairment in the brain. One possibility is disturbance of neurovascular coupling, as neuronal-activity-dependent astrocytic calcium transients are thought to be involved in triggering the release of vasoactive molecules[125]. In the context of β-amyloidopathy, another proposed downstream effect of astrocytic calcium dysregulation is peri-plaque morphological changes: downregulation of astrocytic calcium in the APP/PS1 mouse model caused increases in Aβ plaque compaction and density of astrocytic peri-plaque barrier, potentially limiting neuronal exposure to toxic Aβ fibrils[124]. Other potentially harmful consequences of astrocytic calcium dysregulation include the release of inflammatory cytokines[126].

Beyond calciumCa^{2+}, knowledge of alterations to other second messengers, such as cAMP, is less advanced, possibly because these messengers are more difficult to measure at the single-cell level, especially in vivo. Inducers of cAMP production or cell-permeable analogues of cAMP have long been known to promote functional and morphological maturity, including induction of stellate morphology and increases in glutamate transporter and gap junction expression, in immature or de-differentiated astrocyte monocultures [92,93,127]. Whether this pathway is important in astrocyte maturation in vivo is less clear. Signalling by cAMP is also an important regulator of astrocytic glucose metabolism, mediating neuronal-activity-dependent changes in astrocytic glycolysis and lactate release via CREB activation[91], and also promotes glycogenolysis[128]. In addition to controlling energy metabolism, cAMP signalling has been shown to inhibit activation of the inflammatory mediator NFκB and release of inflammatory cytokines[127,129]. Although cAMP signalling promotes many functions that are thought to be impaired in astrocytes in neurodegenerative disease, we do not know whether deficits in cAMP signalling are the cause of these impairments and, if so, whether non-cell-autonomous effects

(for example, levels of G protein-coupled neuropeptides) or cell-autonomous deficits in cAMP production and the intracellular signal transduction machinery are involved.

[H1] Transcriptomic drivers of reactive astrocyte functions

Knowledge of the transcription factors and upstream activators that control groups of genes that mediate functional changes to astrocytes in disease might indicate potential points of therapeutic intervention. Activation of the JAK–STAT pathway (specifically, JAK2–STAT3), which lies downstream of cytokine or growth factor exposure, has been observed in astrocytes in models of AD, HD and PD[130]. This pathway is regarded as an important mediator of several aspects of astrocyte reactivity, as well as the induction of specific astrocytic states and phenotypes. Downstream effects of STAT3 activation on astrocytes include induction of GFAP expression, secretion of inflammatory cytokines, and regulation of morphology, migration and proliferation[130]. However, several phenotypes have only been reported following STAT3 activation by acute insults such as traumatic brain injury, ischaemia, spinal cord injury, and infection, so the precise consequences are likely to be context dependent[131-134]. In addition, the modulation of STAT3 is likely to be problematic, as scRNA-seq and snRNA-seq analyses of astrocytes from multiple models and species indicate that many different reactive astrocyte substates express *STAT3* (or *Stat92e* in the case of *Drosophila* at high levels[40,50,56,72,97,135,136,137]).

The idea of STAT3 as a master regulator of several reactive phenotypes remains important for our understanding of astrocyte biology, but specific targeting of STAT3-mediated reactivity and downstream pathways lacks the fidelity to target individual clusters of astrocytes. Nevertheless, in a mouse model of β-amyloidopathy (APP/PS1ΔE9), astrocyte-specific inhibition of JAK–STAT signalling reduced gross astrocyte reactivity, as well as ameliorating plaque load and associated spatial memory and electrophysiological deficits[138], However, a similar approach failed to reduce plaque load or spatial memory deficits in the 3×Tg model of AD[139]. This discrepancy might be partly attributable to the low fidelity control afforded by STAT3 targeting. Interestingly, in HD models, JAK2-STAT3-induced astrocyte reactivity was shown to be beneficial by promoting mutant huntingtin clearance[140], thereby providing an example of an ‘astroprotective’ response that serves to slow disease progression. Many of the reactive astrocyte substates that have been described following inflammatory insults are also induced by STAT3[8,50,95,97]; however, well-powered scRNA-seq and snRNA-seq studies have uncovered several low-abundance, biologically important substates that are not under STAT3 control[50,56,141]. Alternative regulators of reactive astrocyte phenotypes include the chromatin

