A stocked toolbox for understanding the role of astrocytes in disease

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Abstract | Our understanding of astrocytes and their role in neurological diseases has increased considerably over the past two decades as the diverse roles of these cells have become recognized. Our evolving understanding of these cells suggests that they are more than support cells for neurons and that they play important roles in CNS homeostasis under normal conditions, in neuroprotection and in disease exacerbation. These multiple functions make them excellent candidates for targeted therapies to treat neurological disorders. New technological advances, including in vivo imaging, optogenetics and chemogenetics, have allowed us to examine astrocytic functions in ways that have uncovered new insights into the dynamic roles of these cells. Furthermore, the use of induced pluripotent stem cell-derived astrocytes from patients with a host of neurological disorders can help to tease out the contributions of astrocytes to human disease. In this Review, we explore some of the technological advances developed over the past decade that have aided our understanding of astrocyte function. We also highlight neurological disorders in which astrocyte function or dysfunction is believed to have a role in disease pathogenesis or propagation and discuss how the technological advances have been and could be used to study each of these diseases.

Tripartite synapse
A site at which three-way communication occurs between presynaptic, postsynaptic and astrocytic processes during synaptic transmission.

Gliotransmission
The release of glutamate, ATP, D-serine and other neurotransmitters that are essential for synaptic transmission and plasticity from astrocytes.

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Astrocytes have a central role in CNS homeostasis and in response to trauma and disease pathogenesis[1]. However, these cells were long thought to primarily provide support for neurons, and morphological changes in astrocytes that have been noted in neurological disease were considered to be nonspecific, reactive processes secondary to neuronal injury. These changes were often dismissed as physiologically irrelevant because the tools for understanding the pathophysiological contributions of astrocytes to neurological disease had not matured, although most of the changes were accompanied by upregulation of glial fibrillary acidic protein (GFAP), which was once considered to be the primary indicator of astrogliosis.

New insight into the roles of astrocytes has shown that these cells are not homogeneous but are specialized according to the region of the CNS in which they reside and consequently influence the structure and function of surrounding neurons[2,3]. Astrocytes also have critical roles in preserving neurological function, from developmental regulation of synapse formation, elimination and maintenance to synaptic preservation in disease[4]. Under normal conditions, astrocytes are key players in CNS homeostasis and, through the channels and receptors present on their surfaces, act as gatekeepers of water, ions (such as potassium and calcium), glutamate and second messengers. Astrocytes also supply energy to neurons, transporting lactate and amino acids to neurons via shuttles, thereby helping to maintain neuronal energy production[5]. Furthermore, astrocytes are part of the tripartite synapse, where they can modulate synaptic activity through gliotransmission[6,7].

In addition, astrocytes contribute to the blood–brain barrier to maintain the CNS as an immune-privileged site, a function that is critical in the design of drug therapies that are intended to affect the CNS[8]. Increasing evidence suggests that astrocytes communicate not just with neurons but also with other astrocytes via gap junctions, underscoring the importance of astrocyte communication in the CNS. In addition to direct communication via gap junctions, astrocytes send signals through hemichannels, affording crosstalk to surrounding neurons, microglia and oligodendrocytes (FIG. 1).

The focus of this Review is our evolving knowledge of the complex physiological functions of astrocytes that underlie normal neurological function, and their roles in neurological disease. New scientific tools, including dynamic in vivo imaging, optogenetic, chemogenetic and metabolite-sensing platforms, have afforded us a new appreciation of astrocyte complexity (FIG. 2). Perhaps the tool with the greatest potential for increasing our understanding of the role of astrocytes in disease is modelling with human induced pluripotent stem cells (iPSCs) derived from individuals with neurological

Key points

- Astrocytes not only have key homeostatic functions in the CNS but also respond to neuronal injury in both neuroprotective and pathological manners.
- Astrocytes have key roles in a broad spectrum of neurodevelopmental and neurodegenerative diseases.
- New tools have been developed to evaluate the structural, functional and molecular mechanisms by which astrocytes respond to injury.
- The in vivo methods by which astrocytes can be studied have revealed new layers of complexity in astrocyte function, which could not have been appreciated with the use of older experimental approaches.
- The use of induced pluripotent stem cell-derived astrocytes could help with interpretation of preclinical observations as they are used to direct the design of human therapeutics.

diseases. We begin by providing an overview of these different tools for studying astrocytes. We then discuss several neurological disorders, ranging from developmental disorders to neurodegenerative disease, in which astrocyte dysfunction is thought to contribute to either disease pathogenesis or progression and for which the toolbox of the latest approaches to studying astrocytes is providing new insight.

New tools for studying astrocytes

In vivo imaging. Historically, our understanding of astrocytes and their role in the CNS has been based on histological samples prepared from human autopsy samples and rodent tissues. However, in vivo imaging in rodents, which is typically conducted through a cranial window or thin-skull preparation in anaesthetized or awake animals that are monitored over days to months, has provided new research opportunities. The invention of two-photon laser scanning fluorescence microscopy (TPLSM) has enabled high-resolution imaging of astrocytes in living slices and tissues[9]. This method gives a glimpse into the spatial and temporal dynamics of astrocytes as part of a neural circuit in an intact brain at penetration depths of more than 500 µm (REF.[9]). Transgenic animal models in which astrocytes are genetically labelled with fluorescent markers or labelled via injection of fluorescent markers (such as sulforhodamine 101) coupled with in vivo imaging have drastically advanced our understanding of these cells, which were once considered static.

Real-time monitoring of astrocytes has provided evidence for their involvement in synaptic pruning, transmission and plasticity and in other events at the tripartite synapse[10,11]. New imaging techniques have shed light on the physiological role of astrocytes in the regulation of the blood–brain barrier, crosstalk between astrocytes and microglia, the influence of astrocytes on oligodendrocyte formation and the contribution of astrocytes to neural circuits involved in the sleep–wake cycle, circadian rhythms, feeding and other CNS functions[12,13].

With these dynamic imaging techniques, the structure and function of astrocytes can be studied longitudinally, not just in physiological conditions but also in disease. For example, an in vivo imaging study has revealed that astrocytes do not all behave the same in response to acute injury: a subset of astrocytes undergo proliferation in the region next to the injury and contribute to injury-mediated responses[14].

Currently, TPLSM is primarily limited to imaging of neocortical regions and cannot be used to image deep cortical layers. However, three-photon imaging enables visualization of deeper brain regions and will further increase our understanding of astrocyte function[15]. A comprehensive review of in vivo imaging of astrocytes is available elsewhere[9].

