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RESEARCH ARTICLE

Astrocytes: From the Physiology to the Disease

Laura Trujillo-Estrada[1], Angela Gomez-Arboledas[3,4,5], Stefan Forner[1], Alessandra Cadete Martini[1], Antonia Gutierrez[3,4,5], David Baglietto-Vargas[1,2,*], and Frank M. LaFerla[1,2*]

[1]*Institute for Memory Impairments and Neurological Disorders (UCI MIND), University of California, Irvine, CA 92697-4545, USA;* [2]*Department of Neurobiology and Behavior, University of California, Irvine, CA 92697, USA;* [3]*Department of Cell Biology, Genetic and Physiology, Faculty of Sciences, University of Malaga, Malaga, Spain;* [4]*Instituto de Investigación Biomédica de Malaga-IBIMA, Malaga, Spain;* [5]*Networking Research Center on Neurodegenerative Diseases (CIBERNED), Madrid, Spain*

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Abstract: Astrocytes are key cells for adequate brain formation and regulation of cerebral blood flow as well as for the maintenance of neuronal metabolism, neurotransmitter synthesis and exocytosis, and synaptic transmission. Many of these functions are intrinsically related to neurodegeneration, allowing refocusing on the role of astrocytes in physiological and neurodegenerative states. Indeed, emerging evidence in the field indicates that abnormalities in the astrocytic function are involved in the pathogenesis of multiple neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). In the present review, we highlight the physiological role of astrocytes in the CNS, including their communication with other cells in the brain. Furthermore, we discuss exciting findings and novel experimental approaches that elucidate the role of astrocytes in multiple neurological disorders.

Keywords: ??????, ???, ??????

1. INTRODUCTION

Astrocytes represent approximately 40% of the total cells in the brain and are the major cellular constituent of the mammalian central nervous system (CNS) [1]. Since the first description of these cells by Rudolf Virchow in 1856 and a later study by Michael von Lenhossek, who suggested that the star-shaped glial cells should be named spider cells or astrocytes, substantial progress in understanding the intrinsic role of astrocytes in the physiology of the CNS has been achieved (for review, see [2]). However, for a long time, the exclusive role of astrocytes was thought to be the provision of support to neurons, and reactive processes were considered to be secondary to neuronal injury. Recent technological advances in research tools (including the use of induced pluripotent stem cells (iPSC), chemogenetics, single-cell RNA sequencing (scRNA-seq) and optogenetics) have enabled scientists to discover new astrocytic pathways and molecular mechanisms and to achieve a better understanding of their critical functions and their dynamic role in the healthy brain and during disease progression (for review, see [3]).

*Address correspondence to these authors at the Department of Neurobiology and Behavior, 5120 Biological Sciences II, Irvine CA 92697-1450, USA; Tel: 949.824.5315; Fax: 949.824.7356; E-mail: laferla@uci.edu and Institute for Memory Impairment and Neurological Disorders (UCI MIND), 3400A Biological Sciences III, Irvine, CA 92697-4545, USA;
Tel: 949.824.3471; Fax: 949.824.7356; E-mail: d.baglietto@uci.edu

Some proposed critical functions of astrocytes include neurovascular coupling, stabilization of the blood-brain barrier (BBB) to maintain the CNS as an immune-privileged site, regulation of synaptic function (formation, elimination, maintenance and plasticity), ion homeostasis (for example, avoiding of extracellular K^+ overload), regulation of the concentration of neurotransmitters at synapses by participating in neurotransmitter clearance (protecting neurons against glutamate excitotoxicity), metabolic and neurotrophic support of neurons by influencing the structure and function of surrounding neurons, regulation of action potential waveforms, and regulation of specific behaviors such as sleep [4-12]. For these functions, a close molecular and morphological relationship between astrocytes and neurons is essential. This contact is made through the end feet of astrocyte. Moreover, astrocytes and neurons constitute the "tripartite synapse", in which presynaptic and postsynaptic terminals are in intimate association with surrounding astrocytes [13]. In addition, it is now well established that astrocytes also respond to all forms of injury, trauma, and infection via a process termed astrogliosis [9]. Under these pathological circumstances, the astrocytic response is referred to as "reactive" or "activated" astrocytes and is followed by important changes in their morphological and physiological features [14]. These changes may include the hypertrophy of cell bodies and processes [15], reorganization of the astrocyte's arborization with a change in the number of primary proc-

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esses [15] and polarization toward the site of injury [16], increased expression of glial fibrillary acidic protein (GFAP), vimentin, nestin, and chondroitin sulfate proteoglycans (CSPGs), changes in the rate of glutamate uptake, release of inflammatory cytokines, production of reactive oxygen species (ROS) and formation of glial scars [17].

A recently increased body of data shows that the effects of astrogliosis on neural tissue and its functions are not uniform. Thus, astrocyte function may vary depending on the context, indicating that astrogliosis may be an adaptive beneficial response to a deleterious process such as a neurodegenerative disease (ND) [12]. In this regard, there is a growing body of evidence for the concept of astrocytopathies in which the disruption of normal astrocyte functions, dysfunctional astrogliosis or astroglial degeneration is the primary cause of neurological dysfunction and disease [12]. The loss of the critical astroglial functions described above exacerbates the progression of various diseases, of which amyotrophic lateral sclerosis (ALS) or Alzheimer's disease (AD) are prominent examples. Although our understanding of astrocytes has increased considerably over the past two decades, we still know very little about how astrocytic pathways are involved in the progression and onset of these diseases. In this review, we summarize recent studies of astrocyte functions in the normal healthy brain. Furthermore, we highlight neurodegenerative diseases in which astrocyte function or dysfunction is believed to play a key role in disease onset or progression. We also discuss new technological methods that make it possible to dissect novel astrocyte functions during disease, and we provide an overview of future directions in research on astrocytes as a therapeutic target in the treatment of neurological disorders.

2. HETEROGENEITY OF ASTROCYTES AND CELL INTERACTION

Given the indicationof their functions, it is evident that astrocytes comprise a heterogenous group of cells [18]. Astrocytes have been divided into two generally recognized categories: fibrous astrocytes, which are present in white matter and express GFAP, and protoplasmic astrocytes of gray matter, which may express less GFAP [18]. However, emerging evidence indicates embryonic patterning as a principle in astrocyte organization, suggesting that distinct subtypes of these cells originate from separate domains in the developing spinal cord [19]. Three subtypes of fibrous astrocytes can be distinguished based on the expression of Reelin and Slit1, which are derived from progenitor domains expressing the homeodomain transcription factors Pax6 and Nkx6.1 (Table **1**) [19]. Moreover, Nkx6.1 is expressed by ventral astrocytes after their migration from the ventricular zone, and its ablation leads to abnormal specification and disorganized morphology of ventral astrocytes [20]. While investigating how astrocytes become restricted to particular regions of CNS, Tsai *et al.* [21] demonstrated that an appropriate domain-specific population of astrocytes is required to support correct synaptogenesis and/or synaptic maintenance; this cannot be achieved even when there is immigration of astrocytes from adjacent regions.

In addition to the traditional classification of astrocytes into two different populations and the more recent views on the developmental diversification of these cells, recent advances in imaging and genetic tools have contributed to elucidating the morphological and functional diversity of astrocytes (for review, see [22]). Since astroglial cells are involved in numerous and different functions in different regions of the brain, it is plausible to speculate that these functions are accomplished by different astroglial subpopulations. In regard to this, Lin and collaborators recently described five distinct astrocyte subtypes that expressed both GFAP and Aldehyde Dehydrogenase 1 Family Member 1 (ALDH1L1) with molecular and functional differences in the mouse brain. Their studies which were based on a novel fluorescence-activated cell shorting (FACS) approach, allowed them to identify a subset of astrocytes that showed an increased capacity to support synapse formation and another subtype that showed an upregulation of genes related to phagocytic functions, among others [23]. In recent years, numerous authors have suggested classifying astroglial subpopulations based on their activation phenotypes under pathological conditions such as neurodegenerative diseases [24]. As an example, Liddelow and colleagues suggested the division of astrocytes into two different phenotypes, A1 and A2, similar to the proposed M1 and M2 classification of macrophages. According to this scheme, A1 astrocytes upregulate the expression of numerous genes related to the complement inflammation signaling pathway and can be classified as "harmful" astrocytes. Because A2 astrocytes upregulate the expression of some neurotrophic factors, they could act as neuroprotective cells [24]. The studies demonstrated that neurotoxic astrocytes (A1) could be induced by activated neuroinflammatory microglia under pathological conditions, as a result losing their homeostatic functions and gaining new neurotoxic functions that lead to the death of both neurons and oligodendrocytes [24, 25]. The development of harmful astrocytes immediately following injury opens up a new scenario in which not only the homeostatic subpopulation of astroglia but also the phenotypic changes they undergo during disease need to be taken into consideration. Unraveling the functional and molecular characteristics of astrocyte populations throughout the different regions of the CNS would provide us with a better comprehension of their functions in both health and disease. Therefore, understanding the astroglial cellular heterogeneity will provide the critical knowledge necessary to further elucidate their roles in many physiological processes in the brain as well as their roles in multiple neurodegenerative disorders.

2.1. Astrocytes-neurons

The close relationship between astrocytes and neurons enables astrocytes to coordinate synaptic networks; such signaling occurs through interactions between signaling molecules and ligand receptors (Fig. **1**, Table **2**). In recent years, several mechanisms have been identified through which these cells might modulate synaptic transmission [26-28]. Astrocytes exhibit a number of properties that suggest that they modulate neuronal activity *in vivo*. These properties include their ability to 1) buffer extracellular potassium, 2) rapidly take up neurotransmitters following release at neuronal synapses, 3) release neurotransmitters and neuromodulators (including glutamate, ATP and D-serine), 4) release neurotrophic factors, and 5) regulate the volume of the ex-

Table 1. Classification of the astrocytes. This classification has been made based on literature studies [19, 23, 25] and described according to their phenotype characteristics.

Type	Phenotype
Fibrous astrocytes	Found in the white matter, with thin and straight processes
Protoplasmic astrocytes	Found in the grey matter, are short branched with thick processes
VA1	Pax6 expression in the absence of Nkx6.1 ($Reelin^+$, $Slit1^-$)
VA2	Co-expression of Pax6 and Nkx6.1 ($Reelin^+$, $Slit1^+$)
VA3	Nkx6.1 expression in the absence of Pax6 ($Slit1^+$, $Reelin^-$)
"A"	$GFP^+CD51^-CD71^-CD63^-$
"B"	$GFP^+CD51^+CD71^+CD63^-$
"C"	$GFP^+CD51^+CD71^-CD63^-$
"D"	$GFP^+CD51^-CD71^+CD63^-$
"E"	$GFP^+CD51^-CD71^-CD63^+$
A1	"Neuroinflammatory"; upregulated complement cascade components drive synapse destruction;
A2	"Ischemic"; upregulated neurotrophic factors

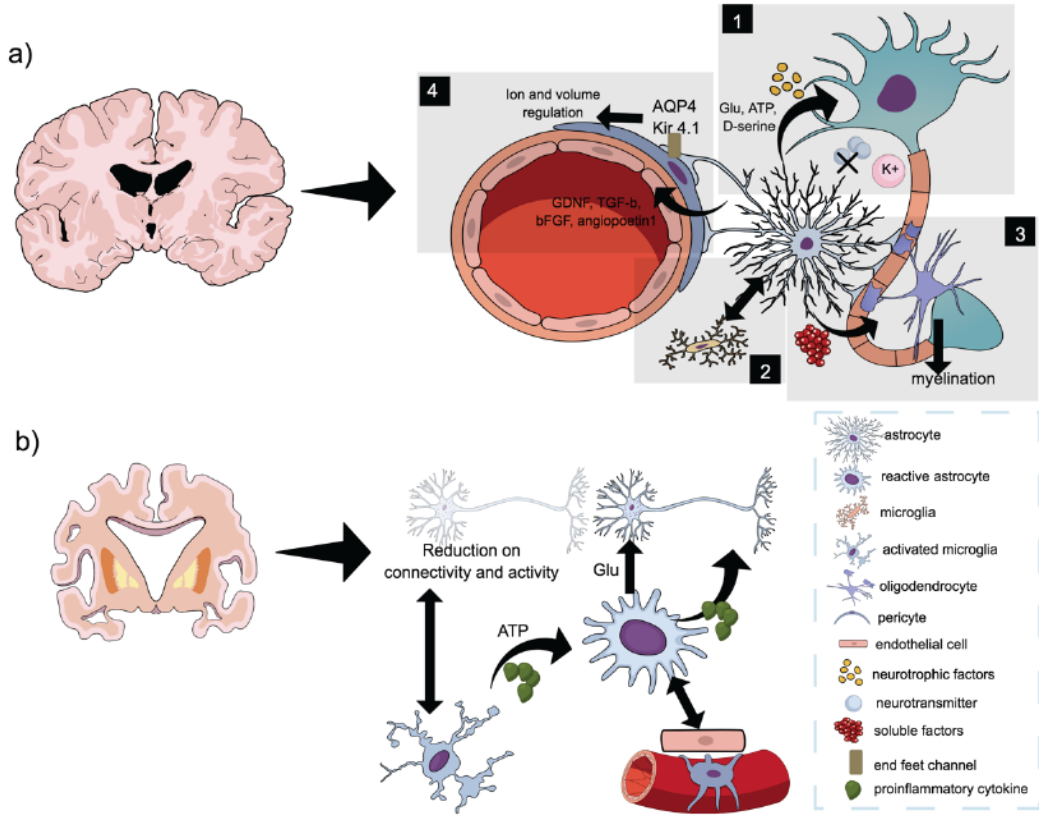

Fig. (1). Astrocytes in health and disease. a). In the healthy brain state, homeostatic and balanced relationships exist among neurons, astrocytes, oligodendrocytes, endothelial cells and microglia. Astrocytes remove neurotransmitters from synaptic space and release glutamate, ATP, D-serine and neurotrophic factors to support neurons (1). Astrocytes also communicate with microglia through a variety of signals (2). The secretion of soluble factors by astrocytes induces myelination by oligodendrocytes (3). Finally, astrocytes express various channels in their end feet that regulate ions and volume, and they also release GDNF, TGF-β, bFGF and angiopoetin 1 to stimulate the BBB (4). **b).** In a disease state such as a neurodegenerative disorder or trauma, there is damage to neurons, leading to their reduced outgrowth, activity and connectivity and causing microglia to become active. The microglia-activated signals (*e.g.,* cytokines) activate astrocytes, which then release glutamate and proinflammatory molecules. Moreover, there is a dysfunction of the BBB.

Table 2. Summary of astrocyte-cell interactions.

Cell Type	Interaction Mechanisms
Neurons	Buffer extracellular potassium; Take up of neurotransmitters following release at neuronal synapses; Release of neurotransmitters, neuromodulators and neurotrophic factors; Regulation of the volume of the extracellular space; Calcium signaling through gap junctions; LTP modulation and neuronal differentiation promoted by ephrins; Synaptic transmission facilitated by endocannabinoids.
Microglia	Activated microglia can secrete IL-1α, TNF and C1q, inducing the activation of A1 astrocytes; Synaptic pruning; Glutamatergic neurotransmission
Oligodendrocyte	Myelination promotion
Endothelial cells	Modulating the brain-blood barrier

tracellular space [29, 30]. Signaling molecules and ligand receptors can also interact in such a way as to facilitate the communication between astrocytes and neurons. These interactions can alter the physiology of both types of cells, thereby impacting many physiological processes, such as synaptic plasticity. Here, we summarize some of these interactions.