remodeller SMARCA4[72] (although whether this factor induces similar gene expression changes to STAT3 remains unclear) and the microRNAs (miRNAs) miR-146a[142], miR-145[143], and miR-125b[144]. Although miRNAs might not directly drive the upregulation of 'reactivity genes', they are thought to be important in stabilizing such transcriptomic changes[144] and are, therefore, integral to the continued switch from physiological to reactive astrocyte states.

Further interrogation of these substates at the functional level, and a move towards understanding the specific drivers of other non-STAT3-induced substates, will be vital for continued progress in the field. A further example of an astroprotective response mediated by the stress-responsive transcription factor NRF2 was recently reported in both tauopathy and β-amyloidopathy models[145]. Activation of this factor in astrocytes was found to be sufficient to slow pathological progression in both disease models. We cannot rule out the possibility that concurrent activation of neurotoxic (or neuro-neglect) and neuroprotective functional changes can take place in the same astrocyte, mediated by different transcription factors. From a therapeutic perspective, strategies that enhance the adaptive–protective responses of astrocytes while blocking pathways that promote neuro-neglect or neurotoxicity might change the balance of astrocytic substates such that reactive astrocytes have a net disease-slowing effect.

[H1] Pathology-associated reactive astrocyte substates

Several other issues remain unresolved in understanding functional changes in astrocytes that might contribute to the initiation and/or progression of inflammatory responses and neurodegenerative disease. We require a greater understanding of whether astrocytes change in a manner that is dependent on brain region or distance from pathological features. For example, are reductions in glutamate transporter currents and other neurosupportive functions observed predominantly in astrocytes near to Aβ plaques? In some models of β-amyloidopathy, cortical synapse loss has been shown to depend on plaque proximity[146,147], and any functional differences between plaque-distal and plaque-proximal astrocytes could point to how these cells contribute to synapse loss. Some studies have putatively attributed the expression of certain plaque-associated genes to astrocytes[75,76]. However, these studies used low-resolution spatial transcriptomic approaches, and additional research using high-resolution and cell-type-specific approaches will be required to confirm the findings. We already know, however, that astrocyte-specific transcripts, particularly those involved in inflammatory responses (for example, *Igtp, Iigp1* and other interferon-responsive genes) show enrichment in regions closely associated with plaques[50,75]. Other inflammation-responsive transcripts are difficult to attribute to a single

cell type; for example, *CXCL10* is upregulated in both microglia[148] and astrocytes[50] following acute inflammatory insult, as well as in human post-mortem brain samples from patients with AD[149] or multiple sclerosis (MS)[150].

The importance of astrocytic perturbations in processes involving more than one cell type, and the contributions of those processes to inflammatory responses and chronic disorders such as AD-associated cognitive decline, have historically been difficult to disentangle. For example, few would disagree that deficits in astrocyte glutamate uptake capacity are likely to affect the fidelity of excitatory synaptic transmission. However, in AD, reduced glucose metabolism in the brain could be attributable to a lowering of astrocyte metabolism or to other factors, such as impaired glucose delivery by the vasculature, or reduced energy demand by hypoactive neural circuits.

Analogous questions could be asked regarding the role of astrocytes in impairment of the neuron–astrocyte–vasculature signalling pathways that underlie neurovascular coupling. Moreover, it is important to know which astrocytic changes have the greatest effect on cognitive performance so that we can identify the phenotypes that should be targeted by future generations of therapies. These questions might be too simplistic, however, as different functional alterations might lead to circuit dysfunction, synapse loss or neuronal death, and the disease stage might be an important factor in determining whether a particular type of intervention will be effective.

