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Astrocytes in the central nervous system and their functions in health and disease: a review

Astrocytes and their functions in health and disease

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Abstract

Astrocytes are key cells in the central nervous system. They are involved in many important functions under physiological and pathological conditions. As part of neuroglia, they have been recognised as cellular elements in their own right. The name astrocyte was first proposed by Mihaly von Lenhossek in 1895 because of the finely branched processes and star-like appearance of these particular cells. As early as the late 19th and early 20th centuries, Ramon y Cajal and Camillo Golgi had noted that although astrocytes have stellate features, their morphology is extremely diverse. Modern research has confirmed the morphological diversity of astrocytes both *in vitro* and *in vivo* and their complex, specific, and important roles in the central nervous system. In this review, the functions of astrocytes and their roles are described.

Key Words: astrocytes; morphology; astrocyte functions; molecular markers

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Core Tip:

Astrocytes are a part of neuroglia with extremely diverse morphology and functions. They participate in numerous physiological processes, such as regulation of axonal growth and support, blood-brain barrier formation, immune responses as well as functioning in higher cognitive functions, including memory. Since modern research has confirmed the morphological diversity of astrocytes both *in vitro* and in vivo, they remain in the centre of investigation.

INTRODUCTION

Astrocytes are key cells in the central nervous system [1]. They are involved in many important functions under physiological and pathological conditions [2, 3]. The name astrocytes or astroglia is derived from the Greek root "astro', which means star. They are

so named because they look like "stars in the night sky" on a sample stained with Golgi. As part of neuroglia, they have been recognised as cellular elements in their own right (Figures 1 and 2). In the past, the term neuroglia was used to refer to all supporting cells of the central nervous system, and it is still used today. The name astrocytes was first proposed by Mihaly von Lenhossek in 1895, based on the finely branched projections and star-like appearance of these specific cells [2-4]. In the late 19th and early 20th centuries, Ramon y Cajal and Camillo Golgi had already established that astrocytes have stellate features but their morphology is different. Since then, it has long been thought that astrocyte function is limited only to supportive and structural tasks and that they respond involuntarily and in a largely stereotyped manner to disease or injury. Modern research has confirmed the morphological diversity of astrocytes both *in vivo* and in vitro. Recent findings have confirmed that astrocytes play much more complex, specific, and important roles in the central nervous system. Because of their diverse forms and functions, these cells are now identified as primary responders to physiological and pathological conditions, and interest in astrocytes has increased dramatically in recent decades, particularly because of advances in cell culture and more specific ways to identify these cells [3-5].

Astrocytes represent the key class of glial cells. Thus, they are the most important cellular component of the central nervous system. In humans, it is estimated that in some brain regions, 25% to 50% of the total tissue volume is composed of astrocytes, thus outnumbering neurons [2-5]. According to their name, these astrocytes sport a characteristic form with star-shaped and finely branched processes. Based on the differences in morphological appearance and distribution of astrocytes, they are divided into two main subtypes: protoplasmic and fibrous astrocytes. Protoplasmic astrocytes are star-shaped and have multiple truncal branches that divide into numerous, finely branched projections. They are predominant in all areas of grey matter, whereas filamentous astrocytes are most numerous in white matter and morphologically have many long filamentous projections. Although this classification

dates back to the end of the 19th century, it is still absolutely valid today [6, 7]. In addition, other types of astrocytes have been described, including radial astrocytes of the retina and cerebellum, velate astrocytes of the cerebellum and olfactory bulb, and special forms of astrocytes described only in certain species, such as interlaminar astrocytes in the cortex of higher primates [6-8]. Astrocytes that reside in various brain regions differ considerably. They diverge according to their morphology and expression of surface cell markers, production of chemokines and cytokines [9-12].

THE NEED FOR ASTROCYTE INVESTIGATION AND THEIR CELL CULTURE ISOLATION

There are many sources of astrocyte isolation. Classically, astrocyte cultures are most commonly obtained from rodent brains because these tissues are easily accessible and widely available, and quite simple to maintain in the cell culture [9, 13-15]. In addition, there are other animal sources [16, 17]. Despite their usefulness, these animal-derived cells are completely different from their human counterparts. The results obtained from experimental cell models in which astrocyte cell cultures were used to conduct *in vitro* research cannot be directly applied to the study of similar processes in humans because of interspecies differences [16, 18]. Therefore, culture of human astrocytes is desirable despite rare reports of their isolation. Human sources for astrocyte isolation include adult and neonatal brain [19, 20].