Astrocytes can communicate with neurons through calcium signals that propagate via gap junctions [31]. Astrocyte intracellular Ca^{+2} signals, which are referred to as Ca^{+2} fluctuations, may be spontaneous or generated by neurotransmitters and are related to both physiological function and neurological disorders [32, 33]. Recently, Otsu and collaborators [34] showed that activated olfactory sensory neuron (OSN) terminals may trigger Ca^{+2} increases in astrocyte processes but not in astrocyte somata. These Ca^{+2} fluctuations guide the onset of functional hyperemia, pointing to astrocytes as potential regulators of neurovascular coupling. Importantly, many studies with $IP_3R_2^{-/-}$ mice have demonstrated the role of these receptors in all Ca^{+2} signaling and the role of such signaling in neuronal and blood vessel function. On the other hand, Srinivasan and colleagues [35] demonstrated that these calcium fluctuations are not entirely dependent on IP_3R_2 receptors, as most of them occur in astrocyte processes. Moreover, it appears that there is a lack of effect on long-term potentiation (LTP) when astrocyte calcium signaling is impaired, especially through the IP_3 signaling pathway [36, 37]. Astrocyte Ca^{+2} fluctuations *in vivo* have two phases: an early component mediated by α1-adrenoceptors [38, 39] and a late component that is independent of α1-adrenoceptors and IP_3R_2-mediated signaling [35]. Notably, astrocytic calcium transients are slower than the neuronal calcium transients that underlie fast synaptic transmission [5].

It has been established that ephrins, by binding to their specific receptors, can control the spatial organization of cells by modulation of attractive and repulsive forces. Recently, ephrins have also gained attention for their role in the signaling between astrocytes and synapses during the LTP process [40]. Ephrin-A3 can be found in astrocytic processes, while its binding partner, EphA4, is expressed in neurons. LTP is attenuated in the CA1 region of hippocampus lacking ephrin-A3 or EphA4 [41] and elevated in transgenic mice whose astrocytes overexpress ephrin-A3 [42]. Moreover, ephrin-B2 from hippocampal astrocytes promotes the neuronal differentiation of adult neural stem cells (NSCs), also through cell-cell signaling [43].

Additionally, endocannabinoids released by neurons can activate CB1 receptors that are expressed in astrocytes, playing key roles in brain function and memory. The CB1 receptor promotes calcium elevation in astrocytes, thereby stimulating the glutamate release that ultimately activates presynaptic metabotropic glutamate receptors. Therefore, astrocyte signals evoked by cannabinoid release from neurons are directly involved in synaptic transmission [44]. Moreover, astrocytic CB1Rs, but not the receptors expressed by glutamatergic or GABAergic hippocampal neurons, are directly involved in endocannabinoid-induced synaptic depression and in the impairment of spatial working memory induced by cannabinoids, as shown by work with conditional transgenic mice that lack CB1R selectively in astrocytes, glutamatergic neurons or GABAergic neurons [45, 46].

2.2. Astrocytes-microglia

The other major glial cell type in the brain is the microglial cells, which are the brain's resident immune cells. Microglia exert a wide array of functions; in addition to their main role as surveillants of the brain parenchyma, they interact with synapses, playing an important physiological role in learning and memory [47, 48]. Although some of the long-term effects of microglial activation and inflammation are already known, the consequences of their interaction with astrocytes are still being explored. During the homeostatic stage (non-activated), microglia constantly extend and retract their dynamic processes while monitoring the environment, including synaptic astrocytes, presynaptic boutons and post-synaptic spines. When activated, microglial cells remove damaged synapses [48-50].

Astrocytes and microglia play a significant role in removing or "pruning" synaptic connections, a fundamental process of structural formation and elimination that controls and refines the connectivity of neuronal circuits [29, 51, 52] (Fig. **1**). Specifically, astrocytes can phagocytose synapses through the multiple EGF-like-domains 10 (MEGF10) and MER receptor tyrosine kinase (MERTK) phagocytic pathways; mice deficient in both pathways retained an excess of functional synapses with their primary targets [53]. Additionally, it has been demonstrated that A1 astrocytes, which display a harmful reactive state [24], can be induced by IL-1α, TNF and C1q secreted by activated microglia. These astrocytes lose most of the normal astrocytic functions but gain a new neurotoxic function and rapidly killing neurons and oligodendrocytes [24, 54].

In brain slices and cell cultures, microglia activated by LPS, a pro-inflammatory molecule, release ATP that stimulates astrocytes to release glutamate. Acting through mGluR5, glutamate then modulates neuronal activity, suggesting a possible role of microglia in excitatory neurotransmission [55]. Recently, the interaction of LPS-activated microglia with astrocyte connexins was also investigated. Abudara and collaborators [56] demonstrated that microglial stimulation with LPS promotes the release of two proinflammatory cytokines, interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α), which, in turn, activate astrocyte hemichannels, mainly through Cx43 hemichannel activity. Consequently, there is an increase in calcium levels in astrocytes and enhanced glutamate release associated with a reduction in excitatory synaptic activity in pyramidal neurons.

2.3. Astrocytes-oligodendrocytes

Oligodendrocytes and astrocytes originate from a common lineage of neuronal progenitor cells within the neuroectoderm [57]. In the CNS, myelination is performed by oligodendrocytes. The myelin sheath is a modified and extended glial plasma membrane that enwraps around the axons, enabling fast saltatory nerve conduction and maintaining axon integrity [58]. In the healthy adult brain, oligodendrocytes are generated continuously, and myelin is produced constantly throughout life [59].

It is well known that astrocytes support oligodendrocyte function (Fig. **1**). In 1984, Noble and Murray demonstrated for the first time the interaction between astrocytes and oligodendrocytes and its impact on myelination [60]. Several soluble factors secreted by astrocytes have been implicated in enhancing myelination. Astrocytes stimulate oligodendrocyte differentiation, supporting the concept of a positive effect of astrocytes in myelination [59, 61]. However, astrocytes may also have detrimental effects on oligodendrocyte differentiation, mostly within the glial scar. Astrocytes within the glial scar inhibited regeneration and negatively impacted remyelination [62-64].

Connexins are a family of proteins that create gap junctions, which are intercellular channels, and are responsible for coupling oligodendrocytes and astrocytes. Astrocytes express connexins 43 and 30, while oligodendrocytes express connexins 32 and 47. Astrocytes and oligodendrocytes can also have heterotypic gap junctions composed of connexins 47 and 43 and connexins 32-30 [65-68]. Genetic mutations in the genes encoding these connexins demonstrate their fundamental role in the proper function of myelin and oligodendrocytes.

In summary, astrocytes play an active role in promoting myelination by modulating the brain-blood barrier, regulating peripheral immune cell trafficking, and acting as a source of several factors such as chemokines and cytokines, thereby facilitating the interaction with oligodendrocytes.

2.4. Astrocytes-endothelial Cells

In the physiological state, the brain endothelium derives many of its properties from intimate interaction with astrocytes and neurons (Fig. **1**). It is well known that astrocytes play a highly important role in neurovascular coupling [69, 70]. The main interaction between astrocytes and endothelial cells occurs at the BBB, a selective barrier formed by brain endothelial cells that line the cerebral vasculature and form complex tight junctions. Given the complexity of BBB induction by glial cells, it is imperative to have communication between the endothelium and glial cells [70-72].

Analysis of the brain microvasculature demonstrates that the end feet of astrocytic glia form a lacework of thin lamellae that are closely attached to the surface of the endothelium. Nonetheless, the endothelial cells have a reciprocal active influence on astrocytes [72-74]. The perivascular end feet of astrocytes, which are closely attached to the microvessel wall, show several specialized features characteristic of this location, including a high density of orthogonal arrays of particles (OAPs) containing the water channel aquaporin 4 and the Kir4.1 K+ channel, channels that are involved in ion and volume regulation [73, 75-77].

Astrocytes secrete several chemical agents and many glial-derived factors, including glial-derived neurotrophic factor (GDNF), transforming growth factor-β (TGFβ), basic fibroblast growth factor (bFGF) and angiopoietin 1, that may activate the BBB phenotype in endothelial cells *in vitro* [71, 72, 78-80]. Overall, the interactions between astrocytes and the brain endothelium within neurovascular units influence the BBB under both physiological and pathological conditions.

In summary, astrocytes are important glial cells with many important functions and fundamental cell interactions that affect the development and function of the CNS in health and disease.

3. NEW SCIENTIFIC APPROACHES TO THE STUDY OF ASTROCYTES

Thanks to technological advances that have occurred in recent decades, new scientific tools have been developed that can be used to advance our knowledge of the complex role of astrocytes in the biology of the CNS. Here, we will highlight some of the novel techniques that can be used to achieve a better understanding of the dynamic role of these cells in the CNS and uncover their involvement in the onset and progression of multiple NDs (Fig. **2**).

3.1. *In Vivo* Imaging

Traditional imaging methods (*i.e.,* imaging of fixed brain tissue from human autopsy or rodent samples) have provided

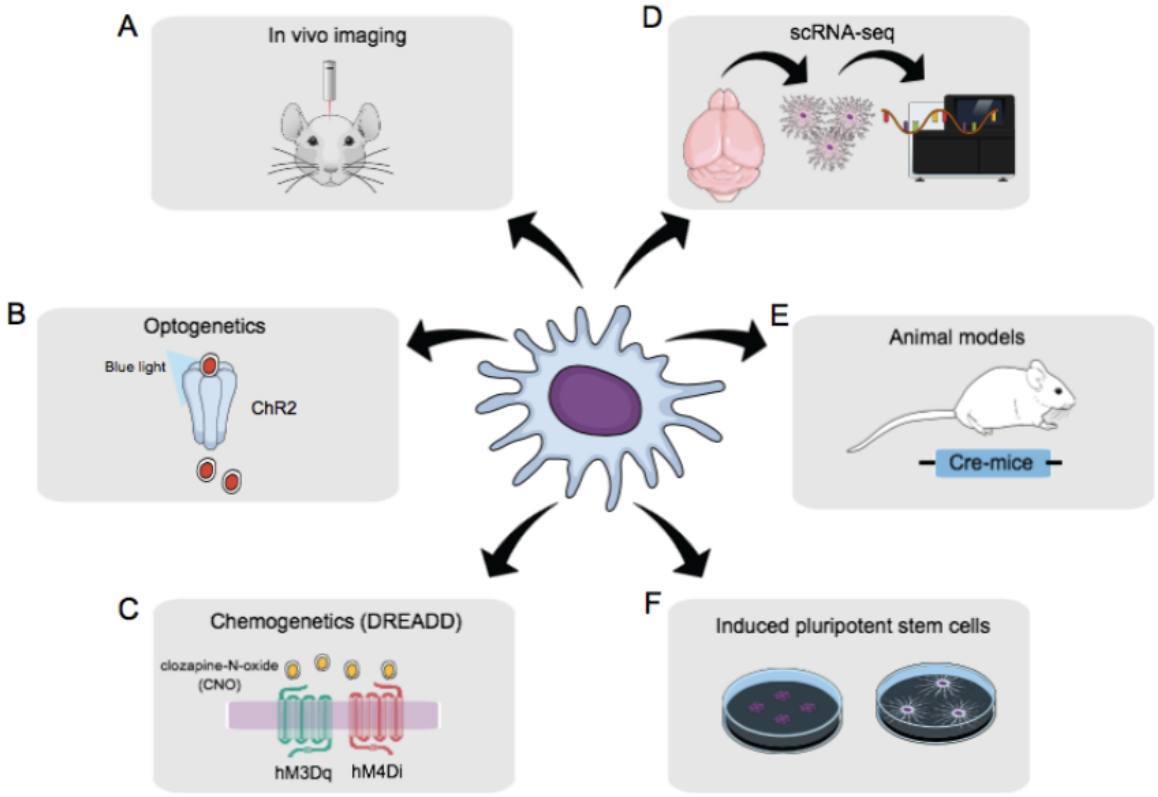

Fig. (2). Novel technical approaches to the study of astrocytes. Over the past few years, novel approaches have been developed to assess the role of astrocytes in the brain under physiological and pathological conditions. These approaches include *in vivo* imaging (A), optogenetics (B), chemogenetics (C), sc-RNA-seq (D), animal models (E) and induced pluripotent stem cells (F).

important data for investigating the involvement of astrocytes in multiple NDs [81-84]. Most of these analyses have focused on bright-field, confocal microscope and electron microscope images. Most recently, we have experienced an important advance in these traditional techniques and three-dimensional fluorescent imaging has been used to determine density and morphological changes in astrocytes during disease progression, and the ion beam scanning electron microscope technique has been recently used to explore ultrastructural changes in astrocytes [81-85].

Despite these important advances in the traditional techniques, the new imaging approaches can provide more relevant insights into the dynamic changes that occur in astrocytes in the CNS *in vivo* [3, 86, 87]. The development of two-photon laser scanning fluorescence microscopy (TPLSM), which uses an imaging technique in an open-skull or thinned-skull craniotomy, makes it possible to visualize and monitor dynamic and structural changes in astrocytes under a variety of physiological and pathological conditions [3, 86, 87]. This technique has drastically advanced our understanding of these cells in the regulation of several of the important physiological functions that were previously discussed, such as their role in the regulation of the BBB, crosstalk with microglia and oligodendrocytes and their contribution to neuronal circuits [3, 86, 87]. Indeed, researchers have used two-photon imaging to truly understand the role of astrocytes in the development and progression of various NDs. For example, two-photon microscopy imaging combined with topical application of sulforhodamine 101 (SR-101), a fluorescent dye widely used in the *in vivo* labeling of astrocytes, in APPswe/PS1dE9 mice has revealed that astrocytes remain in their exclusive and highly organized domains in the presence of Aβ plaques, indicating that Aβ plaques do not exert chemotactic effects on astrocytes in the AD mouse brain [88]. Moreover, astrocytes associated with plaques in APP/PS1 mice displayed hyperactive calcium waves that were partially mediated by purinergic signaling [89, 90]. Two-photon imaging has also been used to investigate astrocyte function in ALS; the results showed that these cells mediated a rapid response to the affected spinal cord area by projecting branches in the direction of the injured axons within minutes to hours post-lesion [91]. Thus, this technique provides a valuable tool for elucidating the structure and function of astrocytes at specific time points during disease progression as well as for performing longitudinal studies. Interestingly, the recent three-photon fluorescence microscope also makes it possible to study astrocytic function in subcortical areas, overcoming the limitation of the two-photon microscope, which only allows imaging in cortical areas [92]. The technological advance represented by the three-photon fluorescence microscope will provide exciting insights into the critical role of astrocytes in subcortical areas *in vivo*.

3.2. Optogenetic Approach

Optogenetics is a powerful technique that uses optics and genetics to manipulate the activity of a target cell [93]. This novel approach allows the investigator to control the activity of a specific cell with high precision both spatially and tem-

porally [93]. Initially, this technique was used to activate or inhibit neuronal cells through light-sensitive opsins, which are microbial channels that allow the flux of ions that depolarize or hyperpolarize neurons when stimulated by light [93]. The most commonly used channels in optogenetics research are channelrhodopsin (ChR) and halorhodopsin (NpHR), which are sensitive to blue and yellow light, respectively. ChR-1 and ChR-2 are cation-selective channels that allow ion influx; this influx depolarizes the membrane, resulting in action potential firing in neurons. NpHR is a light-sensitive chloride pump that hyperpolarizes the membrane and inhibits neuronal activity [3, 93, 94]. An incipient number of studies have focused on activating astrocyte signaling *in vivo* by using ChR2; this leads to an increase in the calcium influx that further activates downstream signaling pathways, inducing the release of cytokines and gliotransmitters that influence the activity of neighboring neurons [95-97]. However, further studies are needed to determine the impact of manipulating astrocytes in the progression of several NDs and to enlighten our understanding of the disease mechanism.