Another important consideration is how underlying genetic mutations or common variants associated with disease might alter neuronal susceptibility, or inflammatory and reactive responses in astrocytes (Fig. 4). In acute insults like infection and trauma, that are often associated with inflammation, but are also important in disease-pathology-associated reactivity in astrocytes and microglia, future investigation should pay careful attention to both the activation and the resolution stages of the reactive response. Unfortunately, current methods only provide a ‘snapshot in time’ at the transcriptomic, proteomic and often functional levels, and some substates defined by a single metric might only be transitory and not representative of a stable or end-stage reactive substate that is responding to or driving inflammation and neurodegeneration. Such transitory states are beginning to be uncovered using methods that derive dynamic information from single-cell experiments, either by using repeated measures across time or specific analysis pipelines that enable gene-specific kinetics to be calculated (for example, velocyto[151] or scVelo[152]). Such computational methods rely on the ratio of mRNA splicing and degradation rates, and have caused some experts to raise concern about their widespread use without additional validation[153].

We will need to ensure that blocking of specific astrocyte functions is beneficial at the particular stage of disease at which it is being targeted, as a particular function could be beneficial at earlier stages but detrimental at later stages of pathology. For example, during the early inflammatory stage of AD when neuronal stress pathways are first engaged, removal of individual neurons from a network might help to preserve network stability while giving affected neurons time to recover, so interventions that reduce the synaptic maintenance functions of astrocytes could be beneficial. Later in AD progression, however, when synapse density has declined considerably owing to excessive microglial pruning[107], synaptic maintenance might be an important functional role. For these complex but subtle reasons, targeting of gross drivers of astrocyte reactivity is unlikely to prove clinically beneficial, and specific substates or individual functions could represent more relevant therapeutic targets.

[H1] Reactive versus disease states

The precise nature of the relationship between reactive and disease astrocytes — that is, those expressing a mutation in a disease-associated gene such as superoxide dismutase 1 (*SOD1*) in ALS [11,12] or *GFAP* in Alexander disease[154] — has remained elusive, and some functional changes in disease astrocytes might overlap with those in reactive astrocytes. Specifically, it is unclear whether disease-causing or disease-associated mutations drive astrocyte responses or whether extrinsic signals from neighbouring cells induce reactive astrocyte reactivity. A further important consideration is whether the mutation in question changes the repertoire of responses of astrocytes to extrinsic stimuli, including pro-inflammatory cues (Figs 1, 4). With these issues in mind, a strength of the hiPSC modelling approach is the ability to capture authentic cell-autonomous phenotypes and conduct molecular interrogation of the pathogenic cascade. Furthermore, serum-free directed differentiation paradigms allow modelling of patient-derived astrocytes, which bypass the need to artificially overexpress or knock down disease genes.

In the context of preclinical testing, hiPSC-based approaches have considerable advantages over animal models, which often rely on gene overexpression. Numerous studies have taken advantage of this approach to generate astrocytes from patients with neurodegenerative diseases and examine cell-autonomous effects. For example, specific cell-autonomous astrocyte phenotypes have been reported in astrocytes carrying ALS-causing mutations in *TARDBP*, which encodes TAR DNA-binding protein 43 (TDP34), or *VCP*, which encodes valosin-containing protein[155,156]. Evidence for cell-autonomous reactive transformation was also reported in a meta-analysis of all available RNA-seq datasets from ALS hiPSC-derived astrocytes, including those carrying ALS-causing mutations in *C9ORF72*, *SOD1*, fused in

sarcoma (*FUS*) or *VCP*[43] Interestingly, reactivity-related changes in gene expression derive, at least in part, from the post-transcriptional splicing of poised (intron retaining) transcripts in astrocytes.[157]