Optogenetic manipulation. Optogenetics is a powerful technique that harnesses light-sensitive microbial channels called opsins to manipulate cellular activity in vitro and in vivo[16]. The most commonly used channel is channelrhodopsin 2 (ChR2). ChR2 is activated with blue light and becomes permeable to cations, resulting in neuronal depolarization. Selective expression and photostimulation of ChR2 in astrocytes leads to increased calcium influx over milliseconds, which results in activation of downstream signalling pathways in astrocytes and the subsequent release of cytokines and gliotransmitters that in turn influence activity of adjacent neurons in slice cultures and in vivo models. In a rat model, optogenetic activation of astrocytes in the brainstem chemoreceptor areas that express ChR2 under the control of the astrocyte-specific GFAP promoter causes a pH-dependent calcium influx and triggers release of ATP, leading to a corresponding increase in breathing responses[17]. Similarly, photoactivation of astrocytes in the primary visual cortex of mice evokes both inhibitory and excitatory responses in neurons, demonstrating that astrocytes influence processing and integration of sensory visual stimuli in vivo[18]. In vivo stimulation of spinal cord astrocytes with an optogenetic fibre in rats induces mechanical and thermal pain[19] via release of ATP and cytokines from astrocytes, which causes neuronal excitability and pain hypersensitivity.

Use of ChR2 expressed under the control of the GFAP promoter is useful for teasing out in vivo contributions of astrocytes to neural circuitry, but the resulting activation of astrocytes is greater than physiological levels[13]. Therefore, the use of alternative opsin channels is now being developed to target astrocytes in a regional and temporal manner. For example, archaerhodopsin (Arch) is a light-driven proton pump that is a hyperpolarizing channel in neurons but has been used to elicit increases in intracellular calcium in astrocyte processes at amplitudes and durations similar to physiological levels[20]. Work by the same researchers has further illustrated that astrocytes release extracellular glutamate, thereby generating slow neuronal oscillations that are critical for sleep and memory consolidation[20]. In vivo photostimulation of astrocytic ChR2 leads to intracellular acidification and release of glutamate, whereas activation of Arch leads to intracellular alkalization and blocks glutamate release, which limits ischaemic brain damage[21].

Optogenetic tools will need further testing to determine the nuances of optimal approaches for specific circuits or diseases. Nonetheless, the technique of manipulating astrocytes to induce release of gliotransmitters such as ATP, glutamate and cytokines that influence neural circuitry in a temporal and spatial manner holds great promise for improving our understanding of

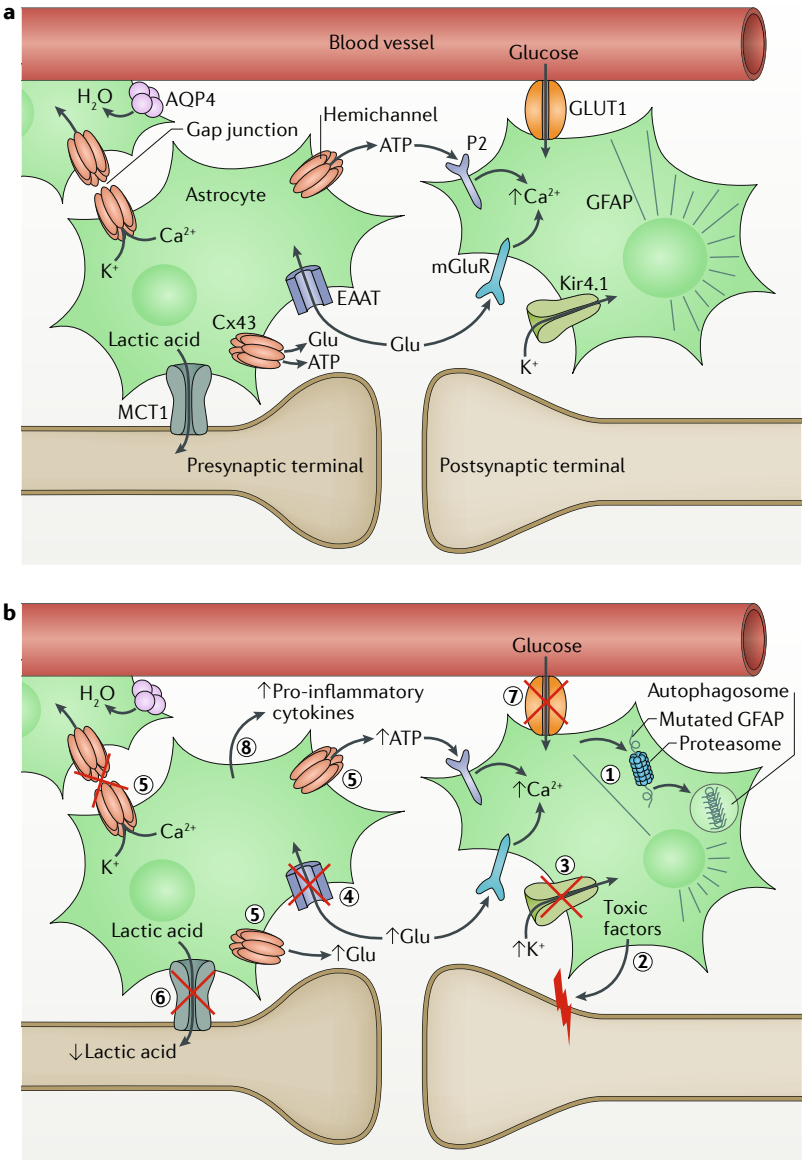

Fig. 1 | **Astrocyte function in health and disease.** **a** | Normal function of astrocytes and their contribution to homeostatic functions at the tripartite synapse. Intercellular communication between astrocytes occurs via gap junctions and purine (ATP) signalling. Astrocytes interact with presynaptic and postsynaptic neurons via release of glutamate (Glu) and ATP, which modulate synaptic function via hemichannels (comprising six connexin 43 (Cx43) subunits). Glucose, which is transported via glucose transporter type 1 (GLUT1), and the metabolite lactic acid, which is transported via monocarboxylate transporter 1 (MCT1), provide energy to astrocytes and neurons. Transport of glutamate at synaptic sites occurs via excitatory amino acid transporters (EAAT), and the influx of potassium occurs via ATP-dependent inwardly rectifying potassium channel Kir4.1 (Kir4.1). Interaction of astrocytes with the blood–brain barrier occurs via the water channel aquaporin 4 (AQP4) and is necessary for cell volume regulation. **b** | Changes to astrocyte function in disease states. Mutations in glial fibrillary acidic protein (GFAP) result in accumulation of the protein (1) and induction of autophagy, leading to astrocyte death. Release of incompletely identified toxic factors (2) in several neurodegenerative disorders mediates a non-cell-autonomous effect of astrocytes on neuron death. Mutations in Kir4.1 or reductions in its expression (3) contribute to seizure activity and epilepsy. Reduced expression or activity of the glutamate transporter EAAT (4) leads to excitotoxicity in several neurodegenerative disorders and epilepsy. Alteration of gap junction and hemichannel (Cx43) activity (5) in several models of neurodegeneration might reduce the capacity for ions to be buffered or result in the release of molecules that induce neuronal toxicity. Reduced lactic acid transport via MCT1 (6) and reduced glucose uptake via GLUT1 (7) might result in neuronal hypometabolism. Release of pro-inflammatory cytokines from astrocytes (8) induces neuroinflammatory cascades in astrocytes and neurons. mGluR, metabotrophic glutamate receptor; P2, ATP receptor.

disease mechanisms and for aiding the development of therapeutic approaches.