First, according to literature reports, neonatal astrocytes are more suitable compared to adult ones. Cultures of neonatal astrocytes show signs of ageing relatively late, after 4 to 6 mo in culture, and initially grow and proliferate at a high rate. Adult brain-derived astrocytes are known to have very limited proliferative activity, do not grow for a long time in culture, and are difficult to subculture. Therefore, these cultures have restricted value [19].

Second, the tissue for isolation procedures of adult astrocytes is relatively easy to obtain in comparison to neonatal brains. Neonatal brains can be obtained from foetuses that are usually 9 to 12 or 22 weeks old and are harvested during elective abortions [19, 22]. There are not many patients, who undergo such a procedure, so the question of tissue source arises. Therefore, a good cooperation between the surgical team and the cell laboratory must be present. In addition, not all foetal brains are appropriate for isolation procedures. According to the literature, only foetuses collected after the surgical procedure of vacuum aspiration are used. The brains of the foetuses that were aborted with the use of medications (foeticides) are not appropriate since the pharmaceutical agents employed to cause foetal death may affect the cell viability and therefore hamper the establishment of the cell culture [19, 22, 23]. Conversely, adult brain tissues are more suitable. Brain surgeries that provide the source of tissue for experiments are frequent. The tissue is gathered in gross resections during open surgery and in numerous types of open or closed (needle) biopsies. Most frequent source of adult brain tissue is the cerebral cortex. The tissue is taken during tumour, trauma, vascular, and epilepsy surgery. Deep brain regions, such as the hypothalamus, basal ganglia, and insula are reachable when performing needle biopsies [19, 20, 23-25].

Factors that may affect the isolation of astrocytes include the differences in age among neonatal donors, as well as variable conditions of donor brain tissue before the preparation of the cell culture. Transport to the laboratory can vary and is usually longer for brain samples collected during abortion. Transport time is usually less than two hours. In adults, however, the tissue is usually more stable as it is collected during the biopsy, and it reaches the laboratory much faster [25, 26].

Neonatal astrocytes, which are different in comparison to adult astrocytes, may also exhibit an incomplete differentiation and their differentiation signals may be absent [10, 19]. Gene expression in neonatal astrocytes is characteristics and these cells are considered more activated than their adult counterparts [17]. The genes that are

expressed in adult and neonatal astrocytes in cell culture is comparable. However, differences in gene classes exist. More genes for proteases, protease inhibitors and metabolic enzymes are expressed in adult astrocytes, pointing to a higher level of metabolic activity. On the other hand, the neonatal astrocytes express more genes that are important for the regulation of the cell cycle, including DNA binding and apoptosis, regulation of cell adhesion, cytoskeleton maintenance, the construction of extracellular matrix, and transduction of signals. Additionally, GFAP genes are expressed only in astrocytes from postnatal brain [13, 17, 27, 28]. This is particularly important when cell culture is used for the purposes of neurodegenerative disease research. Therefore, adult astrocyte cultures are preferred for the study of adult pathophysiology [14, 18, 19, 29-30].

Astrocytes can be isolated from various parts of the human brain, and human tissue samples are usually obtained from neonatal brains [29-31]. In rare cases, adult patients have also been mentioned as donors, mainly those who underwent craniotomy for tumour, trauma, epilepsy surgery, or during surgery for various types of haemorrhage such as arteriovenous malformations, intracerebral hematomas, and aneurysms [32-34]. Numerous neurosurgical procedures from clinical practise provide a welcome laboratory source of healthy and diseased brain tissue [35-37]. Modern neurosurgical procedures offer the possibilities to gather brain tissue samples from diverse neurosurgical pathologies at numerous anatomical locations, which are now more easily and less invasively accessible with minimal potential morbidity, contributing to higher cell yields during the isolation procedure in the laboratory [30, 35]. The aim includes conserving the patient's neurological function, is always first. The collection of tissue for cell isolation is here of secondary importance. Therefore, all samples acquired during surgery represent the surplus brain tissue, which is not utilized for neuropathological diagnosis [38, 39].