A study published in 2022 demonstrated that cell-autonomous transformation of astrocytes extends beyond transcriptomic signature into upregulation of protein markers such as complement component 3 (*C3)*, perturbation of sodium-dependent glutamate uptake and alterations in the basal secretome[158] — changes that are also associated with acute inflammatory responses by astrocytes in both rodents[8] and humans[36]. By comparing *VCP*-mutant and *SOD1*-mutant astrocytes, this study also suggested mutation-dependent differences in early reactive states, which seemed to resolve over time. Importantly, hiPSC-derived astrocytes carrying ALS-associated mutations were also shown to exhibit non-cell-autonomous effects in co-culture with neurons[156]. In human astrocytes, the presence of these ALS-causing mutations alone seems to be sufficient to induce a harmful cell autonomous reactive transformation. By contrast, rodent *SOD1*^{G93A}-expressing astrocytes displayed minimal transcriptomic differences from their wild-type counterparts in the basal state, although the mutant astrocytes reached high levels of reactivity at much lower doses of pro-inflammatory cues, suggesting a degree of cell-autonomous 'priming'[8]. This finding might suggest species-specific responses of astrocytes to harbouring a disease-associated mutation, although alternatively it could be a result of the purification and culture methods used in this study, as astrocyte monocultures might function differently from cells in situ in the CNS.

It is important to remember that disease progression in neurodegenerative conditions caused by specific gene mutations can affect multiple cell types and that astrocyte changes might drive just part of the pathological process. For example, astrocytes derived from hiPSCs harbouring mutant *C9ORF72* expansions cause electrophysiological deficits in co-cultured iPSC-derived motor neurons — an effect that is reversed by CRISPR–Cas9-mediated correction of the expansion[159]. However, motor neurons derived from the same mutant *C9ORF72* hIPSCs also exhibit vulnerability to AMPA receptor-mediated excitotoxicity owing to deregulation of glutamate receptor 1 (*GRIA1*) expression[160], as well as mitochondrial bioenergetic deficits leading to axonal dysfunction[161]. Therefore, *C9ORF72*-associated ALS probably involves several CNS cell types with aberrant phenotypes that combine to drive motor neuron loss.

Given that most cases of neurodegenerative disease are not attributable to monogenic mutations, understanding the acute responses of healthy control astrocytes in a disease-relevant context is also important, as these might differ from the relatively chronic responses of mutation-carrying ALS astrocytes. Seeded aggregation assays, in which healthy control hiPSC-

derived motor neurons and astrocytes were exposed to TDP43 oligomer-containing sarkosyl-insoluble extracts from post-mortem tissue from individuals with sporadic ALS or to highly purified recombinant TDP43 oligomers, revealed that neurons were susceptible to seeded aggregation whereas astrocytes were more resilient, at least initially[162]. In Co-culture experiments have shown that TDP43 proteinopathy can 'spread' from motor neurons to astrocytes and vice versa. However, healthy control astrocytes in this setting were profoundly neuroprotective, improving neuronal survival and decreasing cytoplasmic TDP43 aggregation. Astrocyte-conditioned medium afforded similar neuroprotection to physical co-culture, suggesting that a secreted factor is involved[162]. Taken together, these studies suggest that the response of control astrocytes to adverse conditions is, at least initially, neuroprotective.

With more chronic stressors such as ALS-causing mutations, a more harmful reactive transformation takes place whereby astrocytes lose their homeostatic functions and exhibit an impaired neuroprotective response[163]. The impact of apolipoprotein E ε4 (*APOE* ε4), a strong genetic risk factor for AD was assessed in hiPSC-neural derivatives, an in vivo rodent model and post-mortem brain tissue. This study revealed *APOE* ε4-mediated dysregulation of lipid metabolism and altered matrisome signalling in human but not rodent glia and suggested that *APOE* ε4 incites astrocyte-specific dysfunction in AD[164]. These findings build on earlier studies in familial AD caused by mutations in presenilin 1 (*PSEN1*) or amyloid precursor protein (*APP*) and in sporadic AD, which revealed increased Aβ production, deregulated calcium metabolism, increased reactive oxide species production and/or aberrant cytokine release[165-168]. Analogous studies performed in hiPSC-derived astrocytes have also been performed in PD[169,170] and HD[171,172] and are reviewed elsewhere[163,173]. These studies raise some important open questions for the field, which are listed in Box 3.