Chemogenetic tools. Chemogenetics involves activation of receptors called designer receptors exclusively activated by designer drugs (DREADDs), which are modified G protein-coupled receptors that are activated by agonists with no endogenous targets[22,23]. DREADDs can be excitatory Gq receptors (such as hM3Dq, a modified form of the human M3 muscarinic receptor) or inhibitory Gi receptors (such as hM4Di, a modified form of the human M4 muscarinic receptor), and are expressed in specific cell types, such as astrocytes, through the introduction of astrocyte-promoter-driven viral vectors in a certain region of the CNS or the use of Cre-dependent expression in transgenic mice[24]. These DREADDs can be activated with the selective agonist clozapine *N*-oxide (CNO); CNO is a derivative of the antipsychotic drug clozapine, which is biologically inert in rodents[23]. CNO can cross the blood–brain barrier and so can be delivered through intraperitoneal and oral routes in food and water[25].

When treated with CNO, transgenic mice that express hM3Dq under the control of the GFAP promoter (GFAP–hM3Dq) exhibit changes in parameters that are regulated by the autonomic nervous system, such as increased heart rate and blood pressure and decreased temperature, providing evidence that astrocytes have a role in these important physiological functions[26]. Astrocyte activation via GFAP–hM3Dq in the nucleus accumbens results in release of glutamate and inhibition of cocaine-seeking behaviour in rats[27], making astrocyte-specific Gq DREADDs potential pharmacological targets without off-target effects.

CNO-mediated activation or inhibition of DREADDs is achieved in about 30 minutes and can last up to 2 hours, so this approach is suitable for long-term manipulation of glial cells or neurons. A newly developed DREADD receptor, κ-opioid-derived DREADD (KORD), is coupled to the inhibitory Gi signalling pathway and activated by the selective agonist salvinorin B (SALB)[28]; SALB acts faster than CNO and is cleared from the body more quickly. Therefore, CNO-based and SALB-based DREADDs provide tools to explore the effects of acute and chronic activation of astrocytes.