3.3. Chemogenetic Technology

Chemogenetics has been extensively used as a platform to target and manipulate neuronal and nonneuronal signal transduction in specific cell types such as astrocytes [3, 98]. Multiple different constructs involving kinases, nonkinase enzymes, G protein-coupled receptors (GPCRs) and ligand-gated ion channels have been engineered [94, 98]. Of these constructs, the most widely used chemogenetic tool is Designer Receptors Exclusively Activated by Designer Drugs (DREADDs); these are modified GPCRs that are activated by agonists with no endogenous targets [3, 94, 98, 99]. DREADDs can be excitatory Gq receptors such as hM1Dq, hM3Dq, and hM5Dq, which are modified forms of the human muscarinic receptor. Of these three Cq-DREADDs, hM3Dq is the most frequently used by researchers [3, 94, 98]. DREADDs can also be inhibitory; examples are hM2Di, hM4Di and the κ-opioid-derived DREADD (KORD), of which hM4Di is the most commonly used [3, 94, 98]. Both hM3Dq and hM4Di can be activated by the selective agonist clozapine-N-oxide (CNO), a derivative metabolite of the antipsychotic drug clozapine that is biologically inert in rodents [3, 94, 98]. CNO can cross the BBB and is commonly administered intraperitoneally or orally, thereby enabling a noninvasive manipulation [94, 98]. Currently, genetically engineered mice that express hM3Dq or hM4Di under the control of the tetracycline (*tet-off*) promoter and via Cre-mediated recombination are available, allowing its expression in specific target cells such as astrocytes. In addition, many researchers are also using promoter-driven viral vectors such as modified helper virus (HSVs), adeno-associated virus (AAV) and lentivirus constructs to activate DREADD in specific cells or brain regions [94, 98].

Gq-DREADD was first introduced into astrocytes by Dr. McCarty's laboratory, who generated mice that expressed hM3Dq under the control of the GFAP promoter (GFAP-hM3Dq mice) [94, 100]. The activation of Gq-GPCR in glial cells by CNO in GFAP-hM3Dq mice induces remarkable changes in parameters that are regulated by the autonomic nervous system, such as increased heart rate, blood pressure, saliva formation and a decrease in body temperature [94, 100]. In addition, increased sedation in the presence of a GABA receptor agonist is also observed in these mice [94, 100]. Collectively, these results demonstrate the importance of astrocytes in the modulation of these physiological functions [94, 100]. In addition, astrocyte activation or inhibition via GFAP-hM3Dq and GFAP-hM4Di, respectively, within the medial basal hypothalamus modulates food intake in mice [101]. The mechanism through which astrocytes regulate food intake is mediated by inactivation of the orexigenic agouti-related peptide (AGRP) in neuronal cells in the hypothalamus via adenosine A_1 receptors [101]. Moreover, several studies have demonstrated that activation of astrocytes in different regions of the brain plays a key role in synaptic plasticity. For example, activation of astrocytes via a GFAP-hM3Dq construct in the CA1 modulates memory and learning processes in the hippocampus, and astrocyte activation via viral induction in the central amygdala area (CeM) modulates fear and anxiety behavioral responses in mice [102, 103].

Overall, these studies show that it is possible to manipulate the astrocytes function in multiple neurodegenerative models at different time points of disease progression and in different brain areas, leading to a better understanding of the critical role of the astrocytes in these diseases.

3.4. Single-cell RNA-Sequencing (RNA-seq)

The study of the transcriptome is a powerful technology that has enabled the identification of functional elements of the genome and revealed the molecular components of cells and tissues [104, 105]. Over the years, multiple methodological approaches to studying the transcriptome have been developed; however, the recent development of the high-throughput DNA sequencing method has provided a new approach to both the mapping and the quantification of transcriptomes [104]. This method, termed RNA-sequencing (RNA-seq), is now generally used to analyze gene expression and to identify novel RNA species [104]. Although RNA-seq is a valuable research tool in the study of the transcriptome, important limitations emerge from this approach in terms of studying the brain and neurodegenerative diseases. For example, the brain is composed of multiple cell subtypes (*e.g.,* various neuronal subtypes, astrocytes, microglia and oligodendrocytes), each of which expresses a distinctive set of genes. Under different pathological conditions these cell subtypes can be affected and may respond in various ways. Therefore, it is critical to overcome these intrinsic limitations to gain precise insight into the roles of these different subsets of cells in disease progression. A recent significant technological step forward is the use of single-cell RNA sequencing (scRNA-seq), which makes it possible to study the genetic profiles of these specific cell subtypes under physiological and pathological conditions [106]. Novel studies have demonstrated that astrocytes from multiple brain regions show different genetic profiles related to the inflammatory response and to the expression of synaptic-related genes during aging and when exposed to different pathological stimuli [107, 108]. Moreover, an elegant study conducted in Dr. Bonci's laboratory demonstrated that astrocytes from the ventral midbrain displayed important genetic differences from cortical or hippocampal astrocytes [109].

This relevant study showed that the pathways involved in astrocytic calcium dynamics and electrical membrane properties are significantly different than those in other brain regions [109]. Collectively, these studies support the concept of regional astrocyte heterogeneity, which may contribute to understanding the different degrees of the vulnerability of distinct brain regions to pathological conditions and aging [107-109]. The scRNA-seq approach has also been used to identify the relevant astrocytic pathways involved in NDs. As one example, a recent study of ALS mice highlighted that astrocytes display important alterations in multiple pathways, including glutamine biosynthesis, ion homeostasis, energy metabolism, immune responses, the cell cycle and apoptosis [110]. In summary, this novel approach provides exciting molecular insights into the complexity of the brain that can be applied to the identification of novel molecular mechanisms underlying multiple NDs and to the discovery of novel therapeutic strategies and biomarkers.

3.5. Astrocyte Animal Models

Over the past decades, various strategies for determining the functional significance of astrocytic signaling in the brain and its involvement in the neurodegenerative processes associated with multiple NDs have been developed [94, 111]. Special emphasis has been placed on the generation of multiple knockout (KO) or conditional KO (cKO) models in which specific astrocytic signaling pathways have been deleted; such models make it possible to investigate how these astrocytic pathways may contribute to disease progression.

The inducible Cre system driven by the GFAP promoter (GFAP-CreERT2) has been used extensively to target multiple genes and pathways expressed in astrocytes [45, 112-114]. One of the initial targets was the cannabinoid type-1 receptor (CBIR). GFAP-CBIR cKO mice developed working memory impairment after Cre activation in astrocytes following tamoxifen administration [45]. Astrocytic dopamine D2 receptor (DRD2) floxed mice also showed an increase in secreted proinflammatory mediators compared to wild-type control mice [112]. However, one of the pathways most extensively studied in astrocytes is the Cq-GPCR/PLC/IP3 pathway, which regulates the release of intracellular Ca^{2+} from the endoplasmic reticulum. Depletion of IP3 receptor type 2 (IP3R2) in astrocytes has been achieved by crossing IP3R2 flox mice with GFAP-Cre mice, allowing restriction of the depletion of IP3R2 in astrocytes. This biogenic model reduces the astrocyte response to Gq-GPCR activation or neuronal activity in several brain regions [113, 114]. Recently, other astrocytic models have been generated and used to explore the consequences of altering glutamate reuptake, nitric oxide and gliotransmitter production as well as of buffering potassium ions [94, 111]. These mice showed significant impairments in behavior, synaptic transmission, ion homeostasis and other signs of neurodegeneration [115-117].

More recently, these models have been used to investigate how astrocytic pathways may be involved in the development and progression of several distinct NDs. For example, a recent study demonstrated that disruption of insulin-like growth factor receptor (IGF-1) signaling in astrocytes leads to significant impairment in the hippocampal-dependent working memory test [118]. Moreover, a decrease in mitochondrial energy charge and respiration, as well as impairments of Aβ uptake, are observed in IGF1RKO astrocytes [118]. Interestingly, an innovative inducible mouse model that expresses the human ApoE3 or ApoE4 alleles specifically in astrocytes has been developed [119]. Studies using this model revealed that apolipoprotein E4 (apoE4) facilitates the development of amyloid pathology during the initial seeding stage but not during the rapid growth period, providing important insights into potential ApoE therapeutic strategies for suppressing amyloid pathology [119]. Connexins (Cx) are crucial gap junction proteins that maintain the connections of astrocytic cells in a network [120]. Among the different connexins, Cx43 has received the most attention and has been shown to play an important role in neuronal plasticity [121, 122]. Reduction of astrocytic Cx43 expression in an AD mouse model mitigated cognitive impairment by reducing gliosis and stimulating synaptic function [123]. Furthermore, a recent compelling study performed in an ALS model reported that astrocytic NF-kB activation drives microglial proliferation and leukocyte infiltration in the SOD1 (G93A) ALS mice [124]. The work concluded that activation of this pathway in astrocytes in the presymptomatic phase is neuroprotective; however, in the symptomatic phase, it accelerates the disease progression through microglial activation [124]. Several other astrocytic pathways have been linked to ALS; specifically, astrocytic Kir4.1 has been shown to be critical for maintenance of the function and peak strength of large fast α-motor neurons (FαMNs) [125]. Overall, these innovative rodent models offer a new perspective for assessing the role of glial cells in the mechanisms underlying NDs.

3.6. Human Induced Pluripotent Stem Cells (iPSCs)

Advances in cell reprogramming have made it possible to generate induced pluripotent stem cells (iPSCs) from skin fibroblasts or blood cells and observe their subsequent differentiation into tissue-specific cells [126-128]. This revolutionary technology is a promising tool for disease modeling and drug screening [126-128]. During the 10 years since its creation by Yamanaka's group [129], there have been many advances in modeling NDs using iPSCs generated from familial and sporadic patients [126-128]. These studies provide proof of principle for modeling patient-specific pathology and recapitulate several pathological features of many different NDs *in vitro* [126-128].

Although most human iPSC-based models used to study neurodegenerative conditions are focused on neuronal cells, recent studies have assessed the role of other brain cells in the progression of multiple NDs [126-128]. As such, in the past few years, a cascade of new studies using iPSC technology has focused on determining the contribution of astrocytes to NDs [126-128]. For example, a recent study showed that iPSC-derived astrocytes that harbor the APOE4 allele exhibit profound alterations in gene expression and multiple changes in cellular processes that may be related to AD pathogenesis, including alterations in APOE secretion levels, lipid transport and Aβ clearance [130]. Other studies also investigated the function of astrocytes in Aβ production and clearance and found that, compared to controls, iPSC-derived astrocytes from AD patients secreted higher levels of Aβ42 and showed impaired Aβ uptake [131-133]. In addi-

tion, other aspects of astrocyte biology, such as their metabolism, calcium signaling and responses to inflammatory stimuli, are also altered in iPSC-derived astrocytes from AD patients compared to controls [131-133].

The role of astrocytes in others NDs has also been explored; for example, iPSC-derived astrocytes from HD patients were shown to exhibit an increased cytoplasmic vacuolation compared to cells from control patients, a characteristic that was also identified in primary lymphoblasts harvested from HD patients [134-136]. These results suggest that autophagic processes may be altered and that cellular vacuolation may be an important disease abnormality associated with HD. Thus, further mechanistic studies may provide key findings regarding the relationship of this phenotype to HD pathogenesis. Moreover, iPSC-derived astrocytes from ALS patients are not able to promote the survival of motor neurons (MNs), and these cells exhibit signs of degeneration [137-139]. A recent study conducted by Dr. Lakatos' laboratory suggested that the ephrin-B1 pathway is disrupted in human iPSC-derived astrocytes from ALS patients as well as in animal models of ALS, indicating that disruptions in this pathway block the neuroprotective astrocytic response, facilitating MN degeneration [139]. These important findings provide a better understanding of the molecular events that lead to MN death and of the interaction of MNs with astrocytes and suggest a potential therapeutic target.

Over the years, we have experienced important technological developments that have provided researchers with many advanced tools that can be used to more precisely dissect the physiological functions of astrocytes and to explore their role in the onset and progression of multiple NDs.

4. ROLE OF ASTROCYTES IN NEURODEGENERATIVE DISEASES

Given the important role of astrocytes in supporting neuronal development and homeostasis, it is not surprising that astrocyte dysfunction contributes to a variety of NDs. The tools discussed above all have the potential to provide new insight into the role of astrocytes in specific neurological disorders. In the healthy CNS, astrocytes are uniformly distributed in nonoverlapping domains; however, during the reaction to CNS injury, such as ND, astrocytes undergo changes in morphology and gene expression [1], including hypertrophy and atrophy with loss of function (degeneration) [1, 140, 141]. These changes are summarized in Table **3**. As described above, astrocytes express many receptors that detect abnormal signals in the extracellular space, increased concentrations of some molecules and even the absence of normal signals from neighboring cells. The exact molecular triggers that occur during the initial stages of NDs, before significant neurodegeneration occurs, are unknown. Importantly, in NDs, pathogenic proteins may be directly expressed directly or taken up by astrocytes and activate them. These abnormal proteins can interfere with intracellular signaling pathways by activating or inhibiting various signaling proteins. For example, activation of the STAT3 pathway seems to be a universal feature of astrocyte reactivity in ND models that is shared among disease models, brain regions and animal species (for review, see [142]). In this part of the review, we highlight recent studies that address the contribution of reactive astrocytes to major NDs including Alexander disease, Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (see Fig. **3** and Table **4**).

4.1. Alexander Disease

The clearest example in which astrocytes have been shown to act as the primary culprit in disease is Alexander disease (AxD), a rare genetic disorder that is characterized by astrocyte dysfunction. AxD is a fatal neurodevelopmental disorder; in infants, it causes seizures, megalencephaly, and psychomotor retardation, ultimately leading to death. The early onset form of the disease usually begins in the frontal lobe [143]. In contrast, in adults (late-onset), the disease is delayed and affects mainly the cerebellum and the brain stem, producing conditions such as dysphagia and dysarthria, among others [144, 145].

AxD is an astrocyte-specific disorder that is caused by heterozygous dominant mutations in the GFAP gene [146]. Histopathologically, it is defined by a marked increase in astroglial dimensions, in part due to a vast accumulation of intermediate filaments GFAP and the Rosenthal fibers (RFs). RFs are protein inclusions that consist mainly of GFAP and vimentin, but they can also contain ubiquitinated proteins, 27-kDa heat shock proteins (HSP27) and alpha-B-crystallin [147]. During AxD, astroglial cells not only increase in size but also undergo significant morphological changes in which they lose their finest processes and adopt a dysmorphic phenotype [148].

GFAP mutations could result in the slower formation of intermediate filaments, leading to accumulation of the GFAP protein in an oligomeric conformation [149]. GFAP oligomers appear to directly inhibit the ubiquitin-proteasome system, resulting in impairment of proteasome function. Giving the importance of the proteasome pathway in removing excess GFAP from healthy brains, it appears that astrocytes in AxD are constantly producing and accumulating massive amounts of GFAP filaments. The marked increase in GFAP expression induces astroglial toxicity and alters various cellular pathways such as protein degradation, cellular stress and autophagy (for review, see [150]). In AxD, astroglial cells display a large decrease in the levels of the EAAT2 glutamate transporter, which in turn impairs their capacity to buffer glutamate. This impairment could be the cause of the seizures infants develop during the early stages of the disease. It could also be related to the specific loss of susceptible populations of neurons [148, 151]. As in most NDs, astrocytes in AxD upregulate various genes related to inflammation and secrete pro-inflammatory molecules such as TNFα and IL-1β, thereby contributing to the generation of a pro-inflammatory environment [152, 153].

Although AxD is quite rare, the extent to which we can understand how astrocyte functions are impaired in this disorder and the strategies we can devise to restore astrocyte function will have significant implications for how we deal with the many of the more common neurological diseases that confront us. In this sense, different NDs (Alzheimer's disease and Parkinson's disease) have in common a marked astroglial activation process, and AxD could provide us with substantial information on the effects of the upregulation of

Table 3. Changes in astrocytes related with disease progression.