In addition to *in vivo* experiments, astrocyte morphology can also be studied in vitro, with astrocyte cultures, separately from other cells and influences. To fully understand

the physiology and pathophysiology of astrocytes in the human brain, a method that allows direct purification and analysis of the studied cells is required [38]. The key benefits of astrocyte culture in the *in vitro* conditions comprise the possibility to implement biochemical analysis, especially those including the individual identified cell types, the possibilities to regulate the cellular milieu, and to perform the experiments in an environment with a reduced cell interactions in comparison to whole brain. Additionally, the experiments employing cell electrophysiology, the imaging of individual cells, co-culturing procedures and manipulation of gene expression can be performed [39].

ASTROCYTE FUNCTIONS

The role of astrocytes has long been viewed as primarily passive in the nervous system, serving as structural and support cells for neurons. This view has gradually changed with advances in cell physiology and biology and advances in cell culture techniques, including more precise methods for their identification [40, 41]. The notion that astrocyte dysfunction could trigger mechanisms that lead to pathological changes in the central nervous system and contribute to the expression of clinical symptoms has generally not been considered [42, 43]. Recent evidence has confirmed that astrocytes perform complex roles and various functions in the central nervous system, for example information processing and synaptic transmission in neuronal circuits and functions [2, 44-46]. As the understanding of the form, development, function, and role of astrocytes in health and disease has greatly increased, they are now considered a heterogeneous group of cells with important and diverse functions. This notion of astrocyte heterogeneity is critical to understanding their role and responses to healthy and pathological conditions [46]. Astrocytes have been shown to play an essential role in the formation, function, and elimination of synapses. Fibrous astrocytes use their projections to communicate with nodes of Ranvier, and the projections of protoplasmic astrocytes enclose synapses. They also form gap junctions between distal processes of neighbouring cells, interact with other cell types, and are in extensive contact with blood vessels. In addition, they are

involved in maintaining and nurturing the neuronal microenvironment, helping to guide neuronal migration during development and providing support to neurons. They serve as antigen-presenting cells and are involved in modulating immune responses. Despite these advances in understanding the functions of astrocytes, their development, and their signalling relationships with other cell types, our knowledge of astrocyte functions is still basic [47-50].

As numerous cell populations in the central nervous system, astrocytes perform diverse and important functions not only during normal central nervous system function but also during its development, some of which overlap considerably. Since it is not possible to discuss all aspects of astrocytic functions, the most important ones are described below (Table 1).

The Role in Neuronal Migration

One of the many roles of astrocytes is their interaction with neurons that migrate along with glial cell projections during central nervous system development. Glial cells, also known as radial glia, form scaffolds that provide this migration pathway for neurons. Immunocytochemical studies have confirmed that the processes of radial glial cells contain glial fibrillary acidic protein and vimentin. These cells, thought to be immature astrocytes, gradually lose vimentin during the maturation process and differentiate into mature astrocytes. This process is completed after neuronal migration is finished [46, 47].

The Formation of Extracellular Matrix Proteins and Adhesion Molecules

Adhesion molecules and various extracellular matrix proteins are important for the development and maintenance of the structural integrity of the central nervous system at the cellular level and play an important role in repair and regeneration after injury. Some of these molecules are laminin, fibronectin, neuronal cell adhesion molecules, and cytotoxin J1. The main production source of these molecules is astrocytes, which also have surface receptors for the matrix proteins and adhesion molecules [46-49].

The Production of Neurotrophic and Neurite-Promoting Factors

As support and regulatory cells for neurons, astrocytes are required for neuron survival and are involved in neurite formation. They serve as a source of soluble factors required for neuron support and survival, as well as substrate-bound matrix proteins important for neurite formation and expansion. These neurotrophic and neurite-promoting factors include low molecular weight molecules such as pyruvate and others required for neuronal energy metabolism and extracellular matrix proteins such as laminin, respectively. Astrocytes are cells that produce nerve growth factor and protein S100, which are important for neurite elongation and growth. In addition, astrocytes are a source of neuroactive steroids, including progesterone, estradiol, and various metabolites with synaptic effects [50, 51].