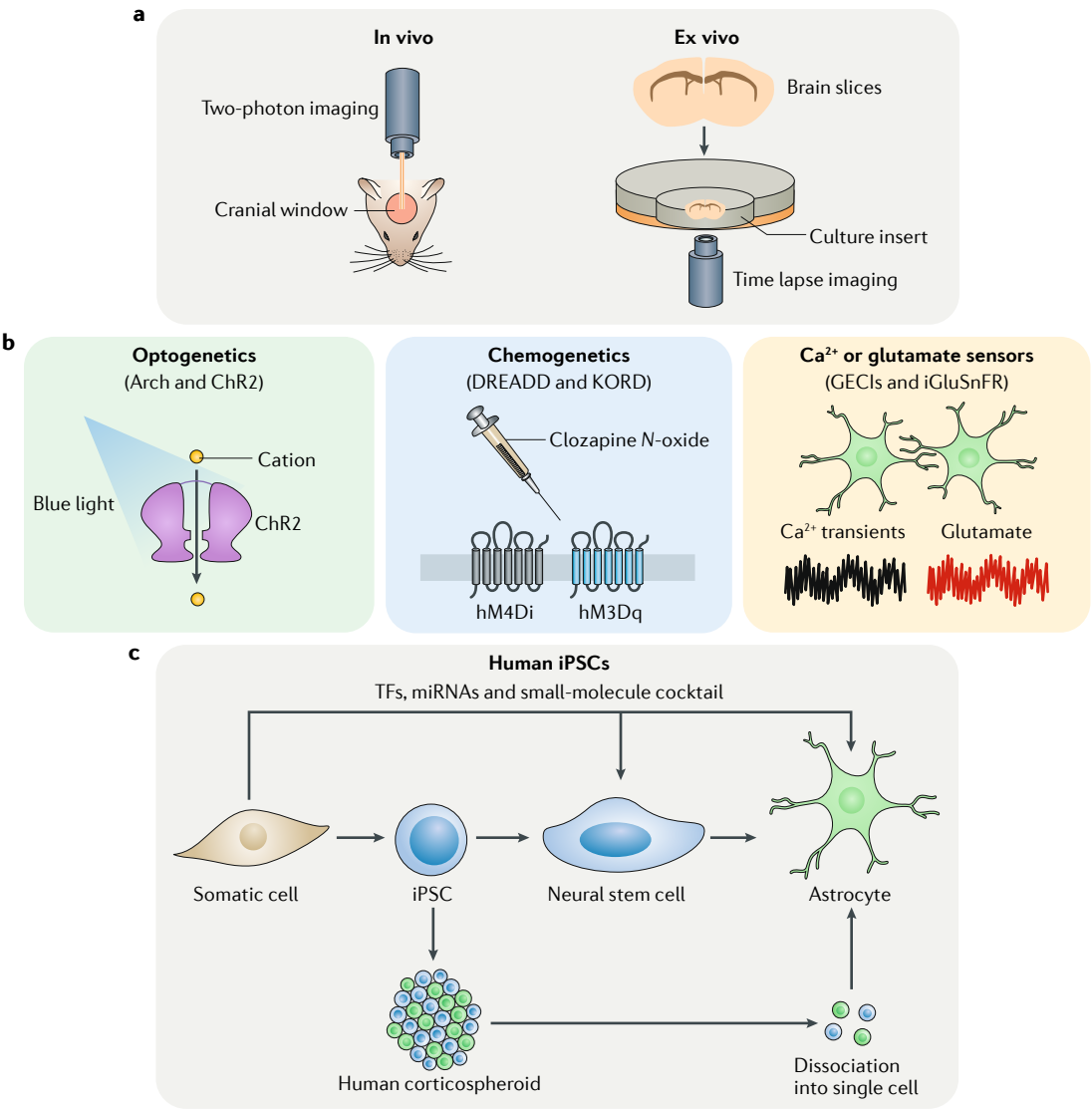

Fig. 2 | **Tools for studying astrocytes in health and disease. a** | Tools have been developed to explore and study the properties of astrocytes using in vivo, ex vivo and in vitro systems. Transgenic mice that express fluorescent proteins under the control of an astrocyte-specific promoter or in which dye loading has been used to label astrocytes can be used for in vivo imaging through a cranial window. Live cell imaging of ex vivo and in vitro (rodent and human) cultures enables examination of morphological changes in pathological conditions. **b** | Optogenetics and chemogenetics enable manipulation of astrocytes via control of light-activated channels, such as archaerhodopsin (Arch) and channelrhodopsin 2 (ChR2) and via chemically activated designer receptors exclusively activated by designer drugs (DREADDs), such as κ-opioid-derived DREADD (KORD), which can be introduced using transgenic methodologies or viral vectors. Functional measurements in astrocytes can be conducted with the use of sensors to observe changes in glutamate, such as intensity-based glutamate-sensing and fluorescent reporter (iGluSnFR), and changes in calcium signals (via genetically encoded calcium indicators (GECIs)). **c** | Human astrocytes can be derived from somatic cells via several methods. Somatic cells can be converted into induced pluripotent stem cells (iPSCs) then driven towards a neural stem cell fate and finally differentiated into astrocytes. Fibroblasts can also be converted directly into neural stem cells to form astrocytes or directly into induced astrocytes. Finally, after reprogramming of somatic cells to form iPSCs, these cells can be driven to form human corticospheroids, in which a combination of astrocytes and neurons form a circuit. The tools discussed above can also be used to examine human iPSC-derived astrocytes. CNO, clozapine *N*-oxide; hM3Dq, modified human M3 muscarinic receptor; hM4Di, modified human M4 muscarinic receptor; miRNA, microRNA; TFs, transcription factors.

Measurement of calcium dynamics. Calcium dynamics in astrocytes are complex, and intracellular calcium signalling is a major pathway that drives astrocyte excitability and communication with the surrounding milieu. Organic membrane-permeable dyes, such as Fluo-4, Fura-2, Oregon Green and BAPTA-1-AM, have been used for bulk loading of astrocytes to study calcium signalling, but these dyes are inadequately taken up in tissues and cannot be used to detect calcium signals in the finer distal processes of astrocytes[12,13]. Technological

advances such as genetically encoded calcium indicators (GECIs) now enable imaging of calcium transients in different parts of astrocytes, including the soma and thin distal astrocyte processes called microdomains[29]. These GECIs are particularly valuable for studies in vivo and in brain slices. GCaMP3, GCaMP6s and GCaMP6f are GECIs targeted to the cytosolic compartment of astrocytes, and Lck-GCaMP3 is a membrane-targeted GECI widely used in astrocytes[20,29,30]. Use of GECIs has also demonstrated neuronal regulation through astrocytic calcium responses[12,31]. Furthermore, the same technique has shown that, although the soma of the astrocyte remains relatively inactive unless a large wave of neuronal stimulation occurs, dynamic calcium transients occur in the proximal and distal processes of astrocytes that receive information from dendrites and axons[12,31].

3D calcium imaging of astrocytes with the use of GECIs has captured the local calcium transients and heterogeneous signals that occur in different subregions of the astrocyte and cannot be completely captured with 2D imaging; these experiments have shown that ~5.1% of transients occur in the cell body, ~85% occur in the processes and ~9.7% occur in the endfeet[32]. In addition, 3D imaging has shown that the astrocytic calcium responses measured in awake animals are stronger than in anaesthetized animals. Although in vivo astrocytic calcium responses are similar to those observed in slice cultures, calcium transients in vivo occur at a higher frequency.

Use of GECIs provides an understanding of the contribution of individual cells in a circuit rather than detecting a global increase in intracellular calcium levels. In the context of neurological diseases, intracellular calcium signalling is a ubiquitous factor in neuronal cell death, and GECIs allow the contribution of astrocyte calcium pathways to disease pathology to be tested.

Development of glutamate sensors. Measurement of glutamate in CNS tissue has typically been conducted with microdialysis using freshly prepared samples from the brain and/or spinal cord. These methods have gradually evolved to the use of optical sensors: glutamate biosensors coupled with fluorescent indicators[33]. In 2013, an ingenious method called intensity-based glutamate-sensing fluorescent reporter (iGluSnFR) was published[34]. This method uses a single-wavelength glutamate sensor to measure the intensity of glutamate responses in neurons and astrocytes and provides a considerably better signal-to-noise ratio than previous methods. The iGluSnFR technique enables detection of small and rapid changes in glutamate levels in astrocytes in cell culture and slice culture models, but more importantly, noninvasive viral vector delivery of the sensor enables tracking of in vivo glutamate dynamics. The role of astrocytes in the context of physiological functions, such as maintenance of the circadian rhythm[35], can therefore be examined with this technique.

Similar sensors have been developed to measure other second messengers and gliotransmitters released from astrocytes. These sensors include Pink Flamindo cAMP indicators[36] and the potassium-sensitive fluorescent indicator Asante Potassium Green 1 (REF.[37]), which can be used for in vitro and in vivo studies. Glutamate dysregulation is key in neurological diseases, such as amyotrophic lateral sclerosis (ALS), Huntington disease, Alzheimer disease (AD) and epilepsy[38], and the tools discussed will help with directly testing the relevance of this mechanism to astrocyte-mediated disease.

Human astrocytes for disease modelling. Exploration of neurological diseases through human stem cell modelling might help to bridge the translational gap between preclinical studies in rodent models and human clinical trials. Transgenic mice enable investigation of familial genetic diseases, but the technology for reprogramming human somatic cells into pluripotent stem cells has enabled modelling of adult-onset and childhood diseases that have no obvious genetic cause. Similarly, animal models are used extensively to validate therapeutic targets, but confirmation of cellular and molecular mechanisms in human cells could increase the probability of successful target validation and drug development. A plethora of differences exists between rodent and human astrocytes, ranging from morphology, function and molecular signature to the process of astrogliosis under conditions of stress, ageing or disease (BOX 1). Furthermore, human astrocytes cultured in vitro are relatively immature and have different morphological and molecular characteristics to astrocytes in vivo[39]. To better understand the cellular interactions of human iPSC-based astrocyte–neuron cultures, the introduction of methods such as high-content image acquisition[40] provides a platform for longitudinal data collection and rapid analysis of appropriate pathways and molecular targets before committing to clinical trials with therapies directed towards astrocytes.