	Disease Related Changes in Astrocytes	References
Morphological changes	Atrophy: Reduced cell soma and volume. Decreased numbers or loss of processes. Decrease in synaptic coverage, astroglial homeostatic support (compromised uptake of glutamate).	[266, 267]
	Reactive astrogliosis: Upregulation of intermediate filaments like GFAP, vimentin, and nestin. Hypertrophy with enlarged soma, thick primary processes, and tangles of finer processes. Increased volume. Proliferation.	[1, 12, 161, 266, 268-270]
Functional changes	Excitotoxic glutamate release Inflammatory cytokine production Upregulation of aquaporin 4 water channels Reactive oxygen species generation Change in intracellular signaling pathways (cAMP, STAT3, NF-kB, calcium, *etc.*)	[12, 266, 271, 272]

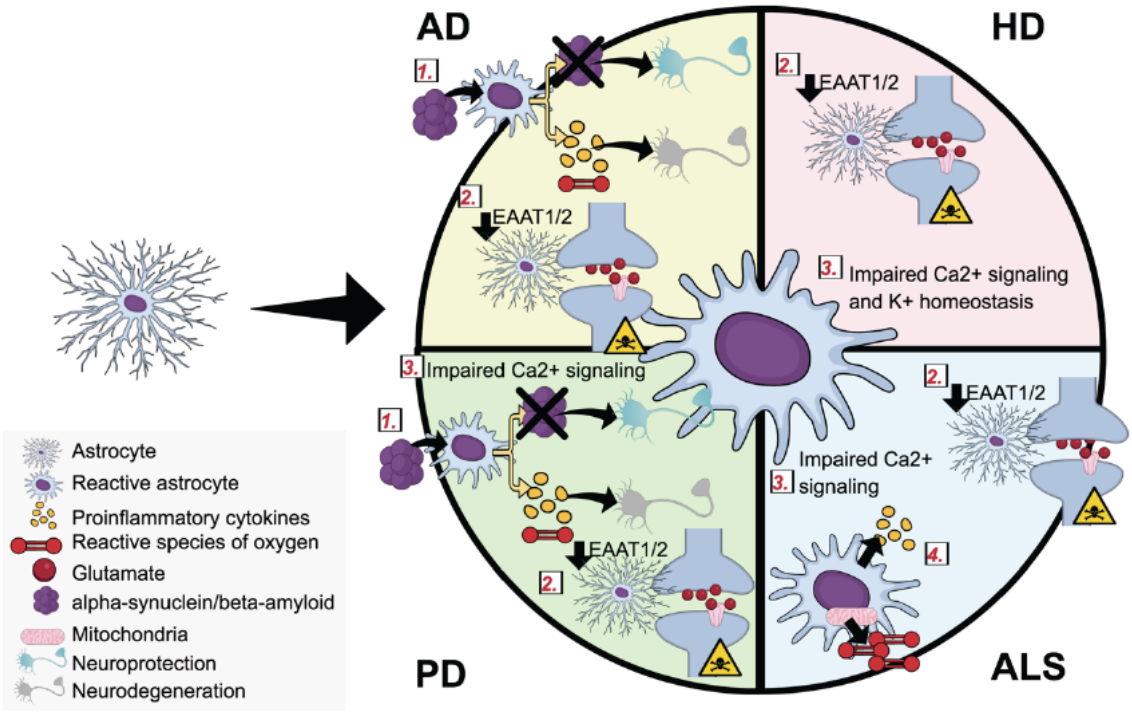

Fig. (3). Mechanisms underlying the role of astrocytes in neurodegenerative diseases. Under pathological conditions, astrocytes undergo activation. Reactive astrocytes contribute to the progression of neurodegenerative diseases through a variety of mechanisms. 1) The accumulation of amyloid-β (in AD) or α-synuclein (in PD) activates phagocytosis by reactive astrocytes. This process can have a neuroprotective role (through the degradation and clearance of these pathological proteins) or contribute to the neurodegenerative processes (through the production and secretion of reactive species of oxygen and proinflammatory cytokines). In AD, it has also been reported that reactive astrocytes are able to eliminate presynaptic dystrophies, thereby contributing to neuroprotective effects. 2) A reduction in the expression of excitatory amino acid transporters 1 and 2 (EAAT1/2) in astrocytes (responsible for most glutamate uptake at synapses) has excitotoxic effects on neurons and contributes to the neurodegenerative process. 3) Astrocytes also experience impairment in Ca^{2+} signaling and K^{+} homeostasis during the neurodegenerative process. 4) Finally, in ALS, reactive astrocytes display mitochondrial dysfunction with release of ROS.

GFAP in pathology. The use of human iPSCs to model different GFAP mutations and/or GFAP overexpression could be used as a novel approach to develop a platform for drug screening or therapeutic development.

4.2. Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, affecting over 35 million people worldwide and causing major progressive deficits in memory

Table 4. Implication of astrocytes in neurological disorders. Table summarizes the astroglial role in AxD, AD, PD, HD and ALS.

Neurodegenerative Disease	Astroglial Implication in the Disease	References
Alexander disease (AxD)	Inhibition of ubiquitin-proteasome	[150]
	Reduction of glutamate uptake	[148, 151]
	Secretion of pro-inflammatory molecules	[152, 153]
Alzheimer's disease (AD)	Release of Pro-inflammatory cytokines/ROS/NOS	[174, 175]
	Aβ generation	[178, 180]
	Aβ phagocytosis and degradation	[168, 181, 183, 184]
	Impaired glutamate uptake	[171]
	Phagocytosis of dystrophic neurites	[84]
Parkinson's disease (PD)	Release of neurotoxic factors/ROS/NOS	[221]
	Impaired glutamate uptake	[210, 211]
	α-synuclein elimination	[215-218]
	Impaired Ca^{2+} signaling	[221]
Huntington's disease (HD)	Impaired glutamate uptake	[232, 238]
	Glutamate release	[239]
	Impaired K^+ and Ca^{2+} signaling	[240, 241]
	Cholesterol metabolism alterations	[242, 243]
Amyotrophic lateral sclerosis (ALS)	Release of pro-inflammatory cytokines/neurotoxic factors/ROS	[256, 258, 259, 261]
	Impaired glutamate uptake	[245, 251-253]
	Impaired Ca^{2+} signaling	[254, 264]

and cognitive function; as yet, there is no effective treatment yet. Neuropathologically, AD is characterized by the extracellular accumulation of senile plaques composed of amyloid-β (Aβ) peptides, the intracellular accumulation of neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau protein, neuroinflammation with active gliosis, and significant synaptic and neuronal loss. AD can be categorized into familial (fAD) and sporadic (sAD) types. Although the two types of AD display similar pathological phenotypes, the factors that trigger the neurodegenerative process in the two types differ. In fAD, the pathological buildup is caused by the presence of autosomal dominant mutations. However, the etiology underlying sAD (which represents ~98% of cases) is complex and multifactorial and results from a combination of genetic, epigenetic, and lifestyle factors [154]. In 1992, Hardy and Higgins suggested amyloid-β protein as the causative event in the pathogenesis of Alzheimer's disease [155]. Since then, other theories (*e.g.,* the cholinergic hypothesis and the neurovascular hypothesis) have been proposed, but none of these theories has managed to replace the amyloid cascade hypothesis. However, in the last decade, the role of glial cells (microglia and astroglia) in the pathogenesis of AD has attracted great interest because these cells are thought to contribute to Alzheimer's pathogenesis as much as amyloid plaques or neurofibrillary tangles themselves [156]. Unlike microglia, little is known about the contribution of astroglial cells to AD.

In animal models [84, 157, 158] and in AD patients [159, 160], a progressive increase in astrocyte reactivity occurring in parallel with the progression of amyloid pathology has been widely described. Recent studies have shown that the number of astroglial cells remains unaltered and that only their level of activation changes during disease progression [82, 160]. Reactive astrogliosis, mainly characterized by the upregulation of GFAP, vimentin and nestin [161] and by the hypertrophy of glial processes [162], can be observed in the hippocampal region even before the onset of the symptomatic phase of AD [163]. However, whether astrogliosis actively contributes to the neurodegenerative processes or whether, in contrast, it exerts beneficial functions during AD progression is still unknown. In this area, contradictory results have been reported. Inhibition/elimination of alpha1 ACT [164], calcineurin [165, 166] or CD40L [167] reverses the formation of amyloid plaques and their associated toxicity, indicating that activation of astrocytes increases their toxicity. In contrast, ablation of GFAP/vimentin expression, instead of decreasing the pathology, results in an increased number of dystrophies around the plaques [168], suggesting a neuroprotective role of the reactive astrocytes.

As previously described, astrocytes play a key role in regulating glutamate levels (for review, see [169, 170]). In AD brains and animal models, the vast amount of Aβ present impairs glutamate uptake by astrocytes, leading to excessive extracellular glutamate levels sufficient to cause toxicity. This phenomenon (excitotoxicity) could be due to an amyloid-dependent reduction in the level of glutamate transporter 1 (GLT-1) and its human analog, EAAT2. Reduced levels of GLT-1/EAAT2 in the hippocampus, mainly in the vicinity of amyloid plaques, have been found in Alzheimer's models and in AD patients [171]. In addition, astrocytes located near Aβ deposits express GLT-1 splice variants that are inert and inhibit normal glutamate uptake, which in turn contributes to an increase in extracellular glutamate levels. On the other hand, it is well established that astrocytes express various types of G protein-coupled receptors (GPCRs) related to Ca^{2+} signaling, including metabotropic glutamate receptors (mGLuRs) and nicotinic acetylcholine receptors (nAchRs). Under pathological conditions such as AD, GPCRs are altered, and Ca^{2+} levels consequently increase. In this sense, Xiu and collaborators described the enhanced expression of α7, α4 and β2-nAchRs in response to amyloid-β [172]. Moreover, Aβ causes sustained astrocytic Ca^{2+} signaling that is mediated by mGluR5 and α7nAchR and finally leads to astroglial glutamate release. Alterations in glutamate uptake combined with astroglial glutamate release can result in excessive extracellular glutamate levels that are toxic to neurons, leading to neuronal death and contributing to AD pathology.

Astrogliosis has been specifically identified in the regions surroundings amyloid plaques [173], and its progression correlates with Aβ pathology with respect to both plaque number and distribution [159]. It is known that reactive astroglia are capable of responding to Aβ by giving rise to the production and secretion of pro-inflammatory cytokines and/or oxidative stress molecules, which in turn have cytotoxic effects. In fact, Aβ42 oligomers directly affect astrocyte activation via the nuclear factor (NF)-κβ signaling pathway and thus cause the up-regulation of cyclooxygenase-2 (COX-2), IL-1β and TNF-α pro-inflammatory mediators [174]. Marked upregulation of some proinflammatory cytokines such as IL-6, IL-1β, interferon gamma (IFN-γ) and TNF-α has been observed in APP transgenic mice [175, 176]. In addition, the release of inflammatory mediators contributes to amyloid production and to its accumulation in extracellular plaques [177]. In this sense, the stimulation of β- and γ-secretase by IL-1β, IL-6 and TNF-α was shown to result in APP cleavage and Aβ generation [178]. Recently, a connection between astroglial aquaporin 4 (AQP4) and Aβ generation and its final accumulation in extracellular plaques has been found [179], whereas AQP4 was traditionally believed to be related to amyloid clearance through the BBB [180].

Contrary to the idea of contributing to amyloid deposition, plaque-associated reactive astrocytes could also have a beneficial role in which they reduce the amyloid burden [162]. Xiao and collaborators showed that enhancing lysosomal function in astrocytes promotes amyloid uptake and consequently reduces Aβ plaque pathology [181]. During the past decade, reactive astrocytes have been proposed as the most important cell type with respect to Aβ internalization and degradation [168, 173, 182-184]. In fact, some authors reported a higher efficiency of Aβ engulfment by astrocytes than by microglia although microglial cells are the main phagocytic population within the CNS [185, 186]. In addition, the capacity of astroglial cells to clear amyloid deposits *in vitro* and *in vivo* has been demonstrated; thus, astroglia not only phagocytose but also degrade Aβ peptides [173, 183]. A subsequent study showed that astrocytes expressing enhanced green fluorescent protein (GFP) transplanted into the hippocampi of APPdE9 transgenic mice were specifically located around amyloid plaques and were capable of internalizing and degrading Aβ [187].

Astrocytes express a vast number of receptors involved in phagocytic pathways that have been related to amyloid-β binding; these include CD36, CD47 [188], brain specific angiogenesis inhibitor-1 (BAI1) [189], lipoprotein receptor-related protein 1 (LRP1) [190], scavenger receptor class B type 1 (SCARB1) [191] and the receptor for advanced glycation end products (RAGE) [188, 192]. Regarding the astroglial degradation process of Aβ, some authors point to an intracellular lysosomal pathway [181], while others suggest extracellular degradation mediated by amyloid-degrading peptidases such as neprilysin [193, 194], metallo-proteinase 2 (MMP-2) and metallo-proteinase 9 (MMP-9) [195]. In addition, the high expression of ApoE by astrocytes [196] has also been linked to Aβ degradation and clearance. Although ApoE does not directly interact with soluble Aβ, it has been suggested that it may compete for the same astroglial uptake pathways [185, 197].

The amyloid phagocytic capacity of astrocytes and their expression of receptors involved in Aβ internalization suggest new physiological roles for astroglial cells. For example, the participation of astrocytes in synaptic pruning in health and disease during development [53] and in the adult CNS [7] has been reported. In relation to AD pathology, the phagocytic clearance of presynaptic dystrophies mediated by reactive astroglia has recently been described in both animal models and AD patients [84]. The accumulation of APP and BACE1 within presynaptic dystrophic neurites and the potential synaptic release of amyloid-β peptides could lead to an increase in amyloid pathology [198-200]. Therefore, the astrocytic clearance of presynaptic dystrophies could, in fact, be a protective mechanism of these glial cells that limits Aβ pathology.

Reactive astrocytes could play a neuroprotective role by releasing neurotrophic factors, or, in contrast, they could act in a cytotoxic manner by mediating the secretion of some pro-inflammatory cytokines. In this sense, the existence of two different functional phenotypes of reactive astrocytes, A1 and A2 (simila to microglial M1/M2 phenotypes), has been demonstrated [2]. A1 astrocytes are essentially cytotoxic and appear to be induced by activated microglia [24]. They lose most of their regular functions and acquire new neurotoxic ones. In contrast, A2 astrocytes seems to be neuroprotective as they upregulate many neurotrophic factors [2, 24]. However, this division of the cells into two opposing reactive astroglial phenotypes may not reflect the reality of the functional diversity among reactive astrocytes in AD. Last year, five distinct subpopulations of astrocytes were identified by transcriptomic analysis, including two subtypes

related to phagocytic activity (types B and C); however, the contributions of the individual astroglial subpopulations to AD are still unknown [23]. In fact, the idea of astroglial loss-of-function and its contribution to neurodegenerative diseases in general and to AD in particular is now emerging [201]. In line with this, microglial dysfunction and degeneration [202, 203] have been linked to AD pathogenesis.

In the last decade, iPSC-derived cells have been used as a novel tool for modeling Alzheimer's disease. Although most studies have focused on neurons generated from iPSCs of AD patients, this technology is now beginning to be used to comprehend the role of neuroinflammation in AD. In this regard, iPSC-derived astrocytes from AD patients showed pathological phenotypes, while astrocytes derived from non-AD subjects displayed normal astroglial phenotypes [131, 204]. Accordingly, the use of iPSC-derived astrocytes could be a novel approach to unraveling the contribution of astroglia to AD pathogenesis.

4.3. Parkinson's Disease

Parkinson's disease (PD) is characterized by the presence of Lewy bodies and the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) [205]. This loss leads to a dopamine deficiency in the striatum and to alterations in the basal ganglia circuitry [205]. As a consequence of these changes, PD patients show major motor symptoms such as akinesia, bradykinesia, tremor, rigidity and postural instability [205] as well as nonmotor alterations such as cognitive fluctuations [206]. Astrocytes have been implicated in the pathogenesis of PD as astrocyte reactivity is detected in the SNpc of PD patients. However, the mechanisms responsible for this neuronal degeneration have not yet been elucidated [207]. Astrocyte activation and the neuroinflammatory process associated with PD have been considered part of a downstream response to the death of dopaminergic neurons. Nevertheless, relevant new evidence suggests that astrocytes play a critical role in the initiation and progression of PD pathophysiology (for review, see [208]).