[H1] Advances in investigation of astrocyte reactivity

Recent advances in synthetic genetic engineering and high-throughput screening of changes in astrocyte function have improved our understanding of how reactivity is induced and of the downstream consequences of this reactivity for the CNS (Fig. 3). These techniques have provided new information about the earliest changes in astrocytes in response to inflammation and during neurodegenerative disease. Examples include new methods that enable rewriting of the mouse genome with entire human gene loci containing disease-associated point mutations. Incorporation of small amounts of DNA is possible using CRISPR–Cas9 and new technologies such as Big-IN[174] and mSWAP-IN[175] allow genome engineering using larger constructs consisting of hundreds or thousands of kB of DNA. These technologies will enable combinatory

interrogation of integrated genomic functional elements at the locus scale, which will be important for studying the enhancer and repressor regions of the genome. Similarly, the use of CRISPR interference (CRIPSRi) in hiPSC-derived astrocytes is enabling deep and systematic interrogation of known inducers of particular reactivity states[176]. Additional advances are afforded by the production of reactive astrocyte-specific reporter mice that enable the tracking of individual cells over long periods of time, including induction of a subset of reactive astrocytes and their resolution back to a physiologically normal state[177]. Further methodological improvements include the widespread adoption of scRNA-seq and snRNA-seq, spatial transcriptomics and single-nuclear ATAC-seq. Subcellular single-organelle sequencing technologies that can incorporate intracellular location[178] will be important for addressing complex questions about reactive astrocytes. These technical advances, in addition the conceptual advances highlighted throughout this Review, continue to improve our understanding of astrocyte reactivity in the context of inflammation and neurodegenerative disease.

[H1] Conclusions and future directions

Astrocytes are abundant in the human CNS and have myriad indispensable roles in neuronal (including synaptic) homeostasis. The long-held view of astrocytes as merely ancillary supportive cells has been challenged through a series of studies across a range of diseases and model systems, and the precise nature of their roles in CNS physiology and pathophysiology, along with intercellular interactions, is increasingly understood, although much remains to be discovered. Astrocyte reactivity is known to be a complex and constantly changing response to inflammation and neurodegenerative disease, but it remains unclear how homogeneous these substates may be across seeminly disparate disease states. Single-cell and single-nuclei transcriptomics have highlighted transcriptomic heterogeneity, but a lack of follow-up studies to validate the functional changes, combined with grossly underpowered sequencing studies, have hampered progress in this area. Beyond their canonical functions, previously unrecognized region-specific functional heterogeneity of astrocytes has been established as an important attribute, again dispelling the traditional assertion of CNS-wide astrocyte homogeneity.

Future priorities for this research field include determination of functional changes in already transcriptomically defined reactive astrocyte substates. What molecules induce these sub-states, and how are their functions altered such that they contribute to disease pathophysiology? Like microglia[73] and oligodendrocytes[74], astrocytes seem to be extremely

heterogeneous within an individual insult or disease, but homogeneous representation of some substates is observed across different diseases, include typical inflammatory responses in interferon-responsive reactive astrocytes[50,76,179], and the neurotoxic substate[8-10,30,36,95,99,180-182]. An appreciation that heterogeneity does not negate the biological inevitability of common responses across diseases will be vital for us to develop novel treatments for neurodegenerative diseases.

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Publisher's note

Key points

- Neurodegenerative diseases are a group of serious and incurable conditions in which astrocytes both cause and respond to neuroinflammation.
- A better understanding of how neuroinflammation and astrocyte function are linked in neurodegeneration is likely to lead to new therapeutic strategies.
- To gain this understanding, researchers are using a range of techniques and datasets, including transcriptomic studies at the single-cell and single-nuclei level, and the findings are being validated using both human and animal models across different stages of neurodegenerative diseases.
- Transcriptomic studies of reactive astrocytes in human and animal models of disease have revealed the co-existence of many pathology-related reactive substates of astrocytes.
- Future priorities for this research field include determination of functional changes in transcriptomically defined reactive astrocyte substates.

Figure 1 | Physiological and reactive functions and of astrocytes. The left-hand side (blue) shows normal physiological functions of astrocytes. and the right-hand side (orange) shows functional changes attributed to different substates of reactive astrocytes. The reactive astrocyte functions shown in the figure were experimentally defined in models of inflammation and neurodegenerative disease. Note that not all functions can be assigned to a single astrocyte, and not all physiological functions are lost in the context of reactivity.

