Astrocyte–neuron interactions in neurological disorders

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Abstract Astrocytes have long been considered as just providing trophic support for neurons in the central nervous system, but recently several studies have highlighted their importance in many functions such as neurotransmission, metabolite and electrolyte homeostasis, cell signaling, inflammation, and synapse modulation. Astrocytes are, in fact, part of a bidirectional crosstalk with neurons. Moreover, increasing evidence is stressing the emerging role of astrocyte dysfunction in the pathophysiology of neurological disorders, including neurodegenerative disease, stroke, epilepsy, migraine, and neuroinflammatory diseases.

Keywords Astrocytes · Neurological diseases · Neurodegeneration · Glial cells

1 Biological bases of astrocyte–neuron interactions

1.1 Introduction

For several neurodegenerative diseases, the pathogenic mechanism remains unknown. Neurons and astrocytes represent a very specialized functional unit, and nowadays it is becoming clear that astrocyte function goes well beyond neurotrophic support, considering many other cell interactions. Recent evidence has stressed the link between astrocytes and several neurological disorders, such as migraine, epilepsy, stroke, inflammatory diseases, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, through pathways that include inflammation, oxidative stress, cell signaling, necrosis, and apoptosis. A better understanding of these interactions could be useful also in view of the possible development of new therapeutical approaches.

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In this paper, we review the principal astrocyte functions and the role they play in many famous neurological diseases.

Astrocytes are the most numerous subtypes of glial cells within the central nervous system (CNS). The cell body and the major processes of astrocytes are enriched with glial fibrillary acidic protein (GFAP) that forms intermediate filaments, whose recognition by Golgi staining is the reason for the classically star-shaped appearance of astrocytes [1]. At least four types of GFAP-positive cells have been identified. The most frequent type of astrocyte in the CNS gray matter is *protoplasmatic astrocyte* that resides in the deeper layers (two to six) of the cortex, which appears progressively more elaborated through the phylogenesis and enrichment of nonoverlapping branched processes. In layer 1 of the cortex, where there is a high number of synapses, primate-specific astrocytes are described, called *interlaminar astrocytes*, whose processes cross the cortical layers, terminating in layers 3 or 4. A third type of cortical astrocyte is *polarized astrocyte*, a unipolar cell that resides in deep layers, near the white matter, with a morphology more similar to a neuron than to an astrocytic cell, despite its intense GFAP labeling. The other main astrocyte type is represented by the *fibrous astrocyte* of the white matter, a class of cells similar in primate and nonprimate mammals, less complex than protoplasmatic astrocytes, probably responsible for the traditional supportive role assigned to glial cells [2].

The distribution of protoplasmatic astrocytes throughout the cortex is highly organized and their processes identify volumetrically defined nonoverlapping compartments of notable uniformity: in each domain, a contiguous cohort of synapses interacts exclusively with a single astrocyte [1, 3]. Furthermore, astrocytes form an extended glial syncytium in which neurons are intimately embedded [4]. In fact, the cytoplasm of astrocytes are strongly coupled to each other by gap junctions and are, occasionally, also coupled to the cytoplasm of neurons and oligodendrocytes (*heterotypic coupling*) [5, 6]. The gap junction channels allow intercellular passive diffusion but are also permeable to endogenous signaling molecules, such as inositol (1,4,5)-triphosphate (IP3) [7], as well as glucose and its metabolites, glutamate, glutamine, and lactate [8]. Therefore, we have to consider astrocytes not as individual elements but as a coordinated group of communicating cells.

In the past, astroglial cells have traditionally been considered as passive elements and satellite cells of the CNS, providing a metabolic support for neurons and regulating the extracellular homeostasis. However, some evidence in the last decade has revealed that astrocytes are also actively involved in the control of neuronal functions, communication pathways, and plasticity, being able to receive signals from neurons and releasing neuroactive substances [4].

Interestingly, almost to confirm their central role, it was observed that the ratio of astrocytes to neurons increases dramatically with phylogenesis and brain complexity [2, 9]. It has been suggested that the greater abundance of astrocytes with evolution and, most importantly, the relative predominance of astrocytes in the human brain cannot be explained only on the basis of different glial metabolic supply but could be a consequence of greater degrees of local modulation and control required by the more sophisticated neuronal network [10].

Astrocytes are electrically nonexcitable, with a constant resting membrane potential. This stability and their low input resistance are likely to reflect the fact that gap junctions effectively couple many of these cells into an electrical syncytium. However, it was observed that their membrane potential is also able to display fluctuations in response to a wide variety of stimuli [11, 12]. In fact, in astrocytes, there are cytosolic calcium oscillations due to a mobilization from calcium stores triggered by mechanical stimulation or exposure to neurotransmitters, including glutamate, gamma-aminobutyric acid (GABA), and adenosine triphosphate (ATP), in a phospholipase-C- and IP3-dependent pathway [13]. In particular,

phospholipase C, activated by G-protein-coupled receptors, generates the second messenger IP₃, responsible for calcium release from intracellular stores, thus producing calcium waves [14]. It has been suggested that changes in glial calcium are essential in the communication between neurons and astrocytes. According to this hypothesis, transmitters released by neurons (including glutamate, norepinephrine, 5-hydroxytryptamine, acetylcholine, ATP, GABA, and endothelin) induce transient elevation of cytosolic calcium in astrocytes; on the other hand, the increase of astrocytic calcium triggers the astrocytes' release of chemical transmitters that can modulate the activity of neighboring neurons [15]. Through the gap junction channels, the endogenous signaling molecules, such as IP₃ and calcium, propagate from astrocyte to astrocyte and can be bidirectional, with the realization of a network of communicating cells.

Therefore, we can briefly regard astrocytes as an integral part of the neuron–glia system through their many housekeeping functions, including structural support, neuronal metabolism, maintenance of the extracellular environment, regulation of cerebral blood flow, stabilization of cell–cell communications, neurotransmitter synthesis, and defense against oxidative stress [16].

The principal astrocytes functions are explained below, focusing specifically on those functions that can have a pivotal role in different processes, such as neuroprotection and neurodegeneration.

1.2 Regulation of cerebral blood flow

The blood–brain barrier (BBB) is a diffusion barrier that prevents the exchange of molecules between the two compartments, in order to defend the brain from harmful substances circulating in the blood [17]. Astrocytes enwrap the vessel wall with a large number of end feet, but their exact role in the BBB is poorly defined [18]. Several factors released by astrocytes might be important for the induction and maintenance of the BBB. According to this hypothesis, previous studies have identified agents released by astrocytes (for example, transforming growth factor alpha (TGF- α) and glial-derived neurotrophic factor (GDNF)) that play the role of supporting the formation of tight junctions in cultured endothelial cells [17]. The tight organization of astrocytes around the vasculature is thought to be due to the necessity of glucose to reach neurons. In fact, it was hypothesized that astrocytes take up glucose since they express a large number of glucose transporters, convert it to lactate, and then deliver lactate to neurons [19]. The expression of the astrocytic glucose transporter GLUT-1 is increased by mechanical, chemical, or electrical stimuli able to trigger calcium waves that are propagated into astrocytes and endothelial cells [7]. It was also observed that the protein aquaporin-A (AQP4), the main water channel in the brain, is markedly expressed in astrocytic end feet that surround capillaries and in ependymal cells. This localization suggests the involvement of AQP4 in water flux throughout the BBB [20], even though little is known about regulation of the protein's expression. Interestingly, it was observed that the expression of AQP4 is negatively modulated by protein kinase C activator in cultured astrocytes [21], according to the hypothetical active role of the astrocytes in BBB functions [14].

1.3 Role of astrocytes in brain metabolism

The brain is one of the most metabolically active organs of the human body and uses about 20% of the glucose and the oxygen consumed by the body [4, 22]. In addition to glucose

metabolism, an intense amino acid metabolism occurs in the brain due to protein turnover and the synthesis and degradation of amino acids and neurotransmitters. The astrocytes are positioned between brain capillaries and neurons and provide nutrition to neurons [23] via an intensive intercellular metabolic exchange. Astrocytes express key enzymes of several metabolic pathways, which are not expressed in neurons, and produce, starting from a variety of exogenous precursors, metabolic intermediates, which are key substrates for the energy metabolism [4].

In particular, lactate, which is terminal product of anaerobic glucose metabolism and of the catabolism of other hexoses [24], has been considered an important precursor for oxidative ATP production in neurons. According to “the astrocyte–neuron lactate shuttle hypothesis,” neural activity increases the extracellular concentration of glutamate, which is taken up by glia, thus stimulating $\text{Na}^+\text{-K}^+\text{-ATPase}$ and glutamine synthetase activity. This promotes glial anaerobic glycolysis, which is responsible for the conversion of glucose to lactate. The latter is then released by glia and converted by neurons to pyruvate to fuel their activity [25]. It was postulated that lactate might be used by both glia and neurons after neural activity when glycolytic rates slow down and provide some substrate during prolonged activity; it might also be important during recovery from pathological insults [26]. Other important energy metabolites released from astrocytes to neurons are ketone bodies, derived from fatty acids [27] or leucine [28].

Astrocytes have also an important role in lipid metabolism and in particular in the cholesterol pathway. Although the CNS accounts for 2% of the whole-body mass, it contains 25% of the total-body cholesterol. Interestingly, glial cells mainly produce brain cholesterol in situ and peripheral tissues regulate its metabolism independently. In the astrocytic compartment, cholesterol is synthesized and secreted bound to apolipoprotein E to be carried to neuronal cells [29].

Finally, astrocytes have also an important role in the regulation of ion concentrations and of acid–base balance in the intracellular and extracellular spaces of the brain. For example, the extracellular potassium, which accumulates from neural activity, is buffered through potassium channels (Kir) expressed by astrocytes at synapses and at end-foot processes around capillaries [30].

1.4 Astrocytes and oxidative stress

Reactive oxygen species (ROS) are continuously generated during cellular oxidative metabolism. The imbalance between generation and elimination of ROS causes “oxidative stress,” which can compromise essential cellular functions and appears to be an important factor influencing aging and the progression of neurodegenerative diseases, as a final common pathogenic mechanism. Compared to other organs, the brain produces ROS at a high rate but apparently has less antioxidant capacity [31, 32]. Astrocytes are considered to play an important role in supporting other brain cells in the defense against ROS-induced toxicity. In fact, they contain the highest levels of various antioxidants in the brain [31]. In particular, it was observed that astrocytes provide the extracellular precursors for the neuronal antioxidant glutathione (GSH) system. The availability of precursors for the synthesis of GSH appears to be crucial. The best extracellular glutamate precursor for neuronal GSH synthesis is glutamine, which is part of the glutamate–glutamine cycle between neurons and astrocytes [33]. However, only the availability of extracellular cysteine limits GSH synthesis in neurons, but cysteine, as well as glycine, can be provided from astrocytes by release of GSH [34, 35] and consecutive cleavage of the tripeptide by the

action of γ -glutamyl transpeptidase and an ectopeptidase [31]. Consequently, precursors for all three amino acids necessary for neuronal glutathione synthesis are provided by astrocytes to neurons.