Pioneering studies led by Shinya Yamanaka[41] demonstrated that somatic cells can be reprogrammed to create iPSCs, a process that has redefined modern studies of disease. Several laboratories have developed protocols for directing iPSCs towards a neural lineage and differentiating them further into a neuronal or astrocytic fate; the most common method for generating astrocytes is inhibition of small mothers against decapentaplegic (SMAD)–transforming growth factor-β (TGFβ) signalling[42]. Following the use of iPSCs to generate neurons and astrocytes, a technique was developed that enables direct conversion of fibroblasts into induced neuronal progenitor cells (iNPCs) and then induced astrocytes[43]. This method bypasses the iPSC stage to generate astrocytes efficiently, with relative ease and in a considerably shorter time frame. Subsequently, several new methods have been reported that bypass even the intermediate iNPC stage and involve direct conversion of fibroblasts into functional astrocytes and neurons through overexpression of transcription factors[44], application of small-molecule cocktails[45] or overexpression of microRNAs[46] (FIG. 2).

Most human iPSC astrocyte cultures are 2D and, on the basis of their cellular morphology, gene expression and function, seem to be reactive and not ideal for long-term studies. However, a 3D method of growing human cortical spheroids (hCSs) that leads to spontaneous generation of astrocytes as part of a cortical neuronal

Microdialysis
A minimally invasive method of sampling in vivo concentrations of various analytes (neurotransmitters, peptides, glutamate, etc.) in the brain and spinal cord using a dialysis probe.

Single-wavelength glutamate sensor
A fluorescent sensor based on a circularly permuted single fluorophore rather than Förster resonance energy transfer (FRET), which is based on ratiometric measurements at two different wavelengths.

Human cortical spheroids
(hCSs). 3D cultures that produce laminated cerebral cortex-like structures that include astrocytes as part of a cortical neuronal circuit.

Box 1 | **Human and mouse astrocytes**

Rodent models have been pivotal in gaining insight into the fundamental roles of astrocytes and in dissecting the disease mechanisms that involve astrocytes because of the relative ease of manipulating these models. However, studies in which human cells have been used have shed light on the structural and functional differences between rodent and human astrocytes. Human astrocytes have a diameter approximately threefold greater than that of rodent astrocytes, have twice as many branches[121] and exhibit greater structural complexity, giving rise to more astrocyte subclasses[122,123]. The vast arborization of human astrocyte processes means that one domain connects with 2×10^6 synapses compared with 1.2×10^5 synapses for rodent astrocytes. Engraftment of human astrocyte precursors into mouse forebrains improved learning and memory in these animals, possibly indicating faster propagation of calcium signals in human astrocytes than in rodent astrocytes[124]. These differences are noteworthy when designing mouse studies of human diseases and for confirming maturation of astrocytes derived from human stem cells.

In a study published in 2016 (REF.[121]), human fetal and adult astrocytes were purified in serum-free conditions and compared with rodent astrocytes under similar conditions. Observations were similar to those in vivo: greater structural complexity and faster calcium dynamics in human astrocytes. The most striking finding, however, was that age-dependent transcriptional profiles were specific to human astrocytes. Although 52% of genes enriched in mouse astrocytes were enriched in human astrocytes, only 30% of genes enriched in human astrocytes were enriched in mouse astrocytes. Furthermore, fetal and adult human astrocytes differ substantially in their proliferative and phagocytic abilities, an important distinction when thinking about neurodevelopmental and adult neurodegenerative diseases[48]. A better understanding of the human transcriptome will provide tangible cellular targets for neurological diseases in the future and help in interpreting preclinical observations as they transition to human therapeutic design.

circuit has been developed[47] and can be used for disease modelling. Use of this model and RNA sequencing analysis of astrocytes in hCSs over 590 days has demonstrated a temporal conversion from fetal astrocytes to adult astrocytes[48]. During this long-term monitoring of astrocytes, immature, intermediate and mature astrocyte pools were identified; functional maturity varied between the groups, recapitulating features of post-mortem human astrocytes.

On the basis of such findings, hCSs seem to have great potential for studying astrocyte-driven molecular mechanisms in the development of neuronal circuitry and aberrant astrocyte functions in disease. However, 20 months are required to grow the cultures, which makes this approach impractical from a drug discovery point of view.

Human iPSCs, therefore, hold the promise of exploring novel roles of astrocytes during human development and in neurodegenerative conditions, but this potential is accompanied by some cautionary tales. For example, variability between different iPSC lines and clonal variations can be large, gene-corrected isogenic lines need to be used to study familial disease, epigenetic influences can be lost during reprogramming of cells, age-associated factors might be lost when modelling adult neurodegenerative diseases and the functional maturity of cultures must be appropriate for the investigation; detailed consideration of each of these pitfalls is available elsewhere[42,49,50].

Studying astrocytes in neurological disease

The tools discussed above all have the potential to provide new insight into the roles of astrocytes in specific neurological disorders. In the remainder of this Review, we discuss several neurological disorders in which astrocytes play a role in disease pathogenesis (FIG. 1; TABLE 1) and highlight examples of how instruments in this toolbox have been and could be applied to improve our understanding of these roles in specific conditions. Notably, human iPSC-derived astrocytes are increasingly being used to study each of these disorders. Given that many of the methods described above are relatively new and rapidly evolving, we anticipate an increase in the use of in vivo imaging, optogenetics, chemogenetics and metabolite sensors to appreciate astrocyte function in the context of neurological disorders.

Alexander disease. Alexander disease is a neurodevelopmental disorder that occurs in infantile, juvenile and adult forms[51]. Young patients have seizures, spasticity and developmental delay, whereas adult-onset disease can include palatal myoclonus, dysphagia and dysarthria. Imaging studies have identified frontal lobe leukodystrophy in early-onset disease, and the brainstem and cervical spinal cord are often affected in late-onset disease[52].

Alexander disease results from mutations in *GFAP* that lead to a toxic gain of function[53]. Reactive astrocytosis, which is defined by upregulation of *GFAP*, is the most documented pathological observation in astrocytes in neurodegenerative diseases, so the effects of *GFAP* mutations in Alexander disease could provide insight into how upregulation of *GFAP* confers pathology. Evidence suggests that mutations in *GFAP* result in slowing of normal polymer formation, leading to large soluble GFAP protein oligomers[54,55]. Astrocytes respond by further increasing *GFAP* expression, leading to GFAP-induced astrocyte toxicity[56]. Indeed, overexpression of wild-type GFAP in mice results in death a few weeks after birth, and these mice exhibit Rosenthal fibres, which are the pathological hallmark of Alexander disease[57]. Possible ways in which these fibres lead to toxicity include proteasome inhibition, activation of c-JUN N-terminal kinase (JNK)-dependent pathways and mislocalization of TAR DNA-binding protein 43 to the cytoplasm[56,58].