Figure 2 | Position of astrocytes within the inflammatory cascade. Astrocytes respond to various stimulatory factors, but mostly to signals from immune cells, both resident (microglia, black arrow) and peripheral (for example, infiltrating macrophages, green arrows). Astrocytes can respond directly to stimuli including changes in blood flow (or extravasation of serum components during ischemic injury, blue arrow), directly or indirectly to trauma or pathogenic proteins (orange arrows), and to changes in neuronal activity or cellular debris (for example, degenerating neurons or axonal processes, yellow arrows). Given their close proximity, numerous interactions occur between astrocytes and microglia (black arrows). The ultimate effects on neurons (purple arrows) can be detrimental or supportive. Astrocyte physiological functions like trophic support, synapse formation and maintenance, and phagocytosis are tightly controlled under both physiological and pathological conditions.

Figure 3 | Methods for studying astrocyte reactivity. Many powerful models exist for the study of astrocytes in inflammatory conditions and neurodegenerative disease. At the functional level, in vitro methods, including monocultures, multi-cell-type or multi-species co-cultures, and microfluidic-based systems, remain the most powerful, as homogeneous populations of reactive substates or physiological subtypes can be cultured at high purity. Other ex vivo techniques, including the use of human post-mortem brain samples for omics and pathological assessments, are important for cross-species validation. In vivo models, such as transgenic or chimaeric rodents, zebrafish, *Drosophila* or *Caenorhabditis elegans*, are required for validation of in vitro assays. New advances that are beginning to be employed include gene editing (for example, using CRISPR–Cas9 or Big-IN), organoids (including connectoids and in vivo implantation to enable vascularization). Gene-editing approaches can be exploited both in vitro for functional testing and in vivo to produce astrocyte-specific models of disease or infection, and can be used to investigate the effects of disease-associated mutations on normal physiology and reactive responses of astrocytes. Integration of different dataset modalities (for example, RNA sequencing and proteomics data) and dissemination of data to other researchers are important. The ability to harness and integrate these datasets for downstream computational modelling and hypothesis generation is a key validation step for understand the role of inflammatory responses across diseases and disease models.

Figure 4 | Disease-associated mutations that alter human astrocyte inflammatory responses and homeostatic functions. Studies in human induced pluripotent stem cells or post-mortem tissue to examine the impact of disease-causing mutations or risk genes on astrocytic functions are converging on several key changes, the relative timings and interdependence of which are not yet fully resolved. The key refers to some of the major subtypes of Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington disease (HD), and the genes are numbered in the coloured boxes of the figure for ease of reference. Lack of experimental evidence for a perturbation in a specific disease does not necessarily mean that the perturbation is absent in that disease, as the appropriate experiments might not yet have been done. *APOE* ε4, apolipoprotein E ε4; APP, amyloid precursor protein; *GBA1*, lysosomal acid glucosylceramidase; *LRRK2*, leucine-rich repeat serine/threonine-protein kinase 2; *PSEN1*, presenilin 1; SOD1, superoxide dismutase 1; TARDBP, TAR DNA-binding protein 43; VCP, valosin-containing protein.

Box 1 | Astrocyte to microglia signalling

Astrocytes and microglia have crucial roles in CNS communication and functionality. Communication between astrocytes and microglia is bidirectional, (Fig. 2), and their interactions can have both positive and negative effects on the immune response. For example, although evidence suggests that many components of the complement system are pro-inflammatory, the astrocyte-derived γ subunit of complement component 8 i interacts with sphingosine 1-phosphate (S1P) receptor 2 to counteract the pro-inflammatory effects of S1P in microglia[183]. The drugs fingolimod and siponimod target this pathway and are used clinically to treat multiple sclerosis. These drugs are thought to act predominantly in the periphery to reversibly retain T cells in the lymphoid tissue[184,185], but they can also cross the blood–brain barrier and act directly on CNS cells[186,187].