1.5 Role of astrocytes in synaptic regulation

Plasticity of synaptic transmission is believed to be the cellular basis for learning and memory. Synaptic plasticity is defined as a change in efficacy of synapses that is mediated by various presynaptic and postsynaptic mechanisms. A wide variety of changes can occur presynaptically to alter transmitter release properties and postsynaptically to modify the responsiveness to transmitter release such as receptor trafficking. These changes can result in increased efficacy, termed *potentiation*, or in a reduction of synaptic efficacy, termed *depression*. Additionally, these changes in synaptic efficacy can be accompanied and supported by morphological changes such as synaptic density and synaptic coverage.

Although they are not excitable cells, astrocytes appear intimately involved with synapses at all stages of development and during adult life. In fact, it was observed that astrocytes can induce the formation of synapses through the secretion of synaptogenic substances and secrete additional signals that regulate both presynaptic and postsynaptic function. They contribute to the maintenance of synaptic structure and to the correct pattern of innervation as well as providing bidirectional signals for neurons to coordinate the network events [36–39]. Astrocytes also have a role in balancing dendritic plasticity and stability.

The process of synapse formation involves structural modifications in both axon terminals and dendrites. For regulating this process, one of the most important candidate signaling systems is the ephrin/Eph receptors. Ephrin ligands and their Eph receptors guide axons during neural development and regulate synapse formation and neuronal plasticity in the adult. It was observed [40] that the protein ephrin is expressed on astrocytic processes and negatively regulates dendritic spine expansion. Therefore, when astrocytes do not surround a dendritic spine, they do not make the necessary contact to initiate Eph signaling, thus allowing further elaboration of the spine [41]. In this way, the astrocytes appear involved also in the regulation of synapse elimination.

It has been found that glial cells not only modify neuronal plasticity, but they are themselves also plastic and can change in response to neuronal activity [42]. In fact, neuronal cells control the gap junctional communication (GJC) in astrocytes in several situations. Neurons release bioactive molecules, including neurotransmitters, peptides, and lipids, which stimulate astrocytic receptors and then modify the activity (short-term regulation) or the expression (long-term regulation) of connexin channels that form the gap junctions [43]. Moreover, functional studies performed with cocultures of neurons/astrocytes and with pure cultures of astrocytes have demonstrated that GJC expression and activity in astrocytes depends on synaptic activity in neurons [6]. Finally, neuronal destruction or nerve injury was also shown to alter astrocytic GJC, with connexin redistribution [44, 45].

1.6 Role of astrocytes in the release of neuroactive substances

The uptake of neurotransmitters released from neuronal terminals in the synapses is an important function of astrocytes [46]. Moreover, astrocytes are also able to release neuroactive substances, including neuropeptides, growth factors, eicosanoids, and steroids [10]. Although the mechanisms involved in neuroactive substance release from astrocytes in

neuronal communications are recognized as being of relevance, the regulation of this astroglial capacity is still poorly understood. It is supposed that astrocyte–neuron communication allows a bidirectional pathway. One of the most important mechanisms by which astrocytes seem to modulate synaptic transmission is the release of glutamate. The release of glutamate can be determined either by application of agents able to increase intracellular calcium in astrocytes (i.e., noradrenalin) or by mechanical or electrical stimulation [4]. Interestingly, previous studies have reported that glutamate released during neuronal activity evokes an intracellular calcium increase in neighboring astrocytes that is itself necessary and sufficient to induce the release of glutamate from astrocytes. This calcium-dependent glutamate release can suggest a regulated exocytotic mechanism. According to this hypothesis, it was observed that glutamate release is blocked in astrocytes after incubation with substances such as bafilomycin A1 and botulinum neurotoxin, which are able to impair the vesicular pathway [47]. Other studies, instead, have provided evidence for channel-mediated release of glutamate. The major arguments for the latter are that several channel blockers reversibly inhibit release of glutamate; furthermore, glutamate is not released in isolation but, rather, together with other amino acids present in high concentration in astrocytes [48].

Moreover, astrocytes are involved also in the “glutamate–glutamine shuttle” that is, the pathway for glutamate to be returned to neurons. During glutamatergic neurotransmission, glutamate is released by neurons into the synaptic cleft and leads to the depolarization of postsynaptic membrane. Extracellular glutamate is rapidly inactivated by astrocytic uptake [49, 50], which is mediated mainly by the glutamate transporter EAAT2/GLT-1. Inside the astrocyte, glutamate is converted to glutamine by the ATP-consuming reaction catalyzed by glutamine synthetase, an enzyme which is present in astrocytes but not in neurons [51]. Glutamine, which lacks neurotransmitter activity is then released from astrocytes and rapidly shuttled back into neurons, where it is converted to glutamate by the phosphate-activated glutaminase [52]. Consequently, regeneration of the neurotransmitter glutamate and metabolic coupling during energy production are important tasks of the cooperation between astrocytes and neurons.

Another important transmitter released from astrocytes is ATP, able to modulate the functions of gap junction channels. The release of ATP seems to be calcium-independent. It was postulated that gap junction channels could function as a release pore for ATP [53]. Subsequently, released ATP propagates a signal wave via activation of purinoceptors [54]. The purinoceptor activation can stimulate trophic signaling pathways, through the activation of protein kinase [55] or changes in gene expression [56].

1.7 Astrocytes and inflammatory response in central nervous system

Astrocytes play a role in neuroinflammatory response in cooperation with the microglial cells. Microglia is a population of brain-resident macrophages that represent the first line of defense during the innate immune response of the CNS [57, 58]. Microglial cells undergo a rapid activation within pathological conditions, including changes in the structural integrity of the brain, but also during very subtle alterations of the microenvironment. Microglial activation is characterized by proliferation, migration into the damaged area, expression of immune-related antigens, and eventual differentiation into phagocytic cells [59, 60]. Activated microglia upregulate a variety of surface receptors, including the major histocompatibility complex (MHC) and complement receptors [61], and release a variety of proinflammatory mediators such as cytokines, chemokine, proteolytic enzymes, reactive oxygen species, and complement proteins [62]. Moreover, some of these factors can also act

on astrocytes or both cell types, thus contributing to perpetuation of ongoing inflammation. Astrocytes, in fact, seem to play an important regulatory role in the processes of microglial differentiation and activation in response to inflammation, also considering their role in the antigen-presenting process [63]. In brain injury, extracellular glutamate levels are increased and astrocytes are stimulated, thus producing calcium wave propagations and releasing nucleosides and nucleotides that are able in turn to stimulate proliferation of microglia [64–67], with a wide production of molecules, including cytokines.

The cytokines interleukin 1β (IL- 1β), interferon- γ (IFN- γ), and tumor necrosis factor (TNF- α) are important for microglial activation because they induce proliferation and immunophenotypical/functional changes in microglial cells; however, they also act on astrocytes, thus creating cascade effects contributing both to perpetuation of inflammation by a vicious cycle and to affecting neuronal function. As examples for that, the following observations can be quoted: (1) the release of IL-1 by microglia leads to the inhibition of calcium waves in the astrocytic network, by a downregulation of connexin 43, the main protein of the gap junction [65]; (2) TNF- α reduces astroglial glutamate uptake capacity, this being important in the buffering of neuronal glutamate excitotoxicity [14].

Astrocytes, together with microglia, also release trophic factors such as basic fibroblast growth factor, nerve growth factor (NGF), ciliary neurotrophic factor, and S100 β , thus promoting neuroplasticity and rebuilding of the nervous system after injury [14]. The release of trophic factors is possibly mediated by ATP, which triggers a form of calcium communication between astrocytes and microglia in tissue repair pathways by stimulating purinergic receptors [68, 69].

2 Aspects of astrocyte involvement in neurological disorders

2.1 Stroke

Cerebral ischemia (stroke) triggers a complex series of biochemical and molecular mechanisms that impair the neurologic functions through breakdown of cellular integrity mediated by excitotoxic glutamatergic signaling, ionic imbalance, and free radical reactions.

The severity of ischemic damage depends on the spreading of the blood flow reduction that induces the deprivation of glucose and oxygen in tissue. The brain is particularly vulnerable to ischemia. Under physiological conditions, the cerebral blood flow (CBF) is strictly regulated and normally maintained around 50 to 60 mL/100 g per minute. The astrocytes play a pivotal role in cerebrovascular regulation and throughout the close relationship with neurons and blood vessels; they can modulate neuronal activity and cerebral blood flow. When the CBF falls to <7 mL/100 g per minute, the ischemic damage becomes irreversible; furthermore, the duration of ischemia is another factor predictive of the degree of damage. The ischemic core is surrounded by a peripheral region called *penumbra*, where the CBF ranges from 7 to 17 mL/100 g per minute. The penumbral region is metabolically active but electrically silent and represents the most successful target for therapy because it is functionally impaired but potentially saveable [70]. During cerebral ischemia, the metabolic activity of cerebral cells is suppressed and, consequently, the structural integrity of cells is damaged, with secondary activation of several mechanisms, including glutamate-mediated excitotoxicity, oxidative stress, stress signaling, and neuroinflammation, and finally cell death. The glutamate-mediated excitotoxicity is caused by an overstimulation of the postsynaptic glutamate receptors which is due to an impaired glutamate uptake by astrocytes

from the synaptic cleft [71, 72]. Glutamate, normally maintained in a strict range, is the major excitatory neurotransmitter in CNS; its metabotropic and ionotropic receptors, once activated, determine initiation of postsynaptic signaling and final mobilization of calcium from internal stores. In turn, calcium is an important regulator of several cellular pathways, responsible for neurotransmitter release, modulation of neuronal excitability, and synaptic plasticity. In pathological conditions, such as ischemic injury, the excitotoxicity mechanism induces a cascade of events, with final activation of proteases, endonucleases, and lipases, leading to breakage of cellular structure and genome and therefore to neurodegeneration. The oxygen and glucose deprivation during ischemia causes mitochondrial dysfunction and blockade of pyruvate oxidation, thus reducing ATP production. In turn, the decrease of ATP also inhibits membrane ion pumps, leading to a loss of cellular and mitochondrial ion gradients with cellular calcium influx that can trigger glutamate release directly, contributing to neuronal excitotoxicity [73].