In this sense, several genes that are expressed in astrocytes and have an important role in their function are known to have a causative role in the development of PD [208]. The most important evidence for a PD-related gene having a role in astrocyte biology has been found for DJ-1, which is encoded by the *PARK7* gene. DJ-1 expression is upregulated in reactive astrocytes in patients with PD [209]. In astrocytes, DJ-1 associates with lipid rafts and mutations in this gene lead to disrupted lipid raft assembly. Astrocytes were found to exhibit impaired glutamate uptake as a consequence of this disruption [210, 211]. Altered glutamate uptake is one of the best documented and earliest described dysfunctions of astrocytes in ND [212]. In this regard, astrocytes are responsible for most glutamate uptake at synapses through transporters encoded by excitatory amino acid transporter genes 1 and 2 (EAAT1 and 2, also called GLAST and GLT1 in rodents) [213]. Inefficient glutamate uptake leads to overstimulation of glutamate receptors, which causes neuronal cell death through excitotoxicity. For instance, the selective expression of α-synuclein A53T in a mouse model of PD reduces GLT1 levels in astrocytes and triggers the death of dopaminergic neurons in the SNpc [214].

One of the pathological hallmarks of PD is the presence of neuronal cytoplasmic inclusion bodies that are mainly composed of α-synuclein fibrils. In postmortem PD brains, α-synuclein-positive inclusions have been found in astrocytes as well as in neurons [215], suggesting that α-synuclein secreted by neurons is taken up by astrocytes via a toll-like receptor 4 (TLR4)-independent endocytosis pathway [216, 217]. It has been shown that endocytosed α-synuclein localizes to lysosomes and that astrocytes have a role in its removal and degradation [218]. Accordingly, if the concentration of α-synuclein in the extracellular space increases above a certain threshold, an inflammatory response is induced, and pathology begins to develop.

Not only have high levels of exogenous α-synuclein been shown to initiate a TLR4 signaling cascade [216, 217], there is also evidence that DJ-1 regulates this signaling pathway [219]. DJ-1 regulates astrocyte activation by IFN-γ [220]. Moreover, in PD pathophysiology, activated glia produce neurotoxic factors such as glutamate, S100B, TNF-α, prostaglandins, and reactive oxygen and nitrogen species, resulting in neuronal injury and leading to cell death [221]. The disruption of inflammatory signaling pathways has been shown to result in changes in essential astrocyte functions, including glutamate transport [210, 214], water transport [214], and neurotrophic capacity [220, 222-224]. All of these functions are important for neuronal health, and it has been demonstrated that changes of this type in astrocytes result in the degeneration of neighboring neurons.

Finally, dopaminergic neurons in the substantia nigra are known to be particularly vulnerable to oxidative stress, presumably due to their lower antioxidant capacity, increased accumulation of ion and oxidation-prone dopamine, and possible defects in mitochondria. In addition to oxidative and nitrative stresses, Ca^{2+} ion release in astrocytes is an important event and is involved in the regulation of a number of mechanisms including the release of S100B and other neurotoxins, which may contribute to the dopaminergic loss [221, 225].

To summarize, recent data demonstrate that astrocytes play an essential role in the onset and progression of PD as several genes expressed by this cell type have been implicated in the development of the disease. The role of astrocytes involves a variety of mechanisms such as glutamate uptake, α-synuclein elimination and impaired Ca^{2+} signaling.

4.4. Huntington's Disease

Huntington's disease (HD) is a fatal genetic ND caused by an autosomal dominant mutation that involves the expansion of a polyglutamine-encoding CAG repeat (>36) in exon 1 of the huntingtin (HTT) gene [226]. HD patients present psychiatric, cognitive and motor symptoms, the most characteristic of which is progressive chorea [227]. HD is also characterized by the extensive loss of GABAergic neurons in the caudate and putamen and by the presence of intracellular mutant HTT (mHTT) aggregates [228]. These inclusions are present in neurons [229] as well as in cortical and striatal astrocytes [230], although the neuronal inclusions are generally larger. Moreover, astrocyte reactivity is an early feature of HD (for review, see [231]), and GFAP immunoreactivity is detected in the striatum of presymptomatic carriers and

increases with disease progression [232]. In corroboration of this, astrogliosis has been reported in the striatum and cortex of many of the mouse models in which mHTT is expressed, suggesting that astrogliosis may be a major factor contributing to disease progression in HD [233, 234].

Several intriguing studies in animal models that express mHTT specifically in astrocytes suggest that astrocyte dysfunction directly contributes to HD pathology [230]. Expression of mHTT in astrocytes resulted in the death of cocultured striatal neurons [230], and expression of an mHTT N-terminal fragment led to reduced GLT1 expression, astrogliosis, and HD-like pathology [232, 235]. Moreover, lentiviral overexpression of the N171-Q82 mHTT fragment in cynomologus monkeys triggered neuronal death and astrogliosis via activation of the JAK/STAT3 pathway [236]. These data support the idea that astrocytes play a major role in HD pathogenesis, as we described below.

In HD patients, the levels of EEAT2 mRNA [237] and protein [232] in the caudate and putamen are decreased. Moreover, the uptake of glutamate is impaired in the prefrontal cortex [238]. Importantly, selective expression of mHTT in striatal astrocytes reduces GLT1 expression and alters glutamate uptake and is also associated with neuronal dysfunction [232]. As previously discussed for PD, the alteration of glutamate uptake is the earliest astrocyte dysfunction detected in HD patients. Additionally, HD is characterized by pathological glutamate release [239] due to an increased expression of pyruvate carboxylase (an enzyme involved in glutamate synthesis) by astrocytes. The increase in glutamate production leads to an elevated level of glutamate accessible for vesicular packaging and therefore to an increase in glutamate release, again leading to high excitotoxicity. On the other hand, astrocytes displayed changes in K^+ homeostasis and Ca^{2+} signaling during disease progression. In the R6/2 mouse model, astrocytes showed decreased expression of Kir4.1 K^+ channels, leading to deficient K^+ buffering [240]. This reduction in the levels of Kir4.1 and GLT1 has several aftereffects for astrocytes. Reduced GLT1 levels mean that astrocytes display robust mGluR2/3-mediated Ca^{2+} signals during stimulation of corticostriatal axons. This gain of evoked Ca^{2+} signals is followed by a loss of spontaneous Ca^{2+} signals. The elevated glutamate and K^+ levels in the extracellular space may contribute to increased excitability of medium spiny neurons [241].

Moreover, in the adult brain, astrocytes have a critical role in the *de novo* synthesis of cholesterol, which regulates neuronal function. Related to this, a dysregulation of cholesterol metabolism is detected in astrocytes in HD [242]. A recent study showed that enhanced degradation of cholesterol in the striatum of R6/2 mice via AAVrh10 expression of CYP46A1 significantly modifies the progression of the disease [243], supporting the idea that cholesterol pathways have a pathogenic impact in HD.

Finally, an additional role of astrocytes in HD is related to the fact that reactive astrogliosis may contribute to pericyte death by causing a reduction in pericyte coverage of cerebral blood vessels, a factor that could also contribute to the progression of the disease [244].

In summary, astrocytes are a contributing factor in the disease progression of HD through the JAK/STAT3 pathway, glutamate excitotoxicity, K^+ homeostasis, Ca^{2+} signaling and cholesterol metabolism.

4.5. Amyotrophic Lateral Sclerosis

Patients suffering from amyotrophic lateral sclerosis (ALS) display progressive muscle atrophy, spasticity and weakness due to the progressive loss of upper motor neurons in the motor cortex and lower motor neurons in the spinal cord and brainstem [245]. Ten percent of ALS cases are due to known familial mutations, whereas 90% of ALS cases are of sporadic in onset. The progression of the disease is rapid, leading to death due to respiratory failure within 1-5 years after disease onset (for review, see [246]). New evidence indicates that astrocytes also have an important role in ALS pathogenesis in which they play deep roles in the process of neurodegeneration [247]. Recent studies using cellular and animal models of ALS indicate that there is a complex interplay between motor neurons and astrocytes. Reactive astrocytes are observed in both ALS patients and models [247]. The appearance and degree of reactivity correlate with the level of neurodegeneration [246, 247].

Dominant mutations in the *SOD1* gene are the second most frequent cause of inherited ALS, comprising 20% of familial ALS cases [248]. According to this, rodent models that overexpress the human SOD1 gene carrying this mutation are extensively used in research on this disease (for review, see [246]). Ubiquitous expression of the mutant human SOD1 gene in mice leads to progressive motor neuron degeneration and gliosis with abnormal accumulation of misfolded, ubiquitinated proteins. SOD1 protein inclusions have been observed in motor neurons and in glial cells, including astrocytes [246].

Experiments using cell transplantation have revealed a detrimental role of mutant astrocytes. Transplantation of wild-type astrocyte precursor cells into the cervical spinal cord slowed the progression of the disease in mutant SOD1 rats [249]. An opposite effect was observed when mutant $SOD1^{G93A}$-expressing astrocyte precursor cells were transplanted [250]. This indicates that transplantation of healthy glial cells in an attempt to improve the cellular environment surrounding motor neurons is a feasible ALS therapy.

As discussed previously, one important function of astrocytes is controlling and reducing the concentration of the neurotransmitter glutamate. Regarding this function, glutamate transport is also impaired in ALS patients [245]. EAAT2 protein levels are low in the spinal cord and motor cortex of patients with familial or sporadic ALS, and animal models expressing mSOD1 showed lower EAAT2 levels even before neuronal loss appeared [245, 251-253]. Moreover, astrocytes expressing mutant SOD1 have been shown to lose the ability to control the expression of the AMPA glutamate receptor subunit GluR2 in motor neurons. This loss leads to an elevated influx of calcium into motor neurons and to their degeneration [254].

Astrocytes also secrete neurotrophic factors that support the maintenance and survival of motor neurons. These factors include glial-derived neurotrophic factor (GDNF), brain-

derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF) (for review, see [246]). An increase in neurotrophin levels might contribute to neuronal protection in ALS. Relevant to this, a recent study showed that TNF receptor 1 (TNFR1) signaling promotes GDNF synthesis and release by astrocytes, and the ablation of TNFR1 hastens neurodegeneration in SOD1-ALS mice [255].

In ALS, mitochondrial dysfunction is a key mechanism for the degeneration of motor neurons. In addition to motor neurons pathology, mitochondrial alterations in astrocytes that result in elevated levels of reactive oxygen species (ROS) have been described. This increase in ROS might contribute to neuron loss in SOD1-ALS mice [256]. Another observation related to mitochondrial dysfunction is that astrocytes contribute to energy production by providing lactate to neurons, and this production is reduced in ALS astrocytes [256, 257].

Another mechanism associated with astrocyte-mediated toxicity in ALS is the production of proinflammatory cytokines. For example, astrocytes expressing mutant SOD1 induce neurodegeneration in motor neurons *in vitro* through secretion of IFNγ [258]. In ALS, astrocytes can also secrete transforming growth factor β1 (TGF-β1), which is toxic to motor neurons [259, 260]. Moreover, astrocytes derived from ALS patients expressed elevated levels of proinflammatory cytokines [261] and caused necroptosis in motor neurons through the receptor-interacting protein 1 (RIP1) [262]. Motor neurons in ALS also show a reduction in HLA and MHC class I molecules that might contribute to neurodegeneration [263].

Finally, astrocytes are also involved in calcium homeostasis, and this process is impaired in ALS [264]. To maintain homeostatic function, astrocytes communicate through gap junctions, through which they exchange glucose, ions, and other components. Connexin 43 is the predominant connexin (a family of proteins associated with gap junctions) in astrocytes and is upregulated in SOD1 mice, sporadic ALS patients, and human ALS iPSC-derived astrocytes. The increased levels of connexin 43 lead to elevated intracellular calcium levels and neuronal toxicity [265].

The foregoing findings show that astrocytes also play a critical role in ALS pathogenesis through the release of cytokines/neurotoxic factors/ROS, the impairment of glutamate uptake and Ca^{2+} signaling. Actually, the transplantation of healthy glial cells appears as a feasible therapy for this disease.

FUTURE DIRECTION AND CONCLUSIONS

In the past few years, significant progress has been made in understanding the critical role of astrocytes in multiple physiological functions in the brain (including vascular function, immunological response, synaptic transmission, metabolic support and brain development) as well as their involvement in the pathological mechanisms that underlie several major NDs. Increasing evidence suggests that astrocyte dysfunction may drive neurodegenerative disorders. Despite this important advance, more insight into astrocyte heterogeneity and into the functional properties of these different subsets of astrocytic populations and their roles in disease progression is necessary. In this sense, single-cell RNA-seq studies will provide relevant information on the molecular and functional signatures of astrocyte subtypes that will enhance our understanding of the pathophysiological mechanisms underlying NDs. Finally, the use and application of IPSC-derived astrocytes from patients with NDs will shed new light on the contribution of this specific glial cell type to the pathogenesis of these diseases and will provide a powerful tool for astrocyte-based therapeutic discovery.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Sofroniew M V, Vinters H V. Astrocytes: biology and pathology. Acta Neuropathol. 119(1): 7-35 (2010).
[2] Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. Immunity. 46(6): 957-967 (2017).
[3] Almad A, Maragakis NJ. A stocked toolbox for understanding the role of astrocytes in disease. Nat Rev Neurol. 14(6): 351-362 (2018).
[4] Khakh BS, Sofroniew M V. Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci. 18(7): 942-52 (2015).
[5] Shigetomi E, Patel S, Khakh BS. Probing the Complexities of Astrocyte Calcium Signaling. Trends Cell Biol. 26(4): 300-312 (2016).
[6] Araque A, Carmignoto G, Haydon PG, Oliet SHR, Robitaille R, Volterra A. Gliotransmitters travel in time and space. Neuron. 81(4): 728-39 (2014).

[7] Chung W-S, Allen NJ, Eroglu C. Astrocytes Control Synapse Formation, Function, and Elimination. Cold Spring Harb Perspect Biol. 7(9): a020370 (2015).

[8] Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol. 72(1): 335-55 (2010).

[9] Burda JE, Sofroniew M V. Reactive gliosis and the multicellular response to CNS damage and disease. Neuron. 81(2): 229-48 (2014).

[10] Alvarez JI, Katayama T, Prat A. Glial influence on the blood brain barrier. Glia. 61(12): 1939-58 (2013).

[11] Molofsky AV, Deneen B. Astrocyte development: A Guide for the Perplexed. Glia. 63(8): 1320-9 (2015).

[12] Pekny M, Pekna M, Messing A, Steinhäuser C, Lee J-M, Parpura V, *et al.* Astrocytes: a central element in neurological diseases. Acta Neuropathol. 131(3): 323-45 (2016).

[13] Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci. 32(8): 421-31 (2009).

[14] Sofroniew M V. Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci. 16(5): 249-63 (2015).

[15] Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M, *et al.* Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc Natl Acad Sci U S A. 103(46): 17513-8 (2006).

[16] Bardehle S, Krüger M, Buggenthin F, Schwausch J, Ninkovic J, Clevers H, *et al.* Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat Neurosci. 16(5): 580-6 (2013).

[17] Kang W, Hébert JM. Signaling pathways in reactive astrocytes, a genetic perspective. Mol Neurobiol. 43(3): 147-54 (2011).

[18] Gallo V, Deneen B. Glial development: the crossroads of regeneration and repair in the CNS. Neuron. 83(2): 283-308 (2014).

[19] Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ. Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. Cell. 133(3): 510-22 (2008).

[20] Zhao X, Chen Y, Zhu Q, Huang H, Teng P, Zheng K, *et al.* Control of astrocyte progenitor specification, migration and maturation by Nkx6.1 homeodomain transcription factor. PLoS One. 9(10): e109171 (2014).

[21] Tsai H-H, Li H, Fuentealba LC, Molofsky A V, Taveira-Marques R, Zhuang H, *et al.* Regional astrocyte allocation regulates CNS synaptogenesis and repair. Science. 337(6092): 358-62 (2012).

[22] Ben Haim L, Rowitch DH. Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci. 18(1): 31-41 (2017).

[23] John Lin C-C, Yu K, Hatcher A, Huang T-W, Lee HK, Carlson J, *et al.* Identification of diverse astrocyte populations and their malignant analogs. Nat Neurosci. 20(3): 396-405 (2017).

[24] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 541(7638): 481-487 (2017).

[25] Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. Immunity. 46(6): 957-967 (2017).

[26] Haydon PG. GLIA: listening and talking to the synapse. Nat Rev Neurosci. 2(3): 185-93 (2001).

[27] Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci. 6(8): 626-40 (2005).

[28] Ben Achour S, Pascual O. Astrocyte-neuron communication: functional consequences. Neurochem Res. 37(11): 2464-73 (2012).