Blood-Brain Barrier

The blood-brain barrier is a highly selective boundary of endothelial cells, pericytes, and astrocytes that acts as a diffusion barrier, preventing the entry of certain molecules into the brain parenchyma according to their size and polarity. Astrocytes contribute to the formation and maintenance of the blood-brain barrier. They provide structural support and influence the transport of molecules between the vasculature and glial cells by altering the transport properties of endothelial cells. Astrocytes can alter enzyme activity in the cerebral endothelium, such as alkaline phosphatase and Na+-K+-ATPase activity, modify the transport of neutral amino acids, and increase the capacity of neutral amino acid transport and glucose transport systems in the cerebral endothelium [52, 53].

Angiogenesis

Angiogenesis is a complex process involving several steps, such as endothelial cell activation, basement membrane dissolution, endothelial cell replication and migration, and formation of hollow cords and tubes with final maturation and restoration of the

basement membrane. Astrocytes are also active during this process, inducing endothelial cells to form capillary-like structures. Their involvement in angiogenesis is important for central nervous system development and repair. This interaction requires physical contact between astrocytes and endothelial cells. Endothelial cells separated from astrocytes do not form such structures [54, 55].

Neurotransmission

Neurotransmission, one of the main functions of the nervous system, involves the storage and release of transmitter molecules in synapses and the interaction of these transmitters with postsynaptic receptors. Neurons have a high-affinity uptake system for neurotransmitters that releases them from the synaptic cleft. Astrocytes also exhibit such properties and play a key role in neurotransmission by taking up the transmitters and supporting the neurons. The capacity of these uptake systems varies widely, including their localization in different brain regions and for different transmitters [56, 57].

The Energy Metabolism and the Regulation of Central Nervous System Microenvironment

Astrocytes make an important contribution to metabolism in the central nervous system. Astrocytes are the major sites of accumulation of glycogen granules, and the highest glycogen stores in astrocytes are in the areas of high synaptic density. These glycogen stores are used to maintain neuronal activity during high neuronal activity and during episodes of hypoglycemia. Through contact between blood vessels, axons at nodes of Ranvier, neuronal perikarya, and synapses, astrocyte processes are well positioned to take up glucose from blood vessels and provide energy metabolites to various neuronal elements in the white and grey matter [58, 59].

In addition, astrocytes are involved in the regulation of pH, ion concentration, and osmolarity in the central nervous system. For normal neuronal activity, changes in the cerebral microenvironment must be tightly controlled. The cellular depolarization that occurs during neurotransmission results in noticeable changes in ion concentrations,

extracellular pH, and osmolarity [5-7]. Astrocytes and also oligodendrocytes play an important role in maintaining the extracellular environment by regulating pH with the help of the enzyme carbonic anhydrase. They contain ion channels for potassium, sodium, calcium, chloride and bicarbonate. For example, when a strong flux of potassium ions into the extracellular space occurs during neurotransmission, astrocytes accumulate potassium and remove it from the extracellular space. Via gap junctions, potassium is shifted from areas of high neuronal activity to areas of low activity, into the CSF and blood. This effect is referred to as spatial buffering and highlights the importance of the glial syncytium in regulating and maintaining the microenvironment [60, 61].

Detoxification

Astrocytes are important for detoxification and removal of toxic substances from the central nervous system. In particular, their role in the uptake and metabolism of excitatory amino acid neurotransmitters is well known, preventing the accumulation of their neurotoxic concentrations that would otherwise impair neurotransmission [3, 7]. The best known excitatory amino acid neurotransmitter is glutamate. Glutamine synthetase in astrocytes is involved in ammonia metabolism and prevents toxic concentrations of this ion. Because astrocytes contain metal-binding proteins such as metallothionein, they are also involved in the uptake and sequestration of some heavy metals. These proteins are involved in the removal of some metals, such as lead, and prevent their accumulation in the central nervous system to toxic levels [62, 63].

The response of astrocytes in various central nervous system insults

In addition to trauma per se (i.e., brain injury), numerous conditions in the central nervous system conditions may result in astrocyte swelling. These take into account the metabolic disorders, such as hyperammonemia and hypoglycemia, and some other insults, including ischemia, hypoxia, and epileptic seizures are associated with astrocyte swelling. In particular, hyperammonemia is of great interest with regard to astrocytes

[64]. Hepatic encephalopathy, which occurs as the main complication of acute or chronic liver failure, is the clinical consequence of increased ammonia concentrations in the brain leading to cerebral dysfunction [65, 66]. In clinical practise, it is recognised as a spectrum of neuropsychiatric and neurologic symptoms ranging from minimal abnormalities such as attention and memory deficits to seizures, cerebral edema, intracranial hypertension, coma, and death. Hyperammonemia in the brain is associated with disturbances in cerebral metabolism and leads to a cascade of secondary effects and encephalopathy. An important morphological feature of hyperammonemia is Alzheimer-type astrocytes II [64-66].