Another key event in pathophysiology of stroke injury is oxidative stress, caused by the reduced availability of oxygen or, conversely, by the reperfusion of ischemic areas [74]. A free radical overproduction exceeds the physiological cellular capacity of detoxification, with secondary irreversible damage of proteins, lipids, and nucleotides that culminate in cell death. Moreover, astrocytes are known to be the main source of antioxidant defense in preventing neuronal death after ischemic insult. In fact, during ischemia/reperfusion, one of the major changes observed in rat brain is a loss of GSH [75] due to a prolonged glucose deprivation, as observed in previous studies *in vitro* [76]. This depletion of astrocytic GSH has been shown to correlate with an increase in neuronal death [77].

During ischemia, also observed are BBB breakdown, loss of endothelial tight junctions, and secondary possible hemorrhagic transformations of the ischemic zone [78]. In addition, the altered integrity of the BBB can allow the extravasations of inflammatory vascular cells and molecules, which are in turn harmful to brain cells [79] and contribute to ischemic injury. The inflammatory response, generated at the BBB interface few hours after the onset of stroke, is mediated by several cytokines and adhesion molecules released from leukocytes, endothelial cells, astrocytes, and microglia [70, 80, 81].

For all these reasons, the astrocytes are believed to be critical players in pathophysiology of ischemia, as well as for their capacity to produce and release growth factors and other molecules that are able to support the neuroregeneration [82]. Interestingly, astrocytes seem to be less sensitive to injury than neurons. In fact, it was demonstrated that, after temporary ischemia, a portion of astrocytes within the ischemic core remains metabolically active in the early phases after reperfusion [83]. Additionally, the astrocytes are responsible for glial scar formation, which is an attempt to isolate the damaged zone and protect the surviving cells [82].

Astrocytic involvement in degeneration pathways after stroke also appears interesting for the development of new therapeutic targets and strategies. Additionally, tamoxifen, a selective estrogen receptor modulator that is used in the treatment of breast cancer, has been recently studied for its neuroprotective action during ischemic insult [84, 85]. In fact, this drug seems able to act as inhibitor of the astrocytic swelling that occurs early after stroke [86], supporting the hypothesis of the central role of astrocytes in modulating the susceptibility to ischemic injury.

Indeed, one of the most hoped-for therapeutic goals is to preserve or, even improve the functional recovery after ischemic damage of the surviving neuronal networks. The involvement of astrocytes in synaptic regeneration and cortical plasticity can represent a possible target for regenerative and rehabilitative strategies [87].

2.2 Migraine

Migraine is a neurological syndrome characterized by unilateral and pulsating headaches lasting from 4 to 72 h, associated with nausea, photophobia, and hyperacusis. Approximately, in one third of patients, migraine is preceded by an array of symptoms, including visual, olfactory, and sensitive deficits, called aura [88].

Astrocytes are thought to play a role in the pathogenesis of migraine with aura, in particular in the genesis of cortical spreading depression (CSD), which is the result of a propagating wave of neural hyper-excitability throughout the cortex, followed by a transient long-lasting suppression [89]. CSD, which is associated with neurovascular modifications such as meningeal blood vessel vasodilatation and activation of perivascular trigeminal afferents, is believed to be related to the basis of aura and headache. CSD that can be also involved in the pathogenesis of other cerebral pathologies such as ischemia and trauma [90] involves both neurons and glial cells. During CSD, transient ion redistributions between the extracellular and intracellular space take place; in particular, there is an increase in extracellular potassium (K^+) concentration and sodium (Na^+) and calcium (Ca^{2+}) flux toward the intracellular compartment. For this reason, astrocytes, contributing to K^+ regulation, are supposed to play a role in CSD pathogenesis by liberating intracellular components to the extracellular space and by facilitating the propagation of Ca^{2+} waves, which are crucial for neural depolarization [91, 92]. The following restoration of ionic homeostasis is energy dependent. CSD could possibly propagate through astrocyte gap junctions, which are useful for intercellular K^+ and Ca^{2+} movement [93]. Depolarization is also associated with glutamate release from astrocytes.

Further evidence of the importance of astrocyte function in the pathogenesis of CSD is derived from patients with familial hemiplegic migraine type 2, an autosomal-dominant migraine subtype [94], where the threshold for CSD is reduced by a mutation involving the astrocyte $\alpha 2$ subunit of $Na^+-K^+-ATPase$ [95].

2.3 Epilepsy

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive, or synchronous neuronal activity in the brain. There are many different epilepsy syndromes, each presenting with its own unique combination of seizure type, typical age of onset, electroencephalography findings, treatment, and prognosis. In *focal epilepsies*, seizures arise from an epileptic focus, a small portion of the brain that acts as an irritant driving the epileptic response. *Generalized epilepsies*, in contrast, arise from many independent foci or from epileptic circuits that involve the whole brain [96].

Inflammatory reactions, including astrocyte activation, are part of the pathophysiology of human epilepsy and also in animal models of epilepsy [97]. Astrocytes are known to be involved in regulation of BBB permeability and endothelial stability, which can both be impaired in neurological diseases associated with epilepsy, such as focal ischemia, trauma, infections, or autoimmune disorders. Indeed, after brain injury, there is an increase of proinflammatory cytokines like IL- 1β and IL-6 in the cerebrospinal fluid as a consequence of upregulation of endothelial adhesion molecules. Moreover, steroids and immunomodulators like adrenocorticotrophic hormone are used to treat some forms of unresponsive epilepsy in children. In addition, genetic susceptibility to inflammation is linked to increased risk to develop epilepsy.

During brain injury, the BBB is damaged, with consequent exposure of neuronal tissue to albumin [98], which is taken up by astrocytes, leading to a downregulation of astrocyte Kir channels. Kir channels are important for the K^+ buffering in the extracellular space. During neural excitation, K^+ is released in the synaptic cleft, thus increasing extracellular K^+ concentration. If this increase is not corrected by astrocytic Kir-channel-mediated K^+ influx, the neuronal membranes undergo sustained depolarization. In fact, reduced astrocyte Kir expression has been observed to be involved in the mechanism causing neuronal hyperexcitability in some forms of focal epilepsy, such as hippocampal mesial sclerosis [99] and tuberous sclerosis [100]. In patients with these forms of focal epilepsy, some authors also found a reduction of EAAT2 immunoreactivity, meaning that astrocytes could be less effective at glutamate uptake from the synaptic cleft [101].

Astrocytes are also a possible source of stored Ca^{2+} , and it is known that paroxysmal depolarization can be induced by Ca^{2+} release from astrocytes; moreover, the fact that several antiepileptic drugs like valproate, phenytoin, and gabapentin are able to reduce Ca^{2+} signaling suggests that astrocytes could be a possible therapeutic target for epileptic patients [102].

In summary, then, astrocytes are involved in epileptogenesis through mechanisms that include regulation in K^+ , Ca^{2+} , and glutamate homeostasis.

2.4 Inflammatory demyelinating diseases: multiple sclerosis

Multiple sclerosis (MS) is defined as a T-cell-mediated autoimmune demyelinating disorder of the CNS, characterized by inflammatory cell infiltration and focal demyelinated lesions of the white matter. Recent evidences suggest that astrocytes play a double role in MS, by both limiting and enhancing inflammation, depending on the conditions.

Astrocytes exert protective effects in the injured CNS by increasing the production of brain-derived neurotrophic factor (BDNF) and NGF, thus promoting neuronal survival. Astrocytes are also actively involved in regulation of BBB permeability, through cytokine secretion; in particular, the release of IL-6, TNF- α , and IL-1 β by astrocytes during inflammation is able to increase BBB permeability, while astrocyte TGF- β production induces tight junction narrowing [103]. Interwoven astrocytic process contribute to the formation of the glial scar around demyelinated regions, limiting tissue damage. Moreover, cytokines released by astrocytes, like IL-6 and IL-1 β , are actively involved in the remyelination process, by enhancing oligodendrocyte functions and CNS repair, as demonstrated in animal models [104, 105].

On the other hand, astrocyte dysfunction leads to a series of adverse effects in MS. First, they can contribute to both innate and adaptive immunity in the CNS. The former is possible through astrocyte expression of pattern recognition receptors that, when activated, induce a cascade of events that contribute to the immune response. Astrocytes also express complement receptors [106] and they can act as “nonprofessional”-presenting cells (APC) in MS, through MHC class II and B-7 costimulatory molecule expression. The latter event occurs since astrocytes in MS white matter lack the expression of β 2-adrenergic receptors, which are necessary for cyclic adenosine monophosphate (cAMP) production. cAMP is a molecule that in normal conditions prevents the induction of MHC class II molecules by inhibition of the MHC class II transactivator protein in astrocytes [107]. During inflammation, astrocytes produce cytokines like IL-1, IL-6, TNF- α , IL-10, and TGF- β , which are involved in the immune response [108]. Astrocytes contribute to T cell migration in the CNS through chemokine release and production of adhesion molecules like

intercellular adhesion molecule 1 and vascular adhesion molecule 1 [109]. The reduction in cAMP production in MS also leads to harm in astrocytic glycogenolysis, with consequent energy impairment and axonal loss [110].

Astrocyte dysfunction in MS can lead to reduced trophic factor production, in particular a lack of neuregulin, thus contributing to oligodendrocyte apoptosis. Astrocytes in MS are also able to inhibit remyelination by the formation of the glial scar through the production of astrocytic processes held together by tight junctions. The glial scar is formed in order to limit the inflammation region, but, unfortunately, it also inhibits the entering of oligodendrocyte progenitor cells into the demyelinated areas, limiting remyelination [111].

All these data suggest that astrocyte function may be a useful target on which to focus the next generation of MS therapy research.

2.5 Neurodegenerative diseases

2.5.1 *Parkinson's disease*

Parkinson's disease (PD) is a chronic progressive neurodegenerative movement disorder characterized by motor involvement including resting tremor, bradykinesia, postural instability, and rigidity due to selective loss of dopaminergic neurons, mainly in the substantia nigra pars compacta [112]. The most useful treatment for PD remains the administration of a precursor of dopamine, L-DOPA, which is able to reduce almost all PD symptoms, even though with severe side effects. Therefore, there is an urgent need to attain a deeper understanding of the etiopathology of PD, in order to develop new therapies aimed at halting its progress, with fewer side effects.