[29] Barres BA. The mystery and magic of glia: a perspective on their roles in health and disease. Neuron. 60(3): 430-40 (2008).

[30] Perez-Nievas BG, Serrano-Pozo A. Deciphering the Astrocyte Reaction in Alzheimer's Disease. Front Aging Neurosci. 10: 114 (2018).

[31] Fujii Y, Maekawa S, Morita M. Astrocyte calcium waves propagate proximally by gap junction and distally by extracellular diffusion of ATP released from volume-regulated anion channels. Sci Rep. 7(1): 13115 (2017).

[32] Khakh BS, McCarthy KD. Astrocyte calcium signaling: from observations to functions and the challenges therein. Cold Spring Harb Perspect Biol. 7(4): a020404 (2015).

[33] Srinivasan R, Lu T-Y, Chai H, Xu J, Huang BS, Golshani P, *et al.* New Transgenic Mouse Lines for Selectively Targeting Astrocytes and Studying Calcium Signals in Astrocyte Processes In Situ and *In Vivo*. Neuron. 92(6): 1181-1195 (2016).

[34] Tan L, Li Q, Xie XS. Olfactory sensory neurons transiently express multiple olfactory receptors during development. Mol Syst Biol. 11(12): 844 (2015).

[35] Srinivasan R, Huang BS, Venugopal S, Johnston AD, Chai H, Zeng H, *et al.* Ca(2+) signaling in astrocytes from Ip3r2(-/-) mice in brain slices and during startle responses *in vivo*. Nat Neurosci. 18(5): 708-17 (2015).

[36] Agulhon C, Fiacco TA, McCarthy KD. Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca2+ signaling. Science. 327(5970): 1250-4 (2010).

[37] Sherwood MW, Arizono M, Hisatsune C, Bannai H, Ebisui E, Sherwood JL, *et al.* Astrocytic IP3 Rs: Contribution to Ca2+ signalling and hippocampal LTP. Glia. 65(3): 502-513 (2017).

[38] Paukert M, Agarwal A, Cha J, Doze VA, Kang JU, Bergles DE. Norepinephrine controls astroglial responsiveness to local circuit activity. Neuron. 82(6): 1263-70 (2014).

[39] Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, *et al.* α1-Adrenergic receptors mediate coordinated Ca2+ signaling of cortical astrocytes in awake, behaving mice. Cell Calcium. 54(6): 387-94 (2013).

[40] Yang J, Luo X, Huang X, Ning Q, Xie M, Wang W. Ephrin-A3 reverse signaling regulates hippocampal neuronal damage and astrocytic glutamate transport after transient global ischemia. J Neurochem. 131(3): 383-94 (2014).

[41] Carmona MA, Murai KK, Wang L, Roberts AJ, Pasquale EB. Glial ephrin-A3 regulates hippocampal dendritic spine morphology and glutamate transport. Proc Natl Acad Sci U S A. 106(30): 12524-9 (2009).

[42] Filosa A, Paixão S, Honsek SD, Carmona MA, Becker L, Feddersen B, *et al.* Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. Nat Neurosci. 12(10): 1285-92 (2009).

[43] Ashton RS, Conway A, Pangarkar C, Bergen J, Lim K-I, Shah P, *et al.* Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. Nat Neurosci. 15(10): 1399-406 (2012).

[44] Navarrete M, Araque A. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron. 68(1): 113-26 (2010).

[45] Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, *et al.* Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell. 148(5): 1039-50 (2012).

[46] Navarrete M, Díez A, Araque A. Astrocytes in endocannabinoid signalling. Philos Trans R Soc Lond B Biol Sci. 369(1654): 20130599 (2014).

[47] Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, Lafaille JJ, *et al.* Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell. 155(7): 1596-609 (2013).

[48] Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science. 308(5726): 1314-8 (2005).

[49] Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: new roles for the synaptic stripper. Neuron. 77(1): 10-8 (2013).

[50] Tremblay M-È, Lowery RL, Majewska AK. Microglial interactions with synapses are modulated by visual experience. PLoS Biol. 8(11): e1000527 (2010).

[51] Stevens MC, Kiehl KA, Pearlson GD, Calhoun VD. Functional neural networks underlying response inhibition in adolescents and adults. Behav Brain Res. 181(1): 12-22 (2007).

[52] Liddelow S, Barres B. SnapShot: Astrocytes in Health and Disease. Cell. 162(5): 1170-1170.e1 (2015).

[53] Chung W-S, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, *et al.* Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature. 504(7480): 394-400 (2013).

[54] Yates D. Glia: A toxic reaction. Nat Rev Neurosci. 18(3): 130 (2017).

[55] Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A. Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. Proc Natl Acad Sci U S A. 109(4): E197-205 (2012).

[56] Abudara V, Roux L, Dallérac G, Matias I, Dulong J, Mothet JP, *et al.* Activated microglia impairs neuroglial interaction by opening

Cx43 hemichannels in hippocampal astrocytes. Glia. 63(5): 795-811 (2015).
[57] Prinz M, Priller J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat Rev Neurosci. 15(5): 300-12 (2014).
[58] Nave K-A, Werner HB. Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol. 30(1): 503-33 (2014).
[59] Yeung MSY, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K, *et al.* Dynamics of oligodendrocyte generation and myelination in the human brain. Cell. 159(4): 766-74 (2014).
[60] Noble M, Murray K. Purified astrocytes promote the *in vitro* division of a bipotential glial progenitor cell. EMBO J. 3(10): 2243-7 (1984).
[61] Domingues HS, Portugal CC, Socodato R, Relvas JB. Oligodendrocyte, Astrocyte, and Microglia Crosstalk in Myelin Development, Damage, and Repair. Front cell Dev Biol. 4: 71 (2016).
[62] Wang H, Song G, Chuang H, Chiu C, Abdelmaksoud A, Ye Y, *et al.* Portrait of glial scar in neurological diseases. Int J Immunopathol Pharmacol. 31: 2058738418801406 (2018).
[63] Gaudet AD, Fonken LK. Glial Cells Shape Pathology and Repair After Spinal Cord Injury. Neurotherapeutics. 15(3): 554-577 (2018).
[64] Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, *et al.* Astrocyte scar formation aids central nervous system axon regeneration. Nature. 532(7598): 195-200 (2016).
[65] Ghézali G, Calvo C-F, Pillet L-E, Llense F, Ezan P, Pannasch U, *et al.* Connexin 30 controls astroglial polarization during postnatal brain development. Development. 145(4): dev155275 (2018).
[66] Orthmann-Murphy JL, Abrams CK, Scherer SS. Gap junctions couple astrocytes and oligodendrocytes. J Mol Neurosci. 35(1): 101-16 (2008).
[67] Papaneophytou CP, Georgiou E, Karaiskos C, Sargiannidou I, Markoullis K, Freidin MM, *et al.* Regulatory role of oligodendrocyte gap junctions in inflammatory demyelination. Glia. (2018).
[68] Mayorquin LC, Rodriguez A V, Sutachan J-J, Albarracín SL. Connexin-Mediated Functional and Metabolic Coupling Between Astrocytes and Neurons. Front Mol Neurosci. 11: 118 (2018).
[69] Pannasch U, Vargová L, Reingruber J, Ezan P, Holcman D, Giaume C, *et al*. Astroglial networks scale synaptic activity and plasticity. Proc Natl Acad Sci U S A. 108(20): 8467-72 (2011).
[70] Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci. 11(2): 87-99 (2010).
[71] Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat. 200(6): 629-38 (2002).
[72] Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 7(1): 41-53 (2006).
[73] Wolburg H, Lippoldt A. Tight junctions of the blood-brain barrier: development, composition and regulation. Vascul Pharmacol. 38(6): 323-37 (2002).
[74] Ludwin SK, Rao VT, Moore CS, Antel JP. Astrocytes in multiple sclerosis. Mult Scler. 22(9): 1114-24 (2016).
[75] Verkman AS. Aquaporin water channels and endothelial cell function. J Anat. 200(6): 617-27 (2002).
[76] Verkman AS, Yang B, Song Y, Manley GT, Ma T. Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. Exp Physiol. 85 Spec No: 233S-241S (2000).
[77] Verkman AS. Physiological importance of aquaporin water channels. Ann Med. 34(3): 192-200 (2002).
[78] Haseloff RF, Blasig IE, Bauer HC, Bauer H. In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells *in vitro*. Cell Mol Neurobiol. 25(1): 25-39 (2005).
[79] De Bock M, Wang N, Decrock E, Bol M, Gadicherla AK, Culot M, *et al.* Endothelial calcium dynamics, connexin channels and blood-brain barrier function. Prog Neurobiol. 108: 1-20 (2013).
[80] Lee S-W, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, *et al.* SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. Nat Med. 9(7): 900-6 (2003).
[81] Trujillo-Estrada L, Jimenez S, De Castro V, Torres M, Baglietto-Vargas D, Moreno-Gonzalez I, *et al. In vivo* modification of Abeta plaque toxicity as a novel neuroprotective lithium-mediated therapy for Alzheimer's disease pathology. Acta Neuropathol Commun. 1(1): 73 (2013).
[82] Baglietto-Vargas D, Sánchez-Mejias E, Navarro V, Jimenez S, Trujillo-Estrada L, Gómez-Arboledas A, *et al.* Dual roles of Aβ in proliferative processes in an amyloidogenic model of Alzheimer's disease. Sci Rep. 7(1): 10085 (2017).
[83] Trujillo-Estrada L, Nguyen C, da Cunha C, Cai L, Forner S, Martini AC, *et al.* Tau underlies synaptic and cognitive deficits for type 1, but not type 2 diabetes mouse models. Aging Cell. e12919 (2019).
[84] Gomez-Arboledas A, Davila JC, Sanchez-Mejias E, Navarro V, Nuñez-Diaz C, Sanchez-Varo R, *et al.* Phagocytic clearance of presynaptic dystrophies by reactive astrocytes in Alzheimer's disease. Glia. 66(3): 637-653 (2018).
[85] Bagheri M, Rezakhani A, Roghani M, Joghataei MT, Mohseni S. Protocol for Three-dimensional Confocal Morphometric Analysis of Astrocytes. J Vis Exp. (106): e53113 (2015).
[86] Guo D, Zou J, Rensing N, Wong M. *In Vivo* Two-Photon Imaging of Astrocytes in GFAP-GFP Transgenic Mice. PLoS One. 12(1): e0170005 (2017).
[87] Kelly P, Hudry E, Hou SS, Bacskai BJ. *In Vivo* Two Photon Imaging of Astrocytic Structure and Function in Alzheimer's Disease. Front Aging Neurosci. 10: 219 (2018).
[88] Galea E, Morrison W, Hudry E, Arbel-Ornath M, Bacskai BJ, Gómez-Isla T, *et al.* Topological analyses in APP/PS1 mice reveal that astrocytes do not migrate to amyloid-β plaques. Proc Natl Acad Sci U S A. 112(51): 15556-61 (2015).
[89] Kuchibhotla K V, Lattarulo CR, Hyman BT, Bacskai BJ. Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science. 323(5918): 1211-5 (2009).
[90] Shigetomi E, Hirayama YJ, Ikenaka K, Tanaka KF, Koizumi S. Role of Purinergic Receptor P2Y1 in Spatiotemporal Ca2+ Dynamics in Astrocytes. J Neurosci. 38(6): 1383-1395 (2018).
[91] Dibaj P, Steffens H, Zschüntzsch J, Kirchhoff F, Schomburg ED, Neusch C. *In vivo* imaging reveals rapid morphological reactions of astrocytes towards focal lesions in an ALS mouse model. Neurosci Lett. 497(2): 148-51 (2011).
[92] Horton NG, Wang K, Kobat D, Clark CG, Wise FW, Schaffer CB, *et al. In vivo* three-photon microscopy of subcortical structures within an intact mouse brain. Nat Photonics. 7(3): 205-209 (2013).
[93] Vann KT, Xiong Z-G. Optogenetics for neurodegenerative diseases. Int J Physiol Pathophysiol Pharmacol. 8(1): 1-8 (2016).
[94] Xie AX, Petravicz J, McCarthy KD. Molecular approaches for manipulating astrocytic signaling *in vivo*. Front Cell Neurosci. 9: 144 (2015).
[95] Yamashita A, Hamada A, Suhara Y, Kawabe R, Yanase M, Kuzumaki N, *et al.* Astrocytic activation in the anterior cingulate cortex is critical for sleep disorder under neuropathic pain. Synapse. 68(6): 235-47 (2014).
[96] Gourine A V, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, *et al.* Astrocytes control breathing through pH-dependent release of ATP. Science. 329(5991): 571-5 (2010).
[97] Perea G, Yang A, Boyden ES, Sur M. Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons *in vivo*. Nat Commun. 5(1): 3262 (2014).
[98] Roth BL. DREADDs for Neuroscientists. Neuron. 89(4): 683-94 (2016).
[99] Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A. 104(12): 5163-8 (2007).
[100] Agulhon C, Boyt KM, Xie AX, Friocourt F, Roth BL, McCarthy KD. Modulation of the autonomic nervous system and behaviour by acute glial cell Gq protein-coupled receptor activation *in vivo*. J Physiol. 591(22): 5599-609 (2013).
[101] Yang L, Qi Y, Yang Y. Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. Cell Rep. 11(5): 798-807 (2015).
[102] Martin-Fernandez M, Jamison S, Robin LM, Zhao Z, Martin ED, Aguilar J, *et al.* Synapse-specific astrocyte gating of amygdala-related behavior. Nat Neurosci. 20(11): 1540-1548 (2017).
[103] Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, *et al.* Astrocytic Activation Generates De Novo Neuronal Potentiation and Memory Enhancement. Cell. 174(1): 59-71.e14 (2018).