The use of human iPSCs has the potential to provide insight into the pathological mechanisms related to *GFAP* mutations and *GFAP* overexpression and help with the development of therapeutic approaches. The formation of Rosenthal fibres, which are observed in human post-mortem tissue from patients with

Table 1 | **Molecular targets in astrocytes that are altered in neurological disease**

Function of target	Proposed change in function in disease	Molecular target	Disease
Growth factors	Loss of function	IGF1	Rett syndrome[71]
		BDNF	HD[126]
		CCL5	HD[127]
		NGF	ALS[128]
Metabolic regulation	Loss of function	GLUT1 and MCT1	AD[112,113] and ALS[128]
		Energy metabolism	AD[129]
		Mitochondrial dysfunction	HD[130], ALS[131] and Rett syndrome[68]
Homeostatic function	Gain of function	Connexins, gap junctions and hemichannels	Rett syndrome[64], AD[25,115,132], ALS[95] and HD[133]
		AQP4	Epilepsy[134]
	Loss of function	Kir4.1	Rett syndrome[70], HD[135] and epilepsy[134]
Cytoskeleton	Loss of function	Microtubules	Rett syndrome[69]
Glutamate receptor	Gain of function	Metabotropic glutamate receptors	ALS[136] and AD[112]
Glutamate transporter	Loss of function	GLT1	ALS[137], HD[138], AD[139] and PD[140]
Signalling pathways	Gain of function	Calcium signalling	AD[109] and ALS[95]
		Purinergic signalling	HD[141] and AD[142]
		JAK–STAT3	AD[114,143], PD[144] and ALS[145]
	Loss of function	MAPK	AD[101]
		TGFβ	HD[146]
		Cholesterol production	ALS[147], HD[106] and AD[106]
Oxidative stress	Loss of function	NRF2	HD, PD, AD and ALS[148]
Inflammatory pathway	Gain of function	TNF	AD[149] and ALS[150]
		NF-κB	ALS[151] and HD[152]
		IFNγ	ALS[153] and PD[154]

AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; AQP4, aquaporin 4; BDNF, brain-derived neurotrophic factor; CCL5, CC-chemokine ligand 5; GLUT1, glucose transporter type 1; HD, Huntington disease; IGF1, insulin-like growth factor 1; JAK, Janus kinase; Kir4.1, ATP-dependent inwardly rectifying potassium channel Kir4.1; MAPK, mitogen-activated protein kinase; MCT1, monocarboxylate transporter 1; NF-κB, nuclear factor-κB; NGF, β-nerve growth factor; NRF2, nuclear factor erythroid 2-related factor 2 (also known as NFE2L2); PD, Parkinson disease; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.

Alexander disease as well as in the mouse model of this disease, has been recapitulated in iPSCs that were derived from patients with Alexander disease and driven towards an astrocytic fate[59]. This preservation of the disease phenotype of GFAP aggregates, along with elevations in pro-inflammatory cytokines, gives human iPSCs potential as a platform for drug screening and therapeutic development. Given that this disorder appears to be related to a GFAP dosing effect, therapeutic strategies in which *GFAP* is downregulated could yield treatment opportunities.

Rett syndrome. Rett syndrome is a neurodevelopmental disorder caused by mutations in the X-linked methyl CpG-binding protein 2 (*MECP2*) gene with clinical features of respiratory abnormalities, severe autism, reduced brain size and mental retardation[60]. Transgenic mouse and rat models of Rett syndrome recapitulate key features of the disease, including respiratory deficits and behavioural abnormalities[61,62].

MECP2 deficiency in neurons alone results in brain dysfunction and loss of neurons, but studies indicate that astrocytes contribute to Rett syndrome in cell-autonomous and non-cell-autonomous ways. Astrocytes isolated from *Mecp2*-knockout mice exhibit markers of reactive astrogliosis, abnormal glutamate clearance[63], brain-derived neurotrophic factor (BDNF) and cytokine dysregulation[64], reduced calcium responses and deficits in CO_2 and pH sensitivity, which are key for regulating respiratory CO_2 chemosensitivity[65]. In a co-culture model, MECP2-deficient astrocytes exert a non-cell-autonomous effect on neurons that leads to stunted dendritic arborization[64,66]. Reconstitution of *Mecp2* into astrocytes in global *Mecp2*-knockout mice ameliorates Rett syndrome phenotypes, restores locomotion and breathing function, reduces anxiety and prolongs lifespan[67]. Emerging cellular targets for astrocyte-targeted therapy in Rett syndrome include connexin 43 (Cx43)-related gap junctions[64], mitochondrial alterations and redox

imbalance[68], microtubule-dependent vesicle transport[69] and ATP-dependent inwardly rectifying Kir4.1 potassium channels[70].

The use of human iPSC-derived astrocytes has already had an important effect on the development of therapeutics for Rett syndrome. This approach has been used to confirm data obtained in rodents and to show that the effects of astrocytes derived from patients with Rett syndrome on neuronal morphology and function can be rescued by treatment with insulin-like growth factor 1 (IGF1). This evidence in human iPSC-derived astrocytes provides support for the rationale behind an ongoing clinical trial of IGF1 for treatment of Rett syndrome[71].

Epilepsy. In epilepsy, ictal activity occurs over seconds to minutes, but chronic epilepsy can result in secondary clinical phenomena, including psychiatric disorders and cognitive changes. Investigation of astrocytic contributions to epilepsy, therefore, needs to address acute ictal activity and chronic sequelae, which are particularly evident neuropathologically. Acute epilepsy can be modelled in vitro and in vivo with mouse models. The study of human epilepsy, however, has primarily been confined to pathological analysis of tissue after surgical excision of epileptogenic regions[72]. Whether the pathological changes observed in this tissue, which are usually the result of long histories of epilepsy, reflect the acute process is open to debate.