Among the pathogenetic factors supposed to play a role in the propagation of the neurodegenerative process in PD, there is glial reaction, which is present in PD patients as well as in experimental models of PD. Recent evidence suggests that glial cells may have either a neuroprotective or a deleterious effect on dopaminergic neurons in PD depending on their activation status [113, 114]. In particular, astrocytes are supposed to play a protective role for dopaminergic neurons by producing neurotrophic factors, such as GDNF, which has been detected in the human striatum during development [115], and BDNF, which is able to support the survival and process outgrowth of dopaminergic structures in the striatum [116], even though there are no similar data in human PD. Another neuroprotective function of astrocytes is their scavenger activity against oxidative stress, a common feature of neurodegeneration [117]. Several data support the presence of reactive oxygen species (ROS) in the cascade of events leading to dopaminergic neuron degeneration, including increased lipid peroxidation and protein and nucleic acid alteration in the substantia nigra of patients with PD [118]. Moreover, these cells contain neuromelanin, the formation of which is associated with the production of free radicals [119], and high levels of iron (which catalyzes the formation of hydroxyl radicals) have also been reported in the substantia nigra of patients with PD [120]. ROS can be detoxified by glutathione peroxidase, an enzyme that prevents the transformation of hydrogen peroxide to highly toxic hydroxyl radicals, which is present in astrocytes of the human mesencephalon [32]. The role of glutathione in the pathophysiology of PD is stressed by the evidence of decreased levels of glutathione in its reduced state in the parkinsonian substantia nigra [121]. In PD, an important source of ROS production in the substantia nigra derives from the catabolism of dopamine by an autoxidation mechanism. It is demonstrated that astrocytes can protect neurons from ROS effects, as shown in *in vitro* studies [122]. Astrocytes, which express

enzymes involved in the catabolism of dopamine, such as monoamine oxidase type B and catechol-*O*-methyltransferase [123] and also contain high levels of glutathione peroxidase, can manage dopamine metabolism intracellularly, without significant injury and preventing neurons from oxidative damage.

Besides the protective role of glial cells in PD, there are other evidences that the same cells, maybe in different conditions, participate to the progression of the degenerative process. Inflammation is one of the pathogenetic mechanisms hypothesized in PD. Although neuronal damage in the substantia nigra in PD can be initiated by different mechanisms including environmental toxins and genetic factors, there is evidence that activation of neuroinflammatory cells contributes to disease progression. In fact, while dopaminergic neurons of the substantia nigra are particularly vulnerable to oxidative and inflammatory attack, glial response can mediate a variety of deleterious events related to inflammation, such as production of prooxidant reactive species, proinflammatory prostaglandin, and cytokines which contribute to neuronal damage and therefore to disease progression.

It is known that the density of glial cells expressing TNF- α , INF- γ , and IL-1 β is increased in the parkinsonian substantia nigra [124]. Furthermore, in the caudate nucleus and putamen of patients with PD are reported elevated levels of other proinflammatory cytokines, such as the concentrations of IL-2, IL-4, and IL-6 [125, 126]. These data indicate that increased cytokine production is associated with the neurodegenerative process observed in PD.

Another feature of PD is an upregulation of MHC molecules, which are pivotal for modulating the immune response. In particular, in the substantia nigra of PD patients, increased numbers of HLA-DR (MHC class II)-positive microglia have been shown [127], while the light chain of MHC class I, β 2-microglobulin, is increased in the striatum of PD patients [128].

Recent evidence implies involvement of cytokines in the activation of nitric oxide production in glial cells [124]. TNF- α , IL-1 β , and IFN- γ are potent activators of inducible nitric oxide synthase (iNOS) in rodent glia cells, by inducing the expression of a molecule called CD23. CD23 is a low-affinity immunoglobulin E receptor that is expressed on the cell membrane of various cell types after stimulation by cytokines [129]. Recently, cytokine induction of CD23 has been reported in an astrocytoma cell line. CD23 is exclusively expressed in the substantia nigra of PD patients but is not detectable in control subjects [124]. The activation of CD23 induces iNOS expression and leads to the release of high amounts of nitric oxide (NO). In addition, the NO could stimulate the production of proinflammatory cytokines, such as TNF- α and IL-6 in glial cells [130], and it can release iron from the iron-buffering protein ferritin [131], thus exerting a toxic effect, since free iron is itself extremely toxic and it can also strengthen the formation of hydroxyl radicals. ROS can be generated by various reactions, among which is the cyclooxygenase-2 (COX-2) reaction, which generates prostanoids linked to pathological events [132]. In fact, increased levels of COX-2 and of PGE2 are described in PD [133]. Therefore, it seems that the cytokines produced by glial cells may reinforce oxidative stress by producing nitric oxide, activating COX-2, and increasing the levels of free iron.

Cytokines may also participate more directly in the molecular events leading to nerve cell death. Glial-derived cytokines can bind to specific cell surface receptors (e.g., TNF- α receptor) and thus act directly on dopaminergic neurons and activate proapoptotic pathways. Dopaminergic neurons have indeed been shown to possess increased levels of TNF- α receptors in PD [134]. The activation of this receptor leads to a cascade of events that induce the activation of caspase-8 and of caspase-3, which brings to neuronal cell

death. Astrocyte activation, which can be mediated by proinflammatory cytokines such as IL-1 β and TNF- α , is presumed to contribute to the propagation of the neurodegenerative process.

Taken altogether, these data suggest that astrocytes play a role, even though not at the initial stage of disease, in the progression of PD. Therefore, a pharmacological manipulation of these mechanisms could be a possible therapeutic target to follow.

2.5.2 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of age-related cognitive decline, characterized by progressive memory deficits, cognitive impairment, and personality changes. The brain in patients affected by AD shows severe atrophy, with a reduction in brain weight of usually more than 35%, consequentially a progressive neurodegeneration of limbic and cortical brain structures, mainly in the temporal lobe. The histopathological hallmarks include senile plaques, resulting from extracellular deposition of β -amyloid ($A\beta$) and intraneurofibrillary tangles (NT) constituted by abnormally phosphorylated tau protein. According to the "amyloid cascade hypothesis," $A\beta$ can adversely affect distinct molecular and cellular pathways, thereby facilitating tau phosphorylation and aggregation in NT, and it can lead to an acceleration of neurodegenerative mechanisms involved in metabolism, cellular detoxification, mitochondrial dysfunction, and energy deficiency. Senile plaques are also closely associated with activated microglia and astrocytes.

Less than 5% of cases of AD has an early onset and shows an autosomal-dominant pattern of inheritance. The genetic mutations (*APP*, *PS1*, and *PS2*) alter the proteolytic cleavage of the amyloid precursor proteins (APP) and increase the production of more fibrillogenic peptide β -amyloid ($A\beta$ -42), with consequential aggregation in senile plaque and neurodegeneration ("amyloid cascade hypothesis"). Several mechanisms of $A\beta$ -induced neurotoxicity have been proposed, including oxidative stress, free radical formation, disrupted calcium homeostasis, induction of apoptosis, and chronic inflammation. Therefore, most forms of AD are sporadic and probably, like other neurodegenerative diseases, have a complex etiology due to environmental and genetic factors which, taken alone, are not sufficient to cause the disease to develop. Many genes have been investigated for their potential role in modulating the risk to develop AD. The apolipoprotein E (APOE) gene, in particular the APOE ϵ 4 allele, is recognized as a major risk factor for sporadic late-onset cases of AD; in fact, individuals bearing APOE ϵ 4 allele show an increased risk (around 50%) to develop AD [135].

APOE, which is postulated to be a major lipid carrier protein in the brain, is synthesized and secreted primarily by astrocytes and is involved in brain development and repair. It was supposed that the isoform apolipoprotein E4 (APOE4) might contribute to developing AD by reducing the phagocytosis of deposited $A\beta$ by astrocytes. Astrocytes, in fact, are recruited probably through chemotactic molecules generated by $A\beta$ deposits and thereby participate in $A\beta$ clearance and degradation, forming a protective barrier between $A\beta$ plaques and neurons [58]. In contrast, astrocytes are also thought to increase $A\beta$ deposition with the overexpression, probably in response to a chronic stress, of β -secretase BACE1, which cleaves the APP together with γ -secretase [136]. Therefore, the exact role of astrocytes in $A\beta$ clearance and/or deposition remains today unclear [58].

In the literature, several immunohistological and molecular findings are reported to indicate inflammatory processes as constant element in AD pathogenesis. After injuries like $A\beta$ exposure, activated microglia and astrocytes play a pivotal role in the defense

of neuronal tissue but, in turn, may represent a risk for their possible transformation into potentially harmful cells by the release of cytokines such as TNF- α , IL-1 β , and IL-6 that directly impair neuronal functions [137], thus perpetuating neurodegeneration mechanisms.

Inflammatory response is also associated, as previously mentioned, with an increase of oxidative stress. In this regard, astrocytes could have a double action: they contribute to microglia activation, but, on the other hand, they can exert a negative feedback on the microglia NO production, thus limiting membrane homeostasis perturbation and neuronal apoptosis [138]. In addition, glial activation and inflammatory response could also be a very early event in AD, occurring even in the absence of A β deposition, as demonstrated in the APPV717I transgenic mouse model [139].

Another possible mechanism involving astrocyte dysfunction in AD could be BBB impairment. BBB function is critical for A β peptide clearance. The BBB regulates A β transport from and to the brain throughout two main receptors, respectively, the LDL-receptor-related protein 1 (LRP-1) and the receptor for advanced glycation end products (RAGE), which perform opposite functions. These receptors play an important role in the balance between A β synthesis and clearance and are both expressed in endothelial and astroglial cells. Severe AD is associated with significant changes in the relative distribution of RAGE and LRP-1 in human hippocampus, compared with age-matched controls [140, 141].

Astrogliosis and reactive astrocytosis are common features of aging and AD. Interestingly, it was observed that, in most cortical areas of patients with severe AD, interlaminar astrocytes are found to be markedly altered or absent and replaced by other cortical hypertrophic astrocytes. The loss of interlaminar astrocytes, which appear relatively late in phylogeny, are likely to be a distinct pathological event that may alter the neocortical glial–neuronal network in AD, although the underlying meaning remains unknown [142].

Last, an alteration of astrocytic calcium signaling is another important feature of AD that is probably able to exert a pathogenic role in the disease. Calcium is important in regulating several different functions of astrocytes; in particular, calcium waves in astrocytes can transfer signals to neurons and modulate their activity. Exposure of cultured astrocytes to A β alters calcium wave signaling, causing an increase in the amplitude and velocity of evoked calcium waves and thus interfering with the neuron–astrocyte communication pathway [143].

3 Conclusions

On the basis of all this evidence, the physiological role of glial cells in neurodegeneration appears to be partly contrasting. However, glial cells' dysfunction may alter their capacity to regulate CNS homeostasis and secondarily promote development or progression of neurological disorders. For this reason, a possible goal for neuroscience researchers could be a deeper understanding of altered astrocyte–neuron interaction in order to develop therapeutic strategies able to modify the activity of glial cells, reduce their neurotoxic effects, and enhance their neuroprotective action.