[104] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 10(1): 57-63 (2009).
[105] Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, *et al.* A survey of best practices for RNA-seq data analysis. Genome Biol. 17(1): 13 (2016).
[106] Ofengeim D, Giagtzoglou N, Huh D, Zou C, Yuan J. Single-Cell RNA Sequencing: Unraveling the Brain One Cell at a Time. Trends Mol Med. 23(6): 563-576 (2017).
[107] Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ. The Aging Astrocyte Transcriptome from Multiple Regions of the Mouse Brain. Cell Rep. 22(1): 269-285 (2018).
[108] Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA. Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci U S A. 115(8): E1896-E1905 (2018).
[109] Xin W, Schuebel KE, Jair K-W, Cimbro R, De Biase LM, Goldman D, *et al.* Ventral midbrain astrocytes display unique physiological features and sensitivity to dopamine D2 receptor signaling. Neuropsychopharmacology. (2018).
[110] Miller SJ, Zhang P-W, Glatzer J, Rothstein JD. Astroglial transcriptome dysregulation in early disease of an ALS mutant SOD1 mouse model. J Neurogenet. 31(1-2): 37-48 (2017).
[111] Davila D, Thibault K, Fiacco TA, Agulhon C. Recent molecular approaches to understanding astrocyte function *in vivo*. Front Cell Neurosci. 7: 272 (2013).
[112] Shao W, Zhang S, Tang M, Zhang X, Zhou Z, Yin Y, *et al.* Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature. 494(7435): 90-4 (2013).
[113] Petravicz J, Fiacco TA, McCarthy KD. Loss of IP3 receptor-dependent Ca2+ increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. J Neurosci. 28(19): 4967-73 (2008).
[114] Chen N, Sugihara H, Sharma J, Perea G, Petravicz J, Le C, *et al.* Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes. Proc Natl Acad Sci U S A. 109(41): E2832-41 (2012).
[115] Aida T, Yoshida J, Nomura M, Tanimura A, Iino Y, Soma M, *et al.* Astroglial glutamate transporter deficiency increases synaptic excitability and leads to pathological repetitive behaviors in mice. Neuropsychopharmacology. 40(7): 1569-79 (2015).
[116] Abu-Ghanem Y, Cohen H, Buskila Y, Grauer E, Amitai Y. Enhanced stress reactivity in nitric oxide synthase type 2 mutant mice: findings in support of astrocytic nitrosative modulation of behavior. Neuroscience. 156(2): 257-65 (2008).
[117] Djukic B, Casper KB, Philpot BD, Chin L-S, McCarthy KD. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci. 27(42): 11354-65 (2007).
[118] Logan S, Pharaoh GA, Marlin MC, Masser DR, Matsuzaki S, Wronowski B, *et al.* Insulin-like growth factor receptor signaling regulates working memory, mitochondrial metabolism, and amyloid-β uptake in astrocytes. Mol Metab. 9: 141-155 (2018).
[119] Liu C-C, Zhao N, Fu Y, Wang N, Linares C, Tsai C-W, *et al.* ApoE4 Accelerates Early Seeding of Amyloid Pathology. Neuron. 96(5): 1024-1032.e3 (2017).
[120] Chandrasekhar A, Bera AK. Hemichannels: permeants and their effect on development, physiology and death. Cell Biochem Funct. 30(2): 89-100 (2012).
[121] Chever O, Lee C-Y, Rouach N. Astroglial connexin43 hemichannels tune basal excitatory synaptic transmission. J Neurosci. 34(34): 11228-32 (2014).
[122] Chever O, Pannasch U, Ezan P, Rouach N. Astroglial connexin 43 sustains glutamatergic synaptic efficacy. Philos Trans R Soc Lond B Biol Sci. 369(1654): 20130596 (2014).
[123] Ren R, Zhang L, Wang M. Specific deletion connexin43 in astrocyte ameliorates cognitive dysfunction in APP/PS1 mice. Life Sci. 208: 175-191 (2018).
[124] Ouali Alami N, Schurr C, Olde Heuvel F, Tang L, Li Q, Tasdogan A, *et al.* NF-κB activation in astrocytes drives a stage-specific beneficial neuroimmunological response in ALS. EMBO J. 37(16): e98697 (2018).
[125] Kelley KW, Ben Haim L, Schirmer L, Tyzack GE, Tolman M, Miller JG, *et al.* Kir4.1-Dependent Astrocyte-Fast Motor Neuron Interactions Are Required for Peak Strength. Neuron. 98(2): 306-319.e7 (2018).
[126] Zhang W, Jiao B, Zhou M, Zhou T, Shen L. Modeling Alzheimer's Disease with Induced Pluripotent Stem Cells: Current Challenges and Future Concerns. Stem Cells Int. 2016: 7828049 (2016).
[127] Yang J, Li S, He X-B, Cheng C, Le W. Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy. Mol Neurodegener. 11(1): 39 (2016).
[128] Mungenast AE, Siegert S, Tsai L-H. Modeling Alzheimer's disease with human induced pluripotent stem (iPS) cells. Mol Cell Neurosci. 73: 13-31 (2016).
[129] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 126(4): 663-76 (2006).
[130] Lin Y-T, Seo J, Gao F, Feldman HM, Wen H-L, Penney J, *et al.* APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. Neuron. 98(6): 1294 (2018).
[131] Oksanen M, Petersen AJ, Naumenko N, Puttonen K, Lehtonen Š, Gubert Olivé M, *et al.* PSEN1 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease. Stem cell reports. 9(6): 1885-1897 (2017).
[132] Lundin A, Delsing L, Clausen M, Ricchiuto P, Sanchez J, Sabirsh A, *et al.* Human iPS-Derived Astroglia from a Stable Neural Precursor State Show Improved Functionality Compared with Conventional Astrocytic Models. Stem cell reports. 10(3): 1030-1045 (2018).
[133] Santos R, Vadodaria KC, Jaeger BN, Mei A, Lefcochilos-Fogelquist S, Mendes APD, *et al.* Differentiation of Inflammation-Responsive Astrocytes from Glial Progenitors Generated from Human Induced Pluripotent Stem Cells. Stem cell reports. 8(6): 1757-1769 (2017).
[134] Nagata E, Sawa A, Ross CA, Snyder SH. Autophagosome-like vacuole formation in Huntington's disease lymphoblasts. Neuroreport. 15(8): 1325-8 (2004).
[135] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, *et al.* Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci. 13(5): 567-76 (2010).
[136] Juopperi TA, Kim WR, Chiang C-H, Yu H, Margolis RL, Ross CA, *et al.* Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. Mol Brain. 5(1): 17 (2012).
[137] Hall CE, Yao Z, Choi M, Tyzack GE, Serio A, Luisier R, *et al.* Progressive Motor Neuron Pathology and the Role of Astrocytes in a Human Stem Cell Model of VCP-Related ALS. Cell Rep. 19(9): 1739-1749 (2017).
[138] Qian K, Huang H, Peterson A, Hu B, Maragakis NJ, Ming G-L, *et al.* Sporadic ALS Astrocytes Induce Neuronal Degeneration *In Vivo*. Stem cell reports. 8(4): 843-855 (2017).
[139] Tyzack GE, Hall CE, Sibley CR, Cymes T, Forostyak S, Carlino G, *et al.* A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models. Nat Commun. 8(1): 1164 (2017).
[140] Verkhratsky A, Rodríguez JJ, Parpura V. Astroglia in neurological diseases. Future Neurol. 8(2): 149-158 (2013).
[141] Rossi D, Brambilla L, Valori CF, Roncoroni C, Crugnola A, Yokota T, *et al.* Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ. 15(11): 1691-700 (2008).
[142] Ben Haim L, Carrillo-de Sauvage M-A, Ceyzériat K, Escartin C. Elusive roles for reactive astrocytes in neurodegenerative diseases. Front Cell Neurosci. 9: 278 (2015).
[143] Springer S, Erlewein R, Naegele T, Becker I, Auer D, Grodd W, *et al.* Alexander disease--classification revisited and isolation of a neonatal form. Neuropediatrics. 31(2): 86-92 (2000).
[144] Russo LS, Aron A, Anderson PJ. Alexander's disease: a report and reappraisal. Neurology. 26(7): 607-14 (1976).
[145] van der Knaap MS, Ramesh V, Schiffmann R, Blaser S, Kyllerman M, Gholkar A, *et al.* Alexander disease: ventricular garlands and abnormalities of the medulla and spinal cord. Neurology. 66(4): 494-8 (2006).
[146] Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, Messing A. Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nat Genet. 27(1): 117-20 (2001).
[147] Iwaki T, Iwaki A, Tateishi J, Sakaki Y, Goldman JE. Alpha B-crystallin and 27-kd heat shock protein are regulated by stress

conditions in the central nervous system and accumulate in Rosenthal fibers. Am J Pathol. 143(2): 487-95 (1993).
[148] Sosunov AA, Guilfoyle E, Wu X, McKhann GM, Goldman JE. Phenotypic conversions of "protoplasmic" to "reactive" astrocytes in Alexander disease. J Neurosci. 33(17): 7439-50 (2013).
[149] Tang G, Perng MD, Wilk S, Quinlan R, Goldman JE. Oligomers of mutant glial fibrillary acidic protein (GFAP) Inhibit the proteasome system in alexander disease astrocytes, and the small heat shock protein alphaB-crystallin reverses the inhibition. J Biol Chem. 285(14): 10527-37 (2010).
[150] Olabarria M, Goldman JE. Disorders of Astrocytes: Alexander Disease as a Model. Annu Rev Pathol. 12(1): 131-152 (2017).
[151] Tian R, Wu X, Hagemann TL, Sosunov AA, Messing A, McKhann GM, *et al.* Alexander disease mutant glial fibrillary acidic protein compromises glutamate transport in astrocytes. J Neuropathol Exp Neurol. 69(4): 335-45 (2010).
[152] Hagemann TL, Gaeta SA, Smith MA, Johnson DA, Johnson JA, Messing A. Gene expression analysis in mice with elevated glial fibrillary acidic protein and Rosenthal fibers reveals a stress response followed by glial activation and neuronal dysfunction. Hum Mol Genet. 14(16): 2443-58 (2005).
[153] Olabarria M, Putilina M, Riemer EC, Goldman JE. Astrocyte pathology in Alexander disease causes a marked inflammatory environment. Acta Neuropathol. 130(4): 469-86 (2015).
[154] Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 362(4): 329-344 (2010).
[155] Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 256(5054): 184-5 (1992).
[156] Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. Nat Immunol. 16(3): 229-236 (2015).
[157] Moreno-Gonzalez I, Baglietto-Vargas D, Sanchez-Varo R, Jimenez S, Trujillo-Estrada L, Sanchez-Mejias E, *et al.* Extracellular amyloid-beta and cytotoxic glial activation induce significant entorhinal neuron loss in young PS1(M146L)/APP(751SL) mice. J Alzheimers Dis. 18(4): 755-76 (2009).
[158] Yeh C-Y, Vadhwana B, Verkhratsky A, Rodríguez JJ. Early astrocytic atrophy in the entorhinal cortex of a triple transgenic animal model of Alzheimer's disease. ASN Neuro. 3(5): 271-9 (2011).
[159] Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F, *et al.* Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging. 31(4): 578-90 (2010).
[160] Serrano-Pozo A, Gómez-Isla T, Growdon JH, Frosch MP, Hyman BT. A phenotypic change but not proliferation underlies glial responses in Alzheimer disease. Am J Pathol. 182(6): 2332-44 (2013).
[161] Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, *et al.* Genomic analysis of reactive astrogliosis. J Neurosci. 32(18): 6391-410 (2012).
[162] Osborn LM, Kamphuis W, Wadman WJ, Hol EM. Astrogliosis: An integral player in the pathogenesis of Alzheimer's disease. Prog Neurobiol. 144: 121-41 (2016).
[163] Doméné A, Cavanagh C, Page G, Bodard S, Klein C, Delarasse C, *et al.* Expression of Phenotypic Astrocyte Marker Is Increased in a Transgenic Mouse Model of Alzheimer's Disease versus Age-Matched Controls: A Presymptomatic Stage Study. Int J Alzheimers Dis. 2016: 5696241 (2016).
[164] Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, *et al.* High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci. 20(11): 4050-8 (2000).
[165] Furman JL, Sama DM, Gant JC, Beckett TL, Murphy MP, Bachstetter AD, *et al.* Targeting Astrocytes Ameliorates Neurologic Changes in a Mouse Model of Alzheimer's Disease. J Neurosci. 32(46): 16129-16140 (2012).
[166] Fernandez AM, Jimenez S, Mecha M, Dávila D, Guaza C, Vitorica J, *et al.* Regulation of the phosphatase calcineurin by insulin-like growth factor I unveils a key role of astrocytes in Alzheimer's pathology. Mol Psychiatry. 17(7): 705-18 (2012).
[167] Tan J, Town T, Crawford F, Mori T, DelleDonne A, Crescentini R, *et al.* Role of CD40 ligand in amyloidosis in transgenic Alzheimer's mice. Nat Neurosci. 5(12): 1288-93 (2002).
[168] Kraft AW, Hu X, Yoon H, Yan P, Xiao Q, Wang Y, *et al.* Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. FASEB J. 27(1): 187-98 (2013).
[169] Acosta C, Anderson HD, Anderson CM. Astrocyte dysfunction in Alzheimer disease. J Neurosci Res. 95(12): 2430-2447 (2017).
[170] González-Reyes RE, Nava-Mesa MO, Vargas-Sánchez K, Ariza-Salamanca D, Mora-Muñoz L. Involvement of Astrocytes in Alzheimer's Disease from a Neuroinflammatory and Oxidative Stress Perspective. Front Mol Neurosci. 10: 427 (2017).
[171] Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T, Zander N, *et al.* Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. J Alzheimers Dis. 11(1): 97-116 (2007).
[172] Xiu J, Nordberg A, Zhang J-T, Guan Z-Z. Expression of nicotinic receptors on primary cultures of rat astrocytes and up-regulation of the α7, α4 and β2 subunits in response to nanomolar concentrations of the β-amyloid peptide1-42. Neurochem Int. 47(4): 281-290 (2005).
[173] Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang H-Y. Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res. 971(2): 197-209 (2003).
[174] Carrero I, Gonzalo MR, Martin B, Sanz-Anquela JM, Arévalo-Serrano J, Gonzalo-Ruiz A. Oligomers of β-amyloid protein (Aβ1-42) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1β, tumour necrosis factor-α, and a nuclear factor κ-B mechanism in the rat brain. Exp Neurol. 236(2): 215-27 (2012).
[175] Apelt J, Schliebs R. Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. Brain Res. 894(1): 21-30 (2001).
[176] Benzing WC, Wujek JR, Ward EK, Shaffer D, Ashe KH, Younkin SG, *et al.* Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. Neurobiol Aging. 20(6): 581-9.
[177] Lian H, Litvinchuk A, Chiang AC-A, Aithmitti N, Jankowsky JL, Zheng H. Astrocyte-Microglia Cross Talk through Complement Activation Modulates Amyloid Pathology in Mouse Models of Alzheimer's Disease. J Neurosci. 36(2): 577-89 (2016).
[178] Liao Y-F, Wang B-J, Cheng H-T, Kuo L-H, Wolfe MS. Tumor necrosis factor-alpha, interleukin-1beta, and interferon-gamma stimulate gamma-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway. J Biol Chem. 279(47): 49523-32 (2004).
[179] Yang J, Zhang R, Shi C, Mao C, Yang Z, Suo Z, *et al.* AQP4 Association with Amyloid Deposition and Astrocyte Pathology in the Tg-ArcSwe Mouse Model of Alzheimer's Disease. J Alzheimers Dis. 57(1): 157-169 (2017).
[180] Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, *et al.* A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 4(147): 147ra111 (2012).
[181] Xiao Q, Yan P, Ma X, Liu H, Perez R, Zhu A, *et al.* Enhancing astrocytic lysosome biogenesis facilitates Aβ clearance and attenuates amyloid plaque pathogenesis. J Neurosci. 34(29): 9607-20 (2014).
[182] Wegiel J, Wang KC, Imaki H, Rubenstein R, Wronska A, Osuchowski M, *et al.* The role of microglial cells and astrocytes in fibrillar plaque evolution in transgenic APP(SW) mice. Neurobiol Aging. 22(1): 49-61.
[183] Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, *et al.* Adult mouse astrocytes degrade amyloid-beta *in vitro* and in situ. Nat Med. 9(4): 453-7 (2003).
[184] Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, *et al.* Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. Nat Med. 10(7): 719-26 (2004).
[185] Nielsen HM, Mulder SD, Beliën JAM, Musters RJP, Eikelenboom P, Veerhuis R. Astrocytic A beta 1-42 uptake is determined by A beta-aggregation state and the presence of amyloid-associated proteins. Glia. 58(10): 1235-46 (2010).
[186] Söllvander S, Nikitidou E, Brolin R, Söderberg L, Sehlin D, Lannfelt L, *et al.* Accumulation of amyloid-β by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. Mol Neurodegener. 11(1): 38 (2016).

[187] Pihlaja R, Koistinaho J, Malm T, Sikkilä H, Vainio S, Koistinaho M. Transplanted astrocytes internalize deposited beta-amyloid peptides in a transgenic mouse model of Alzheimer's disease. Glia. 56(2): 154-63 (2008).

[188] Jones RS, Minogue AM, Connor TJ, Lynch MA. Amyloid-β-induced astrocytic phagocytosis is mediated by CD36, CD47 and RAGE. J Neuroimmune Pharmacol. 8(1): 301-11 (2013).

[189] Sokolowski JD, Nobles SL, Heffron DS, Park D, Ravichandran KS, Mandell JW. Brain-specific angiogenesis inhibitor-1 expression in astrocytes and neurons: implications for its dual function as an apoptotic engulfment receptor. Brain Behav Immun. 25(5): 915-21 (2011).

[190] Provias J, Jeynes B. The role of the blood-brain barrier in the pathogenesis of senile plaques in Alzheimer's disease. Int J Alzheimers Dis. 2014: 191863 (2014).

[191] Husemann J, Silverstein SC. Expression of scavenger receptor class B, type I, by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and in Alzheimer's disease brain. Am J Pathol. 158(3): 825-32 (2001).

[192] Villarreal A, Seoane R, González Torres A, Rosciszewski G, Angelo MF, Rossi A, *et al.* S100B protein activates a RAGE-dependent autocrine loop in astrocytes: implications for its role in the propagation of reactive gliosis. J Neurochem. 131(2): 190-205 (2014).