The known functions of astrocytes in homeostasis — which include potassium buffering[73], glutamate uptake to prevent neurotoxicity[74], and the delivery of energy substrates[75] — make dysfunction in one or many of these pathways a plausible promoter of hyperexcitability that could lead to epileptic activity. As noted above, astrocytic potassium channels have a key role in homeostasis, and several lines of evidence indicate that loss of Kir4.1 channel function contributes to seizure susceptibility and epileptogenesis[76–78]. Other potassium channel variants have been associated with childhood absence and juvenile myoclonic epilepsy[79,80]. Similarly, loss of aquaporin 4 function is thought to disrupt homeostasis by limiting control of fluid homeostasis in the brain and slowing the decay of extracellular potassium after seizures[81]. Finally, removal of glutamate from the synaptic cleft by astrocytic transporters is well described, and failure of this transport can lead to epileptogenesis. Genetic knockout of the glutamate transporter excitatory amino acid transporter 2 (commonly known as GLT1 in mouse models) results in seizure activity, hippocampal pathology and death[74]. Furthermore, gap junctions that regulate astrocyte coupling are altered in epilepsy and might disrupt ion balance[73,82]. The importance of astrocytes in synaptic maintenance and communication means that other similar mechanisms could contribute to hyperexcitability[83].

A combination of the latest tools for studying astrocytes and modulating their function has provided new insight into the role of these cells in epilepsy. Two-photon imaging, genetically encoded pH reporters and single-cell electrophysiological recordings in a hippocampal slice model of epilepsy have been used in combination to demonstrate that astrocytes undergo rapid alkalinization during seizure-like activity and that this alkalinization is in contrast with the acidification that occurs in neighbouring neurons[84]. These observations suggest that astrocyte-specific and neuron-specific changes in pH occur during seizure activity and that astrocytes regulate network activity under normal and pathological conditions[84]. Optogenetic control of astrocytic depolarization and hyperpolarization has been attempted experimentally in brain slices. Use of depolarizing actuators, such as ChR2, permits sodium, hydrogen and calcium to enter the cell, resulting in depolarization of membrane potential and the release of gliotransmitters, including ATP[17,85]. One study showed that activation of the hyperpolarizing actuator Arch reduced extracellular glutamate, an effect that could be used to reduce glutamate-mediated hyperexcitability of nearby neurons[21]. The ability to optogenetically manipulate astrocytes by altering membrane potential and the subsequent release of factors that modulate neuronal activity could be used as a therapeutic approach to epilepsy. Development of such a therapeutic approach would require the development of systems to exploit promoters that are specific to astrocyte subtypes, improved properties of actuators to reduce damage to brain tissue from the illuminating light, and studies of optogenetics in non-human primates[86].

Amyotrophic lateral sclerosis. Beyond the morphological and pathological changes described in the brains and spinal cords of patients with ALS, several lines of evidence demonstrate astrocyte involvement in disease pathogenesis. A contribution of astrocytes to ALS was first demonstrated by an association of the disease with loss of GLT1 (REFS[87,88]). Since that study, astrocyte contributions to disease, which are mostly thought to relate to progression rather than onset, have been demonstrated in various models.

Most animal modelling of ALS has been done with transgenic mice that overexpress the human mutant superoxide dismutase 1 (SOD1) protein. Selective deletion of mutant human *SOD1* in astrocytes ameliorates disease in these mice, suggesting that *SOD1* mutations in astrocytes contribute to disease[89,90]. Transplantation of astrocytes that were derived from glial-restricted progenitors and expressed mutant SOD1 into the spinal cords of wild-type rats also resulted in focal death and dysfunction of wild-type neurons[91].

Investigation of the mechanisms behind astrocyte toxicity in ALS by use of an in vitro co-culture system has shown that motor neuron death can be induced by the presence of astrocytes that express mutant *SOD1* and that the toxicity is related to a diffusible factor[92]. The same technique has been used with autopsy-derived human neural precursor cell (NPC)-derived astrocytes isolated from the lumbar spinal cords of patients with sporadic or familial ALS[93]. Results from this study suggested that silencing of *SOD1* in human NPC-derived astrocytes attenuated motor neuron cell death, even in patients with sporadic ALS. Another study in which autopsy-derived human astrocytes from the motor cortex and spinal cord were used showed that astrocytes from patients with sporadic or familial ALS were toxic to motor neurons,

but this phenomenon did not seem to be mediated by SOD1 and was instead related to caspase-independent programmed cell death (necroptosis)[94].

Of the latest tools for studying astrocytes in disease, human iPSC-derived models might have the greatest capacity to further our knowledge of ALS-relevant mechanisms by enabling the study of the genetic and clinical phenotypic heterogeneity found in this disease. Transplantation of human iPSC-derived astrocytes from patients with sporadic ALS into the spinal cords of rats has reproduced the results seen with SOD1-mutant glial progenitor cells in rodents: the presence of the astrocytes led to death and dysfunction of the nearby healthy neurons[95]. Various mechanisms for this toxicity have been proposed[96].

The use of human iPSCs has, therefore, confirmed findings from in vitro and rodent models and, together with the fact that many of the pathways identified (TABLE 1) correlate with findings in human brain and spinal cord tissue samples, has cemented astrocyte-mediated toxicity as a factor in ALS propagation. This knowledge presents several astrocyte-relevant therapeutic targets, and other novel tools discussed above will help to tease out the underlying astrocyte-mediated mechanisms in ALS. An existing example of this potential is a study of in vivo calcium dynamics with GECIs. In vitro measurements of calcium dynamics in SOD1-G93A astrocytes showed that these cells exhibited elevated calcium levels[95,97], and the in vivo study confirmed this observation in live mice through imaging of astrocytes that express the GECI GCamP3 (REF.[98]).

Alzheimer disease. AD is the most common form of dementia and is characterized by the deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles, dysfunction of synapses, loss of neurons, and neuroinflammation that involves reactive astrocytes and microglia. With ageing, astrocytes normally start to become senescent and, especially upon exposure to Aβ, develop a senescence-associated secretory phenotype with elevated levels of the cytokines IL-6 and matrix metalloproteinase 1 (MMP1)[99]. Reactive astrogliosis, marked by the hypertrophy of astrocytic processes and upregulation of GFAP, is a hallmark of AD, especially close to Aβ plaques and tau aggregates[100], although astrocyte atrophy also occurs further from the plaques[101]. Astrocytes can take up and degrade Aβ plaques via apolipoprotein E (APOE) receptors[102], scavenger receptor class B member 1 (REF.[103]) and age-dependent upregulation of complement component subunit 1q (C1q)-mediated clearance[104]. Over time, however, astrocytes start producing Aβ plaques owing to upregulation of β-secretase 1 (REF.[105]) and undergo transcriptional changes that subsequently alter signalling pathways such as the Janus kinase (JAK)–signal transducer and activator of transcription (STAT), IGF1 and mitogen-activated protein kinase (MAPK) pathways[101,106]. The latest tools for studying astrocytes have contributed multiple insights into the roles of these cells in AD[107].