References

1. Bushong, E.A., Martone, M.E., Jones, Y.Z., Ellisman, M.H.: Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* **22**, 183–192 (2002)

2. Oberheim, N.A., Wang, X., Goldman, S., Nedergaard, M.: Astrocytic complexity distinguishes the human brain. *Trends Neurosci.* **29**(10), 547–553 (2006). doi:[10.1016/j.tins.2006.08.004](https://doi.org/10.1016/j.tins.2006.08.004)
3. Ogata, K., Kosaka, T.: Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* **113**, 221–233 (2002). doi:[10.1016/S0306-4522\(02\)00041-6](https://doi.org/10.1016/S0306-4522(02)00041-6)
4. Kirchhoff, F., Dringen, R., Giaume, C.: Pathways of neuron–astrocyte interactions and their possible role in neuroprotection. *Eur. Arch. Psychiatry Clin. Neurosci.* **251**(4), 159–169 (2001). doi:[10.1007/s004060170036](https://doi.org/10.1007/s004060170036)
5. Alvarez-Maubecin, V., Garcia-Hernandez, F., Williams, J.T., Van Bockstaele, E.J.: Functional coupling between neurons and glia. *J. Neurosci.* **20**(11), 4091–4098 (2000)
6. Rouach, N., Glowinski, J., Giaume, C.: Activity-dependent neuronal control of gap-junctional communication in astrocytes. *J. Cell Biol.* **149**, 1513–1526 (2000). doi:[10.1083/jcb.149.7.1513](https://doi.org/10.1083/jcb.149.7.1513)
7. Leybaert, L., Paemeleire, K., Strahonja, A., Sanderson, M.J.: Inositol-triphosphate-dependent intercellular calcium signalling in and between astrocytes and endothelial cells. *Glia* **24**, 398–407 (1998). doi:[10.1002/\(SICI\)1098-1136\(199812\)24:4<398::AID-GLIA5>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1098-1136(199812)24:4<398::AID-GLIA5>3.0.CO;2-R)
8. Medina, J.M., Giaume, C., Tabernero, A.: Metabolic coupling and the role played by astrocytes in energy distribution and homeostasis. *Adv. Exp. Med. Biol.* **468**, 361–371 (1999)
9. Bass, N.H., Hess, H.H., Pope, A., Thalheimer, C.: Quantitative cytoarchitectonic distribution of neurons, glia, and DNA in rat cerebral cortex. *J. Comp. Neurol.* **143**, 481–490 (1971). doi:[10.1002/cne.901430405](https://doi.org/10.1002/cne.901430405)
10. Nedergaard, M., Ransom, B., Goldman, S.A.: New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.* **26**, 523–530 (2003). doi:[10.1016/j.tins.2003.08.008](https://doi.org/10.1016/j.tins.2003.08.008)
11. Nett, W.J., Oloff, S.H., McCarthy, K.D.: Hippocampal astrocytes in situ exhibit calcium oscillations that occur independent of neuronal activity. *J. Neurophysiol.* **87**, 528–537 (2002)
12. Parri, H.R., Gould, T.M., Crunelli, V.: Spontaneous astrocytic Ca²⁺ oscillations in situ drive NMDAR-mediated neuronal excitation. *Nat. Neurosci.* **4**, 803–812 (2001). doi:[10.1038/90507](https://doi.org/10.1038/90507)
13. Volterra, A., Meldolesi, J.: Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* **6**(8), 626–640 (2005). doi:[10.1038/nrn1722](https://doi.org/10.1038/nrn1722)
14. Hansson, E., Rönnbäck, L.: Glial neuronal signaling in the central nervous system. *FASEB J.* **17**(3), 341–348 (2003). doi:[10.1096/fj.02-0429rev](https://doi.org/10.1096/fj.02-0429rev)
15. Zonta, M., Carmignoto, G.: Calcium oscillations encoding neuron-to-astrocyte communication. *J. Physiol. (Paris)* **96**(3–4), 193–198 (2002). doi:[10.1016/S0928-4257\(02\)00006-2](https://doi.org/10.1016/S0928-4257(02)00006-2)
16. Maragakis, N.J., Rothstein, J.D.: Mechanisms of disease: astrocytes in neurodegenerative disease. *Nat. Clin. Pract. Neurol.* **2**(12), 679–689 (2006). doi:[10.1038/ncpneuro0355](https://doi.org/10.1038/ncpneuro0355)
17. Abbott, N.J.: Astrocyte–endothelial interactions and blood–brain barrier permeability. *J. Anat.* **200**, 629–638 (2002). doi:[10.1046/j.1469-7580.2002.00064.x](https://doi.org/10.1046/j.1469-7580.2002.00064.x)
18. Del Zoppo, G.J., Hallenbeck, J.M.: Advances in the vascular pathophysiology of ischemic stroke. *Thromb. Res.* **98**, 73–81 (2000). doi:[10.1016/S0049-3848\(00\)00218-8](https://doi.org/10.1016/S0049-3848(00)00218-8)
19. Takano, T., Tian, G.F., Peng, W., Lou, N., Libionka, W., Han, X., Nedergaard, M.: Astrocyte-mediated control of cerebral blood flow. *Nat. Neurosci.* **9**(2), 260–267 (2006). doi:[10.1038/nn1623](https://doi.org/10.1038/nn1623)
20. Rubino, E., Rainero, I., Vaula, G., Crasto, F., Gravante, E., Negro, E., Brega, F., Gallone, S., Pinessi, L.: Investigating the genetic role of aquaporin4 gene in migraine. *J. Headache Pain* **10**(2), 111–114 (2009). doi:[10.1007/s10194-009-0100-z](https://doi.org/10.1007/s10194-009-0100-z)
21. Nakahama, K., Nagano, M., Fujioka, A., Shinoda, K., Sasaki, H.: Effect of TPA on aquaporin 4 mRNA expression in cultured rat astrocytes. *Glia* **25**, 240–246 (1999). doi:[10.1002/\(SICI\)1098-1136\(19990201\)25:3<240::AID-GLIA4>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1098-1136(19990201)25:3<240::AID-GLIA4>3.0.CO;2-C)
22. Clarke, D.D., Sokoloff, L.: Circulation and energy metabolism of the brain. In: Sigel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (eds.) *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, pp. 637–699. Lippincott-Raven, Philadelphia (1999)
23. Somjen, G.G.: Nervenkitzel: notes on the history of the concept of neuroglia. *Glia* **1**, 2–9 (1988). doi:[10.1002/glia.440010103](https://doi.org/10.1002/glia.440010103)
24. Wiesinger, H., Hamprecht, B., Dringen, R.: Metabolic pathways for glucose in astrocytes. *Glia* **21**, 22–34 (1997). doi:[10.1002/\(SICI\)1098-1136\(199709\)21:1<22::AID-GLIA3>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1098-1136(199709)21:1<22::AID-GLIA3>3.0.CO;2-3)
25. Chih, C.P., Roberts, E.L., Jr.: Energy substrates for neurons during neural activity: a critical review of the astrocyte–neuron lactate shuttle hypothesis. *J. Cereb. Blood Flow Metab.* **23**(11), 1263–1281 (2003). doi:[10.1097/01.WCB.0000081369.51727.6F](https://doi.org/10.1097/01.WCB.0000081369.51727.6F)
26. Chih, C.P., Lipton, P., Roberts, E.L., Jr.: Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci.* **24**(10), 573–578 (2001). doi:[10.1016/S0166-2236\(00\)01920-2](https://doi.org/10.1016/S0166-2236(00)01920-2)
27. Auestad, N., Korsak, R.A., Morrow, J.W., Edmond, J.: Fatty acid oxidation and ketogenesis by astrocytes in primary culture. *Neurochemistry* **56**, 1376–1386 (1991). doi:[10.1111/j.1471-4159.1991.tb11435.x](https://doi.org/10.1111/j.1471-4159.1991.tb11435.x)

28. Bixel, M.G., Hamprecht, B.: Generation of ketone bodies from leucine by cultured astroglial cells. *J. Neurochem.* **65**, 2450–2461 (1995)
29. Canevari, L., Clark, J.B.: Alzheimer's disease and cholesterol: the fat connection. *Neurochem. Res.* **32**(4–5), 739–750 (2007). doi:[10.1007/s11064-006-9200-1](https://doi.org/10.1007/s11064-006-9200-1)
30. Kofuji, P., Newman, E.A.: Potassium buffering in the central nervous system. *Neuroscience* **129**(4), 1045–1056 (2004). doi:[10.1016/j.neuroscience.2004.06.008](https://doi.org/10.1016/j.neuroscience.2004.06.008)
31. Dringen, R., Gutterer, J.M., Hirrlinger, J.: Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *Eur. J. Biochem.* **267**, 4912–4916 (2000). doi:[10.1046/j.1432-1327.2000.01597.x](https://doi.org/10.1046/j.1432-1327.2000.01597.x)
32. Schulz, J.B., Lindenau, J., Seyfried, J., Dichgans, J.: Glutathione, oxidative stress and neurodegeneration. *Eur. J. Biochem.* **267**, 4904–4911 (2000). doi:[10.1046/j.1432-1327.2000.01595.x](https://doi.org/10.1046/j.1432-1327.2000.01595.x)
33. Kranich, O., Hamprecht, B., Dringen, R.: Different preferences in the utilization of amino acids for glutathione synthesis in cultured neurons and astroglial cells derived from rat brain. *Neurosci. Lett.* **219**, 211–214 (1996). doi:[10.1016/S0304-3940\(96\)13217-1](https://doi.org/10.1016/S0304-3940(96)13217-1)
34. Dringen, R., Kranich, O., Hamprecht, B.: The gamma-glutamyl transpeptidase inhibitor acivicin preserves glutathione released by astroglial cells in culture. *Neurochem. Res.* **22**, 727–733 (1997). doi:[10.1023/A:1027310328310](https://doi.org/10.1023/A:1027310328310)
35. Dringen, R., Pfeiffer, B., Hamprecht, B.: Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J. Neurosci.* **19**, 562–569 (1999)
36. Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., Malenka, R.C.: Control of synaptic strength by glial TNF alpha. *Science* **295**, 2282–2285 (2002). doi:[10.1126/science.1067859](https://doi.org/10.1126/science.1067859)
37. Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A., Pfrieger, F.W.: CNS synaptogenesis promoted by glia-derived cholesterol. *Science* **294**, 1354–1357 (2001). doi:[10.1126/science.294.5545.1354](https://doi.org/10.1126/science.294.5545.1354)
38. Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., Barres, B.A.: Control of synapse number by glia. *Science* **291**, 657–661 (2001). doi:[10.1126/science.291.5504.657](https://doi.org/10.1126/science.291.5504.657)
39. Pfrieger, F.W., Barres, B.A.: Synaptic efficacy enhanced by glial cells in vitro. *Science* **277**, 1684–1687 (1997). doi:[10.1126/science.277.5332.1684](https://doi.org/10.1126/science.277.5332.1684)
40. Murai, K.K., Nguyen, L.N., Irie, F., Yamaguchi, Y., Pasquale, E.B.: Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat. Neurosci.* **6**, 153–160 (2003). doi:[10.1038/nn994](https://doi.org/10.1038/nn994)
41. Klein, R.: Bidirectional modulation of synaptic functions by Eph/ephrin signalling. *Nat. Neurosci.* **12**, 15–20 (2009). doi:[10.1038/nn.2231](https://doi.org/10.1038/nn.2231)
42. Piet, R., Vargova, L., Sykova, E., Poulain, D.A., Oliet, S.H.: Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 2151–2155 (2004). doi:[10.1073/pnas.0308408100](https://doi.org/10.1073/pnas.0308408100)
43. Giaume, C., McCarthy, K.D.: Control of gap-junctional communication in astrocytic networks. *Trends Neurosci.* **19**(8), 319–325 (1996). doi:[10.1016/0166-2236\(96\)10046-1](https://doi.org/10.1016/0166-2236(96)10046-1)
44. Todd, K.J., Serrano, A., Lacaille, J.C., Robitaille, R.: Glial cells in synaptic plasticity. *J. Physiol. (Paris)* **99**, 75–83 (2006). doi:[10.1016/j.jphysparis.2005.12.002](https://doi.org/10.1016/j.jphysparis.2005.12.002)
45. Allen, N.J., Barres, B.A.: Signaling between glia and neurons: focus on synaptic plasticity. *Curr. Opin. Neurobiol.* **15**(5), 542–548 (2005). doi:[10.1016/j.conb.2005.08.006](https://doi.org/10.1016/j.conb.2005.08.006)
46. Anderson, C.M., Swanson, R.A.: Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32**, 1–14 (2000). doi:[10.1002/1098-1136\(200010\)32:1<1::AID-GLIA10>3.0.CO;2-W](https://doi.org/10.1002/1098-1136(200010)32:1<1::AID-GLIA10>3.0.CO;2-W)
47. Araque, A., Li, N., Doyle, R.T., Haydon, P.G.: SNARE protein-dependent glutamate release from astrocytes. *J. Neurosci.* **20**, 666–673 (2000)
48. Nedergaard, M., Takano, T., Hansen, A.J.: Beyond the role of glutamate as neurotransmitter. *Nat. Rev. Neurosci.* **3**, 748–755 (2002). doi:[10.1038/nrn916](https://doi.org/10.1038/nrn916)
49. Clements, J.D., Lester, R.A., Tong, G., Jahr, C.E., Westbrook, G.L.: The time course of glutamate in the synaptic cleft. *Science* **258**, 1498–1501 (1992). doi:[10.1126/science.1359647](https://doi.org/10.1126/science.1359647)
50. Bergles, D.E., Jahr, C.E.: Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. *J. Neurosci.* **18**, 7709–7716 (1998)
51. Ye, Z.C., Wyeth, M.S., Baltan-Tekkok, S., Ransom, B.R.: Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J. Neurosci.* **23**(9), 3588–3596 (2003)
52. Kvamme, E., Roberg, B., Torgner, I.A.: Phosphate-activated glutaminase and mitochondrial glutamine transport in the brain. *Neurochem. Res.* **25**, 1407–1419 (2000). doi:[10.1023/A:1007668801570](https://doi.org/10.1023/A:1007668801570)
53. Cotrina, M.L., Lin, J.H., Lopez-Garcia, J.C., Naus, C.C., Nedergaard, M.: ATP-mediated glia signaling. *J. Neurosci.* **20**, 2835–2844 (2000)