[193] Mohajeri MH, Wollmer MA, Nitsch RM. Abeta 42-induced increase in neprilysin is associated with prevention of amyloid plaque formation *in vivo*. J Biol Chem. 277(38): 35460-5 (2002).

[194] Apelt J, Ach K, Schliebs R. Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of beta-amyloid plaques. Neurosci Lett. 339(3): 183-6 (2003).

[195] Yin K-J, Cirrito JR, Yan P, Hu X, Xiao Q, Pan X, *et al.* Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. J Neurosci. 26(43): 10939-48 (2006).

[196] Grehan S, Tse E, Taylor JM. Two distal downstream enhancers direct expression of the human apolipoprotein E gene to astrocytes in the brain. J Neurosci. 21(3): 812-22 (2001).

[197] Mulder SD, Nielsen HM, Blankenstein MA, Eikelenboom P, Veerhuis R. Apolipoproteins E and J interfere with amyloid-beta uptake by primary human astrocytes and microglia *in vitro*. Glia. 62(4): 493-503 (2014).

[198] Sanchez-Varo R, Trujillo-Estrada L, Sanchez-Mejias E, Torres M, Baglietto-Vargas D, Moreno-Gonzalez I, *et al.* Abnormal accumulation of autophagic vesicles correlates with axonal and synaptic pathology in young Alzheimer's mice hippocampus. Acta Neuropathol. 123(1): 53-70 (2012).

[199] Torres M, Jimenez S, Sanchez-Varo R, Navarro V, Trujillo-Estrada L, Sanchez-Mejias E, *et al.* Defective lysosomal proteolysis and axonal transport are early pathogenic events that worsen with age leading to increased APP metabolism and synaptic Abeta in transgenic APP/PS1 hippocampus. Mol Neurodegener. 7: 59 (2012).

[200] Sadleir KR, Kandalepas PC, Buggia-Prévot V, Nicholson DA, Thinakaran G, Vassar R. Presynaptic dystrophic neurites surrounding amyloid plaques are sites of microtubule disruption, BACE1 elevation, and increased Aβ generation in Alzheimer's disease. Acta Neuropathol. 132(2): 235-56 (2016).

[201] Masgrau R, Guaza C, Ransohoff RM, Galea E. Should We Stop Saying "Glia" and "Neuroinflammation"? Trends Mol Med. 23(6): 486-500 (2017).

[202] Sanchez-Mejias E, Navarro V, Jimenez S, Sanchez-Mico M, Sanchez-Varo R, Nuñez-Diaz C, *et al.* Soluble phospho-tau from Alzheimer's disease hippocampus drives microglial degeneration. Acta Neuropathol. 132(6): 897-916 (2016).

[203] Navarro V, Sanchez-Mejias E, Jimenez S, Muñoz-Castro C, Sanchez-Varo R, Davila JC, *et al.* Microglia in Alzheimer's Disease: Activated, Dysfunctional or Degenerative. Front Aging Neurosci. 10: 140 (2018).

[204] Jones VC, Atkinson-Dell R, Verkhratsky A, Mohamet L. Aberrant iPSC-derived human astrocytes in Alzheimer's disease. Cell Death Dis. 8(3): e2696 (2017).

[205] Agid Y. Parkinson's disease: pathophysiology. Lancet (London, England). 337(8753): 1321-4 (1991).

[206] Witjas T, Kaphan E, Azulay JP, Blin O, Ceccaldi M, Pouget J, *et al.* Nonmotor fluctuations in Parkinson's disease: frequent and disabling. Neurology. 59(3): 408-13 (2002).

[207] Forno LS, DeLanney LE, Irwin I, Di Monte D, Langston JW. Astrocytes and Parkinson's disease. Prog Brain Res. 94: 429-36 (1992).

[208] Booth HDE, Hirst WD, Wade-Martins R. The Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis. Trends Neurosci. 40(6): 358-370 (2017).

[209] Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, *et al.* The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. Brain. 127(Pt 2): 420-30 (2004).

[210] Kim J-M, Cha S-H, Choi YR, Jou I, Joe E-H, Park SM. DJ-1 deficiency impairs glutamate uptake into astrocytes via the regulation of flotillin-1 and caveolin-1 expression. Sci Rep. 6(1): 28823 (2016).

[211] Butchbach MER, Tian G, Guo H, Lin C-LG. Association of excitatory amino acid transporters, especially EAAT2, with cholesterol-rich lipid raft microdomains: importance for excitatory amino acid transporter localization and function. J Biol Chem. 279(33): 34388-96 (2004).

[212] Soni N, Reddy BVK, Kumar P. GLT-1 transporter: an effective pharmacological target for various neurological disorders. Pharmacol Biochem Behav. 127: 70-81 (2014).

[213] Danbolt NC. Glutamate uptake. Prog Neurobiol. 65(1): 1-105 (2001).

[214] Gu X-L, Long C-X, Sun L, Xie C, Lin X, Cai H. Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol Brain. 3(1): 12 (2010).

[215] Braak H, Sastre M, Del Tredici K. Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. Acta Neuropathol. 114(3): 231-41 (2007).

[216] Rannikko EH, Weber SS, Kahle PJ. Exogenous α-synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. BMC Neurosci. 16(1): 57 (2015).

[217] Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, *et al.* Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. Glia. 61(3): 349-60 (2013).

[218] Lee H-J, Suk J-E, Patrick C, Bae E-J, Cho J-H, Rho S, *et al.* Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem. 285(12): 9262-72 (2010).

[219] Ashley AK, Hinds AI, Hanneman WH, Tjalkens RB, Legare ME. DJ-1 mutation decreases astroglial release of inflammatory mediators. Neurotoxicology. 52: 198-203 (2016).

[220] Kim J, Choi D, Jeong H, Kim J, Kim DW, Choi SY, *et al.* DJ-1 facilitates the interaction between STAT1 and its phosphatase, SHP-1, in brain microglia and astrocytes: A novel anti-inflammatory function of DJ-1. Neurobiol Dis. 60: 1-10 (2013).

[221] Niranjan R. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Mol Neurobiol. 49(1): 28-38 (2014).

[222] Lev N, Barhum Y, Ben-Zur T, Melamed E, Steiner I, Offen D. Knocking out DJ-1 attenuates astrocytes neuroprotection against 6-hydroxydopamine toxicity. J Mol Neurosci. 50(3): 542-50 (2013).

[223] Mullett SJ, Hinkle DA. DJ-1 deficiency in astrocytes selectively enhances mitochondrial Complex I inhibitor-induced neurotoxicity. J Neurochem. 117(3): 375-87 (2011).

[224] Solano RM, Casarejos MJ, Menéndez-Cuervo J, Rodriguez-Navarro JA, García de Yébenes J, Mena MA. Glial dysfunction in parkin null mice: effects of aging. J Neurosci. 28(3): 598-611 (2008).

[225] Niranjan R, Nath C, Shukla R. The mechanism of action of MPTP-induced neuroinflammation and its modulation by melatonin in rat astrocytoma cells, C6. Free Radic Res. 44(11): 1304-16 (2010).

[226] A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 72(6): 971-83 (1993).

[227] Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP. Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol. 44(6): 559-77 (1985).

[228] Waldvogel HJ, Kim EH, Tippett LJ, Vonsattel J-PG, Faull RL. The Neuropathology of Huntington's Disease. In Current topics in behavioral neurosciences bll 33-80 (2014).

[229] Jansen AHP, van Hal M, Op den Kelder IC, Meier RT, de Ruiter A-A, Schut MH, *et al.* Frequency of nuclear mutant huntingtin inclusion formation in neurons and glia is cell-type-specific. Glia. 65(1): 50-61 (2017).

[230] Shin J-Y, Fang Z-H, Yu Z-X, Wang C-E, Li S-H, Li X-J. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol. 171(6): 1001-12 (2005).

[231] Khakh BS, Beaumont V, Cachope R, Munoz-Sanjuan I, Goldman SA, Grantyn R. Unravelling and Exploiting Astrocyte Dysfunction in Huntington's Disease. Trends Neurosci. 40(7): 422-437 (2017).

[232] Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, *et al. In vivo* expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet. 19(15): 3053-67 (2010).

[233] Gu X, André VM, Cepeda C, Li S-H, Li X-J, Levine MS, *et al.* Pathological cell-cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. Mol Neurodegener. 2(1): 8 (2007).

[234] Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, *et al.* Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet. 10(2): 137-44 (2001).

[235] Bradford J, Shin J-Y, Roberts M, Wang C-E, Sheng G, Li S, *et al.* Mutant huntingtin in glial cells exacerbates neurological symptoms of Huntington disease mice. J Biol Chem. 285(14): 10653-61 (2010).

[236] Ben Haim L, Ceyzériat K, Carrillo-de Sauvage MA, Aubry F, Auregan G, Guillermier M, *et al.* The JAK/STAT3 pathway is a common inducer of astrocyte reactivity in Alzheimer's and Huntington's diseases. J Neurosci. 35(6): 2817-29 (2015).

[237] Arzberger T, Krampfl K, Leimgruber S, Weindl A. Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease--an in situ hybridization study. J Neuropathol Exp Neurol. 56(4): 440-54 (1997).

[238] Hassel B, Tessler S, Faull RLM, Emson PC. Glutamate uptake is reduced in prefrontal cortex in Huntington's disease. Neurochem Res. 33(2): 232-7 (2008).

[239] Lee W, Reyes RC, Gottipati MK, Lewis K, Lesort M, Parpura V, *et al.* Enhanced Ca(2+)-dependent glutamate release from astrocytes of the BACHD Huntington's disease mouse model. Neurobiol Dis. 58: 192-9 (2013).

[240] Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD, *et al.* Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci. 17(5): 694-703 (2014).

[241] Jiang R, Diaz-Castro B, Looger LL, Khakh BS. Dysfunctional Calcium and Glutamate Signaling in Striatal Astrocytes from Huntington's Disease Model Mice. J Neurosci. 36(12): 3453-70 (2016).

[242] Valenza M, Marullo M, Di Paolo E, Cesana E, Zuccato C, Biella G, *et al.* Disruption of astrocyte-neuron cholesterol cross talk affects neuronal function in Huntington's disease. Cell Death Differ. 22(4): 690-702 (2015).

[243] Boussicault L, Alves S, Lamazière A, Planques A, Heck N, Moumné L, *et al.* CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. Brain. 139(Pt 3): 953-70 (2016).

[244] Hsiao H-Y, Chen Y-C, Huang C-H, Chen C-C, Hsu Y-H, Chen H-M, *et al.* Aberrant astrocytes impair vascular reactivity in Huntington disease. Ann Neurol. 78(2): 178-92 (2015).

[245] Rothstein JD, Martin LJ, Kuncl RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N Engl J Med. 326(22): 1464-8 (1992).

[246] Yamanaka K, Komine O. The multi-dimensional roles of astrocytes in ALS. Neurosci Res. 126: 31-38 (2018).

[247] Barbeito LH, Pehar M, Cassina P, Vargas MR, Peluffo H, Viera L, *et al.* A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. Brain Res Brain Res Rev. 47(1-3): 263-74 (2004).

[248] Bruijn LI, Miller TM, Cleveland DW. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci. 27(1): 723-49 (2004).

[249] Lepore AC, Rauck B, Dejea C, Pardo AC, Rao MS, Rothstein JD, *et al.* Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. Nat Neurosci. 11(11): 1294-301 (2008).

[250] Papadeas ST, Kraig SE, O'Banion C, Lepore AC, Maragakis NJ. Astrocytes carrying the superoxide dismutase 1 (SOD1G93A) mutation induce wild-type motor neuron degeneration *in vivo*. Proc Natl Acad Sci U S A. 108(43): 17803-8 (2011).

[251] Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, *et al.* Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci U S A. 99(3): 1604-9 (2002).

[252] Pardo AC, Wong V, Benson LM, Dykes M, Tanaka K, Rothstein JD, *et al.* Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1(G93A) mice. Exp Neurol. 201(1): 120-30 (2006).

[253] Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol. 38(1): 73-84 (1995).

[254] Van Damme P, Bogaert E, Dewil M, Hersmus N, Kiraly D, Scheveneels W, *et al.* Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity. Proc Natl Acad Sci U S A. 104(37): 14825-30 (2007).

[255] Brambilla L, Guidotti G, Martorana F, Iyer AM, Aronica E, Valori CF, *et al.* Disruption of the astrocytic TNFR1-GDNF axis accelerates motor neuron degeneration and disease progression in amyotrophic lateral sclerosis. Hum Mol Genet. 25(14): 3080-3095 (2016).

[256] Cassina P, Cassina A, Pehar M, Castellanos R, Gandelman M, de León A, *et al.* Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. J Neurosci. 28(16): 4115-22 (2008).

[257] Madji Hounoum B, Mavel S, Coque E, Patin F, Vourc'h P, Marouillat S, *et al.* Wildtype motoneurons, ALS-Linked SOD1 mutation and glutamate profoundly modify astrocyte metabolism and lactate shuttling. Glia. 65(4): 592-605 (2017).

[258] Das PK, Rambukkana A, Baas JG, Groothuis DG, Halperin M. Enzyme-linked immunosorbent assay for distinguishing serological responses of lepromatous and tuberculoid leprosies to the 29/33-kilodalton doublet and 64-kilodalton antigens of Mycobacterium tuberculosis. J Clin Microbiol. 28(2): 379-82 (1990).

[259] Endo F, Komine O, Fujimori-Tonou N, Katsuno M, Jin S, Watanabe S, *et al.* Astrocyte-derived TGF-β1 accelerates disease progression in ALS mice by interfering with the neuroprotective functions of microglia and T cells. Cell Rep. 11(4): 592-604 (2015).

[260] Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, *et al.* Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci. 10(5): 615-22 (2007).

[261] Haidet-Phillips AM, Gross SK, Williams T, Tuteja A, Sherman A, Ko M, *et al.* Altered astrocytic expression of TDP-43 does not influence motor neuron survival. Exp Neurol. 250: 250-9 (2013).

[262] Re DB, Le Verche V, Yu C, Amoroso MW, Politi KA, Phani S, *et al.* Necroptosis drives motor neuron death in models of both sporadic and familial ALS. Neuron. 81(5): 1001-1008 (2014).

[263] Song S, Miranda CJ, Braun L, Meyer K, Frakes AE, Ferraiuolo L, *et al.* Major histocompatibility complex class I molecules protect motor neurons from astrocyte-induced toxicity in amyotrophic lateral sclerosis. Nat Med. 22(4): 397-403 (2016).

[264] Kawamata H, Ng SK, Diaz N, Burstein S, Morel L, Osgood A, *et al.* Abnormal intracellular calcium signaling and SNARE-dependent exocytosis contributes to SOD1G93A astrocyte-mediated toxicity in amyotrophic lateral sclerosis. J Neurosci. 34(6): 2331-48 (2014).

[265] Almad AA, Doreswamy A, Gross SK, Richard J-P, Huo Y, Haughey N, *et al.* Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. Glia. 64(7): 1154-69 (2016).

[266] Arranz AM, De Strooper B. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. Lancet Neurol. 18(4): 406-414 (2019).

[267] Verkhratsky A, Steardo L, Parpura V, Montana V. Translational potential of astrocytes in brain disorders. Prog Neurobiol. 144: 188-205 (2016).

[268] Robinson SR. Neuronal expression of glutamine synthetase in Alzheimer's disease indicates a profound impairment of metabolic interactions with astrocytes. Neurochem Int. 36(4-5): 471-82 (2000).
[269] Dossi E, Vasile F, Rouach N. Human astrocytes in the diseased brain. Brain Res Bull. 136: 139-156 (2018).
[270] Buffo A, Rolando C, Ceruti S. Astrocytes in the damaged brain: molecular and cellular insights into their reactive response and healing potential. Biochem Pharmacol. 79(2): 77-89 (2010).
[271] Sofroniew M V. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32(12): 638-47 (2009).
[272] Pekny M, Pekna M. Astrocyte Reactivity and Reactive Astrogliosis: Costs and Benefits. Physiol Rev. 94(4): 1077-1098 (2014).