Exposure of in vitro astrocyte cultures to Aβ oligomers leads to increased intracellular calcium concentration and an increased frequency of calcium transients[108]. In vivo time-lapse imaging of calcium dynamics in the APP/PS1 mouse model of AD has also shown that astrocytes have higher basal intracellular calcium levels and exhibit spontaneous calcium transients, particularly in astrocytes that surround Aβ plaques[109]. The astrocytic calcium signals observed were independent of neuronal activity and led to propagation of intracellular calcium waves in AD model mice that was not seen in wild-type mice. These data imply that astrocytes in AD mice exhibit a cell-autonomous effect, leading to elevated intracellular calcium activity.

Excitotoxicity-mediated neuronal death is a key feature of AD and is attributed mainly to increased extracellular levels of glutamate, loss of GLT1 (REF.[110]) and release of GABA through astrocytic bestrophin receptors[111]. Use of the glutamate sensor iGluSnFR has confirmed that clearance of glutamate in the areas surrounding Aβ plaques is impaired[107], which results from loss of GLT1 in astrocytes in the APP/PS1 model of AD[110]. Treatment with ceftriaxone upregulates GLT1, and the use of in vivo imaging of glutamate with iGluSnFR has enabled the consequent dynamic increase in glutamate transport to be observed[107]. Astrocyte-mediated metabolic coupling and neurovascular support are also compromised in AD owing to impaired function of glucose transporter type 1 (GLUT1; also known as SLC2A1) and monocarboxylate transporter 1 (MCT1; also known as SLC16A1) transporters, and these deficiencies have the potential to reduce metabolic support for neurons[112,113]. The development of novel sensors similar to glutamate sensors for detecting other metabolites, including lactate, and glucose will enable real-time monitoring of astrocytes at a functional level.

A variety of functions that involve astrocytic receptors are implicated in AD, so chemogenetic and/or optogenetic techniques have the potential to modulate the function of these receptors in disease. One existing example is the chemogenetic manipulation of the astrocytic Gs-coupled adenosine receptor A_{2A}. In post-mortem tissue from patients with AD, expression of this receptor is increased in astrocytes, and this receptor has also been implicated in the regulation of cognition. A chemogenetic approach to manipulating this receptor in mice has shown that activation of A_{2A} results in long-term memory loss[114]. Other astrocytic receptors involved in various pathways are candidates for similar manipulation. Mounting evidence indicates that gap junctions and hemichannels (composed of connexin proteins), as well as the activity of other channels, enable excitatory molecules, including glutamate, ATP and calcium, to pass through astrocyte networks or to be released into the extracellular space adjacent to neurons[112,115]. In addition, the receptor-mediated interplay between astrocytes and microglia escalates ongoing neuroinflammation in AD via engagement of inflammatory pathways that involve activation of the nuclear factor-κB (NF-κB) pathway, triggering receptor expressed on myeloid cells 2 (TREM2), clusterin and complement receptor type 1 (REF.[116]) and release of pro-inflammatory cytokines, including tumour necrosis factor (TNF), IL-1α, IL-1β and IL-6. In fact,

Box 2 | **Astrocyte heterogeneity in disease**

Astrocytes are a diverse population in the CNS, and the earliest attempts to classify the different types divided them into protoplasmic (grey matter) and fibrous (white matter) astrocytes on the basis of morphology and location[122]. Refinement by region gave rise to the naming of astrocytes such as Bergmann glia in the cerebellum, Müller glia in retinae, radial glia, ependymal glia, marginal glia, perivascular glia, velate glia and tanycytes[122].

Understanding astrocyte heterogeneity is an important step in deciphering the functions of specific astrocyte pools. Approaches for profiling astrocytes include regional isolation of astrocytes, fluorescence-activated cell sorting with astrocyte-specific reporter lines, a bacterial artificial chromosome-translating ribosome affinity purification approach and a single-cell RNA sequencing technique[125]. Investigation of molecular targets in precise subgroups of astrocytes in regions affected by disease will be helped by further definition of the astrocyte transcriptome[125].

A study published in 2017 led to the proposal that two types of reactive astrocytes can be identified in disease and injury: A1 astrocytes and A2 astrocytes. In this study, activated microglia induced the generation of toxic A1 astrocytes through release of IL-1α, tumour necrosis factor and the complement component subunit 1q. A1 astrocytes were unable to perform the normal astrocyte functions of promoting neuronal survival, synapse formation and phagocytosis of synapses and myelin debris. These early data suggest that A1 astrocytes have a role in neurodegenerative disease but that a proportion of trophic A2 reactive astrocytes can provide trophic support and induce synapse formation through release of thrombospondins, as seen in spinal cord injury and ischaemia[117]. Further studies will improve our understanding of astrocytes subtypes during development and disease and address the feasibility of manipulating these phenotypes to provide therapeutic opportunities.

classification of astrocytes as either A1 or A2 has been suggested on the basis of their reactive states and interactions with microglia[117] (BOX 2). Expression of the cytokines TNF, IL-1α and C1q in AD can give rise to pro-inflammatory A1 astrocytes[117] and consequently exacerbate the ongoing cognitive decline.

Finally, the availability of human stem cell-based models of AD now provides evidence that astrocytes in both sporadic and familial AD have cell-autonomous pathological phenotypes, including mislocalization of astrocyte markers, astrocyte atrophy and release of the inflammatory secretome[118]. In a human iPSC-based model of AD, derived from patients with *APP* mutations, accumulation and secretion of Aβ oligomers is observed in astrocytes[119], resulting in increased endoplasmic reticulum stress and production of reactive oxygen species in astrocytes[120]. Given the increasing availability of these human iPSC-based platforms, further evaluation of AD pathomechanisms and new approaches to AD drug discovery are likely to be forthcoming.

Conclusions

The neurological disorders discussed in this Review demonstrate the diversity of astrocyte-mediated contributions to neurodevelopmental disorders, disorders that result in acute physiological changes that manifest as seizures and the chronic processes observed in neurodegenerative diseases. Given that astrocytes have important functions in normal CNS pathophysiology, all neurological diseases are likely to have astrocyte-related pathology to varying degrees. The influences that these cells have on neural activity therefore offer an array of specific targets for therapeutic intervention that can complement other neuroprotective strategies. The variety of new tools available for studying astrocyte function discussed in this Review will aid the dissection of astrocytic contributions to normal CNS biology and disease propagation and will hopefully enable thorough analysis of potential therapeutic pathways that involve astrocytes.

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