54. Fam, S.R., Gallagher, C.J., Salter, M.W.: P2Y(1) purinoceptor mediated Ca(2+) signaling and Ca(2+) wave propagation in dorsal spinal cord astrocytes. *J. Neurosci.* **20**, 2800–2808 (2000)
55. Neary, J.T., McCarthy, M., Cornell-Bell, A., Kang, Y.: Trophic signaling pathways activated by purinergic receptors in rat and human astroglia. *Prog. Brain Res.* **120**, 323–332 (1999). doi:[10.1016/S0079-6123\(08\)63566-9](https://doi.org/10.1016/S0079-6123(08)63566-9)
56. Priller, J., Reddington, M., Haas, C.A., Kreutzberg, G.W.: Stimulation of P2Y-purinoceptors on astrocytes results in immediate early gene expression and potentiation of neuropeptide action. *Neuroscience* **85**(2), 521–525 (1998). doi:[10.1016/S0306-4522\(97\)00653-2](https://doi.org/10.1016/S0306-4522(97)00653-2)
57. Conde, J.R., Streit, W.J.: Microglia in the aging brain. *J. Neuropathol. Exp. Neurol.* **65**, 199–203 (2006)
58. Heneka, M.T., O'Banion, M.K.: Inflammatory processes in Alzheimer's disease. *J. Neuroimmunol.* **184**, 69–91 (2007). doi:[10.1016/j.jneuroim.2006.11.017](https://doi.org/10.1016/j.jneuroim.2006.11.017)
59. Fetter, L., Amigorena, S.: Neuroscience. Brain under surveillance: the microglia patrol. *Science* **309**, 392–393 (2005). doi:[10.1126/science.1114852](https://doi.org/10.1126/science.1114852)
60. Banati, R.B., Gehrmann, J., Schubert, P., Kreutzberg, G.W.: Cytotoxicity of microglia. *Glia* **7**(1), 111–118 (1993). doi:[10.1002/glia.440070117](https://doi.org/10.1002/glia.440070117)
61. Liu, B., Hong, J.S.: Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J. Pharmacol. Exp. Ther.* **304**, 1–7 (2003). doi:[10.1124/jpet.102.035048](https://doi.org/10.1124/jpet.102.035048)
62. Bruce-Keller, A.J.: Microglial–neuronal interactions in synaptic damage and recovery. *J. Neurosci. Res.* **58**, 191–201 (1999). doi:[10.1002/\(SICI\)1097-4547\(19991001\)58:1<191::AID-JNR17>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-4547(19991001)58:1<191::AID-JNR17>3.0.CO;2-E)
63. Frohman, E.M., van den Noort, S., Gupta, S.: Astrocytes and intracerebral immune responses. *J. Clin. Immunol.* **9**(1), 1–9 (1989). doi:[10.1007/BF00917121](https://doi.org/10.1007/BF00917121)
64. Schipke, C.G., Boucsein, C., Ohlemeyer, C., Kirchhoff, F., Kettenmann, H.: Astrocyte Ca²⁺ waves trigger responses in microglial cells in brain slices. *FASEB J.* **16**(2), 255–257 (2002)
65. Ciccarelli, R., Di Iorio, P., D'Alimonte, I., Giuliani, P., Florio, T., Caciagli, F., Middlemiss, P.J., Rathbone, M.P.: Cultured astrocyte proliferation induced by extracellular guanosine involves endogenous adenosine and is raised by the co-presence of microglia. *Glia* **29**, 202–211 (2000). doi:[10.1002/\(SICI\)1098-1136\(20000201\)29:3<202::AID-GLIA2>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1098-1136(20000201)29:3<202::AID-GLIA2>3.0.CO;2-C)
66. Illes, P., Nörenberg, W., Gebicke-Haerter, P.J.: Molecular mechanisms of microglial activation. B. Voltage- and purinoceptor-operated channels in microglia. *Neurochem. Int.* **29**, 13–24 (1996). doi:[10.1016/0197-0186\(95\)00133-6](https://doi.org/10.1016/0197-0186(95)00133-6)
67. John, G.R., Scemes, E., Suadicani, S.O., Liu, J.S., Charles, P.C., Lee, S.C., Spray, D.C., Brosnan, C.F.: IL-1 β differentially regulates calcium wave propagation between primary human fetal astrocytes via pathways involving P2 receptors and gap junction channels. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 11613–11618 (1999) doi:[10.1073/pnas.96.20.11613](https://doi.org/10.1073/pnas.96.20.11613)
68. Verderio, C., Matteoli, M.: ATP mediates calcium signaling between astrocytes and microglial cells: modulation by IFN- γ . *J. Immunol.* **166**, 6383–6391 (2001)
69. Heales, S.J., Lam, A.A., Duncan, A.J., Land, J.M.: Neurodegeneration or neuroprotection: the pivotal role of astrocytes. *Neurochem. Res.* **29**(3), 513–519 (2004). doi:[10.1023/B:NERE.0000014822.69384.0f](https://doi.org/10.1023/B:NERE.0000014822.69384.0f)
70. Mehta, S.L., Manhas, N., Raghubir, R.: Molecular targets in cerebral ischemia for developing novel therapeutics. *Brain Res. Rev.* **54**(1), 34–66 (2007). doi:[10.1016/j.brainresrev.2006.11.003](https://doi.org/10.1016/j.brainresrev.2006.11.003)
71. Giffard, R.G., Swanson, R.A.: Ischemia-induced programmed cell death in astrocytes. *Glia* **50**(4), 299–306 (2005). doi:[10.1002/glia.20167](https://doi.org/10.1002/glia.20167)
72. Mori, T., Tateishi, N., Kagamiishi, Y., Shimoda, T., Satoh, S., Ono, S., Katsube, N., Asano, T.: Attenuation of a delayed increase in the extracellular glutamate level in the peri-infarct area following focal cerebral ischemia by a novel agent ONO-2506. *Neurochem. Int.* **45**(2–3), 381–387 (2004). doi:[10.1016/j.neuint.2003.06.001](https://doi.org/10.1016/j.neuint.2003.06.001)
73. Bambrick, L., Kristian, T., Fiskum, G.: Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. *Neurochem. Res.* **29**(3), 601–608 (2004). doi:[10.1023/B:NERE.0000014830.06376.e6](https://doi.org/10.1023/B:NERE.0000014830.06376.e6)
74. Nita, D.A., Nita, V., Spulber, S., Moldovan, M., Popa, D.P., Zagrean, A.M., Zagrean, L.: Oxidative damage following cerebral ischemia depends on reperfusion—a biochemical study in rat. *J. Cell. Mol. Med.* **5**(2), 163–170 (2001). doi:[10.1111/j.1582-4934.2001.tb00149.x](https://doi.org/10.1111/j.1582-4934.2001.tb00149.x)
75. Anderson, M.F., Sims, N.R.: The effects of focal ischemia and reperfusion on the glutathione content of mitochondria from rat brain subregions. *J. Neurochem.* **81**(3), 541–549 (2002). doi:[10.1046/j.1471-4159.2002.00836.x](https://doi.org/10.1046/j.1471-4159.2002.00836.x)
76. Papadopoulos, M.C., Koumenis, I.L., Dugan, L.L., Giffard, R.G.: Vulnerability to glucose deprivation injury correlates with glutathione levels in astrocytes. *Brain Res.* **748**(1–2), 151–156 (1997). doi:[10.1016/S0006-8993\(96\)01293-0](https://doi.org/10.1016/S0006-8993(96)01293-0)