Interactions between astrocytes and microglia can also be deleterious. For example, in neuroinflammatory conditions such as experimental autoimmune encephalomyelitis in mice or MS in humans[184], cathelicidin-related antimicrobial peptide, an effector molecule of the innate immune system with immunomodulatory roles, is expressed on astrocytes and binds its receptor FPR2 on microglia to drive their IFNγ-induced reactive transformation through the STAT3 pathway. Other such examples of astrocyte–microglial interaction have been reported[60,94,164,188,189], and undoubtedly more remain to be discovered.

Box 2 | Interspecies differences in astrocyte functions

The use of model organisms for the discovery and validation of astrocyte functions, particularly in the context of neurodegenerative disease, has been integral to our understanding of disease initiation and progression. However, key differences exist between astrocytes from laboratory animals and humans. For example, compared with mouse astrocytes, human astrocytes show faster propagation of calcium waves and a more robust glutamate response, possibly reflecting an increased capacity to sense and respond to synaptic activity[8,29,78,190]

In vitro studies using acutely purified rodent and human astrocytes maintained in serum-free conditions have shown that human astrocytes have improved antigen presentation pathway induction under inflammatory conditions, along with enhanced susceptibility to oxidative stress derived from a divergence in mitochondrial physiology, for example, increased expression of *NDUFA7* and *NDUFB7*, which encode components of the mitochondrial respiratory chain[80]. Furthermore, unlike their human counterparts, mouse astrocytes activate a neural repair response programme under hypoxic conditions[80]. In addition to transcriptomic analyses, human astrocytes have been investigated at a more functional level by xenografting their precursors into mouse brains, where they differentiated into mature astrocytes, thereby enabling direct comparison with mouse astrocytes[78]. The grafted human astrocytes seemed to improve network connectivity and learning and memory function in the mouse brain[77,78]. In a subsequent study, however, culture media conditioned by mouse or human astrocytes did not show differential effects on neuronal function in vitro[80]

Interspecies differences have also been observed in the temporal dynamics and levels of reactivity responses of astrocytes following acute trauma[191-194]. In primates, the kinetics of astrocyte reactive transformation are delayed and display a relatively lower degree of activation compared with rodents[49]. In situations where endogenous regenerative capacity is enhanced, such as during development in mammals, or in non-mammalian species[195], reactive astrocytes in extend processes toward the lesion and form a bridge rather than a scar, thereby allowing axonal regrowth through the lesion[51].

Despite these differences, many astrocyte functions, including neuron trophic support, synaptogenic capacity and phagocytosis, are evolutionarily conserved. In addition, validation of the neurotoxic reactive substate identified in rodents[8] at the transcriptomic, proteomic and functional level in human induced pluripotent stem cell-derived astrocytes[36,196] has underlined the utility of rodent models for in vivo confirmation of discoveries made in cell-based models of human disease.

Box 3 | Open questions

The ongoing study of reactive astrocytes in inflammation and a range of neurodegenerative diseases is uncovering considerable transcriptomic and functional overlap across disease states. The concept of common responses has persisted even in the face of continued uncovering of heterogeneity in reactive responses of astrocytes. The development of increasingly complex parallel methods to interrogate reactive astrocytes (Fig. 3) will enable us to address the following questions, among others::

- What molecules induce the wide array of transcriptomically defined reactive astrocyte substates, and how are their functions altered such that they can contribute to the initiation or progression of disease?
- What are the molecular bases for harmful and helpful reactive astrocytes?
- Does the harmful reactive transformation have an evolutionary purpose in terms of preserving neuronal circuit integrity?
- Can this harmful state be reversed, and how heterogenous is the fate of these reactive astrocytes?
- How does spatially restricted functional heterogeneity interface with reactive transformation?
- What are the roles of other glial cell types and neurons in the initiation, potentiation and termination of reactive astrocyte substates?
- Can we predictably manipulate reactive states of astrocytes for patient benefit?
- Are cell therapies with astrocytes that are primed or engineered to be neuroprotective feasible?