77. Chen, Y., Vartiainen, N.E., Ying, W., Chan, P.H., Koistinaho, J., Swanson, R.A.: Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. *J. Neurochem.* **77**(6), 1601–1610 (2001). doi:[10.1046/j.1471-4159.2001.00374.x](https://doi.org/10.1046/j.1471-4159.2001.00374.x)
78. Fukuda, S., Fini, C.A., Mabuchi, T., Koziol, J.A., Eggleston, L.L., Jr., del Zoppo, G.J.: Focal cerebral ischemia induces active proteases that degrade microvascular matrix. *Stroke* **35**(4), 998–1004 (2004). doi:[10.1161/01.STR.0000119383.76447.05](https://doi.org/10.1161/01.STR.0000119383.76447.05)
79. Del Zoppo, G.J.: Stroke and neurovascular protection. *N. Engl. J. Med.* **354**(6), 553–555 (2006). doi:[10.1056/NEJMp058312](https://doi.org/10.1056/NEJMp058312)
80. Huang, J., Choudhri, T.F., Winfree, C.J., McTaggart, R.A., Kiss, S., Mocco, J., Kim, L.J., Protopsaltis, T.S., Zhang, Y., Pinsky, D.J., Connolly, E.S., Jr.: Postischemic cerebrovascular E-selectin expression mediates tissue injury in murine stroke. *Stroke* **31**(12), 3047–3053 (2000)
81. Yenari, M.A., Xu, L., Tang, X.N., Qiao, Y., Giffard, R.G.: Microglia potentiate damage to blood-brain barrier constituents: improvement by minocycline in vivo and in vitro. *Stroke* **37**(4), 1087–1093 (2006). doi:[10.1161/01.STR.0000206281.77178.ac](https://doi.org/10.1161/01.STR.0000206281.77178.ac)
82. Anderson, M.F., Blomstrand, F., Blomstrand, C., Eriksson, P.S., Nilsson, M.: Astrocytes and stroke: networking for survival? *Neurochem. Res.* **28**(2), 293–305 (2003). doi:[10.1023/A:1022385402197](https://doi.org/10.1023/A:1022385402197)
83. Thoren, A.E., Helps, S.C., Nilsson, M., Sims, N.R.: Astrocytic function assessed from 1-14C-acetate metabolism after temporary focal cerebral ischemia in rats. *J. Cereb. Blood Flow Metab.* **25**(4), 440–450 (2005). doi:[10.1038/sj.jcbfm.9600035](https://doi.org/10.1038/sj.jcbfm.9600035)
84. Kimelberg, H.K.: Tamoxifen as a powerful neuroprotectant in experimental stroke and implications for human stroke therapy. *Recent Pat. CNS Drug Discov.* **3**(2), 104–108 (2008)
85. Wakade, C., Khan, M.M., De Sevilla, L.M., Zhang, Q.G., Mahesh, V.B., Brann, D.W.: Tamoxifen neuroprotection in cerebral ischemia involves attenuation of kinase activation and superoxide production and potentiation of mitochondrial superoxide dismutase. *Endocrinology* **149**(1), 367–379 (2008). doi:[10.1210/en.2007-0899](https://doi.org/10.1210/en.2007-0899)
86. Kimelberg, H.K.: Astrocytic swelling in cerebral ischemia as a possible cause of injury and target for therapy. *Glia* **50**(4), 389–397 (2005). doi:[10.1002/glia.20174](https://doi.org/10.1002/glia.20174)
87. Nilsson, M., Pekny, M.: Enriched environment and astrocytes in central nervous system regeneration. *J. Rehabil. Med.* **39**(5), 345–352 (2007). doi:[10.2340/16501977-0084](https://doi.org/10.2340/16501977-0084)
88. Cutrer, F.M., Huertter, K.: Migraine aura. *Neurologist* **13**(3), 118–125 (2007). doi:[10.1097/01.nrl.0000252943.82792.38](https://doi.org/10.1097/01.nrl.0000252943.82792.38)
89. Sanchez-Del-Rio, M., Reuter, U., Moskowitz, M.A.: New insights into migraine pathophysiology. *Curr. Opin. Neurol.* **19**, 294–298 (2006). doi:[10.1097/01.wco.0000227041.23694.5c](https://doi.org/10.1097/01.wco.0000227041.23694.5c)
90. Strong, A.J., Dardis, R.: Depolarisation phenomena in traumatic and ischaemic brain injury. In: Pickard, J.D. (eds.) *Advances and Technical Standards in Neurosurgery*, pp. 3–49. Springer, Wien (2005)
91. Martins-Ferreira, H., Nedergaard, M., Nicholson, C.: Perspectives on spreading depression. *Brain Res. Brain Res. Rev.* **32**, 215–234 (2000). doi:[10.1016/S0165-0173\(99\)00083-1](https://doi.org/10.1016/S0165-0173(99)00083-1)
92. Silberstein, S.D.: Migraine pathophysiology and its clinical implications. *Cephalalgia* **24**(Suppl 2), 2–7 (2004). doi:[10.1111/j.1468-2982.2004.00892.x](https://doi.org/10.1111/j.1468-2982.2004.00892.x)
93. Smith, J.M., Bradley, D.P., James, M.F., Huang, C.L.: Physiological studies of cortical spreading depression. *Biol. Rev. Camb. Philos. Soc.* **81**(4), 457–481 (2006). doi:[10.1017/S1464793106007081](https://doi.org/10.1017/S1464793106007081)
94. Ducros, A.: Familial and sporadic hemiplegic migraine. *Rev. Neurol. (Paris)* **164**(3), 216–224 (2008). doi:[10.1016/j.neurol.2007.10.003](https://doi.org/10.1016/j.neurol.2007.10.003)
95. Capendeguy, O., Horisberger, J.D.: Functional effects of Na⁺, K⁺-ATPase gene mutations linked to familial hemiplegic migraine. *Neuromol. Med.* **6**, 105–116 (2004). doi:[10.1385/NMM:6:2-3:105](https://doi.org/10.1385/NMM:6:2-3:105)
96. Blume, W., Lüders, H., Mizrahi, E., Tassinari, C., van Emde Boas, W., Engel, J.: Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology. *Epilepsia* **42**(9), 1212–1218 (2001). doi:[10.1046/j.1528-1157.2001.22001.x](https://doi.org/10.1046/j.1528-1157.2001.22001.x)
97. Choi, J., Koh, S.: Role of brain inflammation in epileptogenesis. *Yonsei Med. J.* **49**(1), 1–18 (2008). doi:[10.3349/ymj.2008.49.1.1](https://doi.org/10.3349/ymj.2008.49.1.1)
98. Korn, A., Golan, H., Melamed, I., Pascual-Marqui, R., Friedman, A.: Focal cortical dysfunction and blood-brain barrier disruption in patients with postconcussion syndrome. *J. Clin. Neurophysiol.* **22**, 1–9 (2005). doi:[10.1097/01.WNP.0000150973.24324.A7](https://doi.org/10.1097/01.WNP.0000150973.24324.A7)
99. Schroder, W., Seifert, G., Huttman, K., Hinterkeuser, S., Steinhauser, C.: AMPA receptor-mediated modulation of inward rectifier K⁺ channels in astrocytes of mouse hippocampus. *Mol. Cell. Neurosci.* **19**, 447–458 (2002). doi:[10.1006/mcne.2001.1080](https://doi.org/10.1006/mcne.2001.1080)
100. Jansen, L.A., Uhlmann, E.J., Crino, P.B., Gutmann, D.H., Wong, M.: Epileptogenesis and reduced inward rectifier potassium current in tuberous sclerosis complex-1-deficient astrocytes. *Epilepsia* **46**, 1871–1880 (2005). doi:[10.1111/j.1528-1167.2005.00289.x](https://doi.org/10.1111/j.1528-1167.2005.00289.x)

101. Proper, E.A., Hoogland, G., Kappen, S.M., Jansen, G.H., Rensen, M.G., Schrama, L.H., van Veelen, C.W., van Rijen, P.C., van Nieuwenhuizen, O., Gispen, W.H., de Graan, P.N.: Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain* **125**, 32–43 (2002). doi:[10.1093/brain/awf001](https://doi.org/10.1093/brain/awf001)
102. Tian, G.F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., Oberheim, N., Lou, N., Wang, X., Zielke, H.R., Kang, J., Nedergaard, M.: An astrocytic basis of epilepsy. *Nat. Med.* **11**(9), 973–981 (2005)
103. Abbott, N.J., Rönnebeck, L., Hansson, E.: Astrocyte–endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* **7**(1), 41–53 (2006). doi:[10.1038/nrn1824](https://doi.org/10.1038/nrn1824)
104. Willenborg, D.O., Fordham, S.A., Cowden, W.B., Ramshaw, I.A.: Cytokines and murine autoimmune encephalomyelitis: inhibition or enhancement of disease with antibodies to select cytokines, or by delivery of exogenous cytokines using a recombinant vaccinia virus system. *Scand. J. Immunol.* **41**(1), 31–41 (1995). doi:[10.1111/j.1365-3083.1995.tb03530.x](https://doi.org/10.1111/j.1365-3083.1995.tb03530.x)
105. Nair, A., Frederick, T.J., Miller, S.D.: Astrocytes in multiple sclerosis: a product of their environment. *Cell. Mol. Life Sci.* **65**, 2702–2720 (2008). doi:[10.1007/s00018-008-8059-5](https://doi.org/10.1007/s00018-008-8059-5)
106. Farina, C., Aloisi, F., Meinl, E.: Astrocytes are active players in cerebral innate immunity. *Trends Immunol.* **28**, 138–145 (2007). doi:[10.1016/j.it.2007.01.005](https://doi.org/10.1016/j.it.2007.01.005)
107. De Keyser, J., Wilczak, N., Leta, R., Streetland, C.: Astrocytes in multiple sclerosis lack beta-2 adrenergic receptors. *Neurology* **53**, 1628–1633 (1999)
108. Dong, Y., Benveniste, E.N.: Immune function of astrocytes. *Glia* **36**, 180–190 (2001). doi:[10.1002/glia.1107](https://doi.org/10.1002/glia.1107)
109. Gimenez, M.A., Sim, J.E., Russell, J.H.: TNFR1-dependent VCAM-1 expression by astrocytes exposes the CNS to destructive inflammation. *J. Neuroimmunol.* **151**, 116–125 (2004). doi:[10.1016/j.jneuroim.2004.02.012](https://doi.org/10.1016/j.jneuroim.2004.02.012)
110. De Keyser, J., Zeinstra, E., Wilczak, N.: Astrocytic beta2-adrenergic receptors and multiple sclerosis. *Neurobiol. Dis.* **15**, 331–339 (2004). doi:[10.1016/j.nbd.2003.10.012](https://doi.org/10.1016/j.nbd.2003.10.012)
111. Holley, J.E., Gveric, D., Newcombe, J., Cuzner, M.L., Gutowski, N.J.: Astrocyte characterization in the multiple sclerosis glial scar. *Neuropathol. Appl. Neurobiol.* **29**, 434–444 (2003). doi:[10.1046/j.1365-2990.2003.00491.x](https://doi.org/10.1046/j.1365-2990.2003.00491.x)
112. Thomas, B., Flint Beal, M.: Parkinson’s disease. *Hum. Mol. Genet.* **16**, R183–R194 (2007). doi:[10.1093/hmg/ddm159](https://doi.org/10.1093/hmg/ddm159)
113. Sheng, J.G., Shirabe, S., Nishiyama, N., Schwartz, J.P.: Alterations in striatal glial fibrillary acidic protein expression in response to 6-hydroxydopamine-induced denervation. *Exp. Brain Res.* **95**, 450–456 (1993). doi:[10.1007/BF00227138](https://doi.org/10.1007/BF00227138)
114. Kohutnicka, M., Lewandowska, E., Kurkowska-Jastrzebska, I., Czlonkowski, A., Czlonkowska, A.: Microglial and astrocytic involvement in a murine model of Parkinson’s disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology* **39**, 167–180 (1998). doi:[10.1016/S0162-3109\(98\)00022-8](https://doi.org/10.1016/S0162-3109(98)00022-8)
115. Hunot, S., Bernard, V., Faucheux, B., Boissiere, F., Leguern, E., Brana, C., Gautris, P.P., Guerin, J., Bloch, B., Agid, Y., Hirsch, E.C.: Glial cell line-derived neurotrophic factor (GDNF) gene expression in the human brain: a post mortem in situ hybridization study with special reference to Parkinson’s disease. *J. Neural Transm.* **103**, 1043–1052 (1996). doi:[10.1007/BF01291789](https://doi.org/10.1007/BF01291789)
116. Kordower, J.H., Palfi, S., Chen, E.Y., Ma, S.Y., Sendera, T., Cochran, E.J., Cochran, E.J., Mufson, E.J., Penn, R., Goetz, C.G., Comella, C.D.: Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson’s disease. *Ann. Neurol.* **46**, 419–424 (1999). doi:[10.1002/1531-8249\(199909\)46:3<419::AID-ANA21>3.0.CO;2-Q](https://doi.org/10.1002/1531-8249(199909)46:3<419::AID-ANA21>3.0.CO;2-Q)
117. Emerit, J., Edeas, M., Bricaire, F.: Neurodegenerative diseases and oxidative stress. *Biomed. Pharmacother.* **58**(1), 39–46 (2004). doi:[10.1016/j.biopha.2003.11.004](https://doi.org/10.1016/j.biopha.2003.11.004)
118. Dexter, D.T., Carter, C.J., Wells, F.R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., Marsden, C.D.: Basal lipid peroxidation in substantia nigra is increased in Parkinson’s disease. *J. Neurochem.* **52**, 381–389 (1989). doi:[10.1111/j.1471-4159.1989.tb09133.x](https://doi.org/10.1111/j.1471-4159.1989.tb09133.x)
119. Kastner, A., Hirsch, E.C., Lejeune, O., Javoy-Agid, F., Rascol, O., Agid, Y.: Is the vulnerability of neurons in the substantia nigra of patients with Parkinson’s disease related to their neuromelanin content? *J. Neurochem.* **59**, 1080–1089 (1992). doi:[10.1111/j.1471-4159.1992.tb08350.x](https://doi.org/10.1111/j.1471-4159.1992.tb08350.x)
120. Jellinger, K., Kienzl, E., Rumpelmair, G., Riederer, P., Stachelberger, H., Ben-Shachar, D., Youdim, M.B.: Iron melanin complex in substantia nigra of parkinsonian brains: an X-ray microanalysis. *J. Neurochem.* **59**, 1168–1171 (1992). doi:[10.1111/j.1471-4159.1992.tb08362.x](https://doi.org/10.1111/j.1471-4159.1992.tb08362.x)
121. Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., Marsden, C.D.: Alterations in glutathione levels in Parkinson’s disease and other neurodegenerative disorders affecting basal ganglia. *Ann. Neurol.* **36**, 348–355 (1994). doi:[10.1002/ana.410360305](https://doi.org/10.1002/ana.410360305)

122. Desagher, S., Glowinski, J., Premont, J.: Pyruvate protects neurons against hydrogen peroxide-induced toxicity. *J. Neurosci.* **1**, 9060–9067 (1997)
123. Kastner, A., Anglade, P., Bounaix, C., Damier, P., Javoy-Agid, F., Bromet, N., Agid, Y., Hirsch, E.C.: Immunohistochemical study of catechol-O methyltransferase in the human mesostriatal system. *Neuroscience* **62**, 449–457 (1994). doi:[10.1016/0306-4522\(94\)90379-4](https://doi.org/10.1016/0306-4522(94)90379-4)
124. Hunot, S., Dugas, N., Faucheux, B., Hartmann, A., Tardieu, M., Debre, P., Agid, Y., Dugas, B., Hirsch, E.C.: FcεRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-α in glial cells. *J. Neurosci.* **19**, 3440–3447 (1999)
125. Mogi, M., Harada, M., Narabayashi, H., Inagaki, H., Minami, M., Nagatsu, T.: Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci. Lett.* **211**, 13–16 (1996). doi:[10.1016/0304-3940\(96\)12706-3](https://doi.org/10.1016/0304-3940(96)12706-3)
126. Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., Nagatsu, T.: Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci. Lett.* **165**, 208–210 (1994). doi:[10.1016/0304-3940\(94\)90746-3](https://doi.org/10.1016/0304-3940(94)90746-3)
127. McGeer, P.L., Schwab, C., Parent, A., Doudet, D.: Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. *Ann. Neurol.* **54**, 599–604 (2003). doi:[10.1002/ana.10728](https://doi.org/10.1002/ana.10728)
128. Mogi, M., Harada, M., Kondo, T., Riederer, P., Nagatsu, T.: Brain beta 2-microglobulin levels are elevated in the striatum in Parkinson's disease. *J. Neural Transm. Parkinson's Dis. Dement. Sect.* **9**, 87–92 (1995). doi:[10.1007/BF02252965](https://doi.org/10.1007/BF02252965)
129. Dugas, B., Mossalayi, M.D., Damais, C., Kolb, J.P.: Nitric oxide production by human monocytes: evidence for a role of CD23. *Immunol. Today* **16**, 574–580 (1995). doi:[10.1016/0167-5699\(95\)80080-8](https://doi.org/10.1016/0167-5699(95)80080-8)
130. Arock, M., Le Goff, L., Becherel, P.A., Dugas, B., Debre, P., Mossalayi, M.D.: Involvement of FcεRII/CD23 and L-arginine dependent pathway in IgE-mediated activation of human eosinophils. *Biochem. Biophys. Res. Commun.* **203**, 265–271 (1994). doi:[10.1006/bbrc.1994.2177](https://doi.org/10.1006/bbrc.1994.2177)
131. Juckett, M., Zheng, Y., Yuan, H., Pastor, T., Antholine, W., Weber, M., Vercellotti, G.: Heme and the endothelium. Effects of nitric oxide on catalytic iron and heme degradation by heme oxygenase. *J. Biol. Chem.* **273**(36), 23388–23397 (1998). doi:[10.1074/jbc.273.36.23388](https://doi.org/10.1074/jbc.273.36.23388)
132. O'Banion, M.K.: Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. *Crit. Rev. Neurobiol.* **13**, 45–82 (1999)
133. Teismann, P., Tieu, K., Choi, D.K., Wu, D.C., Naini, A., Hunot, S., Vila, M., Jackson-Lewis, V., Przedborski, S.: Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 5473–5478 (2003). doi:[10.1073/pnas.0837397100](https://doi.org/10.1073/pnas.0837397100)
134. Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., Nagatsu, T.: Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *J. Neural Transm.* **107**, 335–341 (2000). doi:[10.1007/s007020050028](https://doi.org/10.1007/s007020050028)
135. Rocchi, A., Pellegrini, S., Siciliano, G., Murri, L.: Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res. Bull.* **61**(1), 1–24 (2003). doi:[10.1016/S0361-9230\(03\)00067-4](https://doi.org/10.1016/S0361-9230(03)00067-4)
136. Rossner, S., Lange-Dohna, C., Zeitschel, U., Perez-Polo, J.R.: Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. *J. Neurochem.* **92**(2), 226–234 (2005). doi:[10.1111/j.1471-4159.2004.02857.x](https://doi.org/10.1111/j.1471-4159.2004.02857.x)
137. Meda, L., Baron, P., Scarlato, G.: Glial activation in Alzheimer's disease: the role of Aβ and its associated proteins. *Neurobiol. Aging* **22**(6), 885–893 (2001). doi:[10.1016/S0197-4580\(01\)00307-4](https://doi.org/10.1016/S0197-4580(01)00307-4)
138. Schubert, P., Ogata, T., Marchini, C., Ferroni, S.: Glia-related pathomechanisms in Alzheimer's disease: a therapeutic target? *Mech. Ageing Dev.* **123**(1), 47–57 (2001). doi:[10.1016/S0047-6374\(01\)00343-8](https://doi.org/10.1016/S0047-6374(01)00343-8)
139. Heneka, M.T., Sastre, M., Dumitrescu-Ozimek, L., Dewachter, I., Walter, J., Klockgether, T., Van Leuven, F.: Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. *J. Neuroinflammation* **2**, 22 (2005). doi:[10.1186/1742-2094-2-22](https://doi.org/10.1186/1742-2094-2-22)
140. Deane, R., Wu, Z., Zlokovic, B.V.: RAGE (yin) versus LRP (yang) balance regulates Alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. *Stroke* **35**(Suppl 1(11)), 2628–2631 (2004). doi:[10.1161/01.STR.0000143452.85382.d1](https://doi.org/10.1161/01.STR.0000143452.85382.d1)
141. Farfara, D., Lifshitz, V., Frenkel, D.: Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease. *J. Cell. Mol. Med.* **12**(3), 762–780 (2008). doi:[10.1111/j.1582-4934.2008.00314.x](https://doi.org/10.1111/j.1582-4934.2008.00314.x)
142. Colombo, J.A., Quinn, B., Puissant, V.: Disruption of astroglial interlaminar processes in Alzheimer's disease. *Brain Res. Bull.* **58**(2), 235–242 (2002). doi:[10.1016/S0361-9230\(02\)00785-2](https://doi.org/10.1016/S0361-9230(02)00785-2)
143. Mattson, M.P., Chan, S.L.: Calcium orchestrates apoptosis. *Nat. Cell Biol.* **5**(12), 1051–1061 (2003). doi:[10.1038/ncb1203-1041](https://doi.org/10.1038/ncb1203-1041)