Hyperammonemia, like other traumatic and metabolic disorders of the nervous system, is an uncontrollable condition. These patients may have marked alterations in the concentrations of extracellular ions, for example a decrease in Na^{+}, C^{1-}, and Ca^{2+}, increase in K^{+} concentration, a drop of extracellular pH, and the buildup of excitatory neurotransmitters. This can lead to various changes in astrocyte function, protein expression, and morphology [2, 53]. Reactive astrocytes of the adult brain, which are generated as a result of various injuries and insults and are then plated and cultivated in cell culture, may re-express some markers that are characteristic for developing astrocytes. Adult astrocytes in normal, quiescent situations, express more genes coding for metabolic enzymes in comparison to neonatal astrocytes [1, 18]. The most striking morphological alteration is the swelling of astrocytes, which is reversible. This morphology changes as soon as the cells are placed in culture [2, 7, 42, 53].

The Role in Immune Response and Phagocytosis

Astrocytes also play an important role in the immune response. They can function as macrophages and act as modulators of immune functions. They are capable of phagocytosis and serve as antigen-presenting cells that are induced to express and produce molecules that contribute to and facilitate immune responses. The central nervous system can be considered an immune privileged site to some degree. It is

isolated from the body's immune system due to the blood-brain barrier and the absence of lymphatic drainage and a significant population of resident lymphoid cells [67-69]. In the resting state, astrocytes do not normally express major histocompatibility complex (MHC) antigens or they express them only at very low levels. Expression of MHC molecules can be induced by a variety of inducers, including viruses and interferon-gamma (IFN-gamma). Adhesion molecules such as intercellular adhesion molecules (ICAM) expressed by astrocytes may facilitate astrocyte-lymphocyte interactions and promote their entry into the central nervous system, contributing to immune responses. The induction of ICAM expression is increased when astrocytes are exposed to certain viruses, bacterial products such as lipopolysaccharide, and IFN-gamma and interleukin-1 [68-70].

Various Functions

Astrocytes perform numerous additional functions in the central nervous system. These include receptor-mediated endocytosis, translocation, and exocytosis of macromolecules. They participate in the transport of large molecules, their translocation and exocytosis. In addition, they protect the brain from oxidative damage through the presence of glutathione in astrocytes. Astrocytes are also involved in the secretion of neurohormones. With their processes, neurohypophyseal astrocytes interact with neuroendocrine cells according to the need for certain hormones, such as during dehydration or lactation [2, 5, 71-73].

ASTROCYTE MORPHOLOGY

Astrocytes, as the name implies, have a special morphology. In hematoxylin and eosin staining, they are seen as cells with little discernible cytoplasm and a pale stained nucleus. The shape of the nucleus varies; it is round to oval in protoplasmic cells and lobed in fibrous astrocytes. Astrocytes are closely related to other cellular and structural components of the central nervous system and therefore play an important role in structure and function. Astrocytic foot processes enclose blood vessels, invest cell

bodies and neuronal processes, surround synapses, and enclose the nodes of Ranvier of myelinated axons [2, 5, 74].

In addition to organisation of the astrocytes according to different regions of the brain and their morphological differences, these cells also vary according to their physiological properties. Phenotypic and functional characterization of astrocytes can be performed using immunocytochemistry and looking for the presence of important astrocyte markers, such as glutamate transporter and GFAP, as well as the variations in membrane potential, and potassium conductance [3, 6].

The astrocytic cytoplasm is filled with glial fibrils. The most important ultrastructural recognition feature of astrocytes are the intermediate filaments. They are much more noticeable in fibrous than in protoplasmic astrocytes. Their major component is GFAP. It is relatively specific for astrocytes and therefore serves as an important astrocytic marker in immunohistochemical identification and is specific for astrocytes both in cell culture and in situ. Immunocytochemical techniques permit the identification of astrocytes' specific molecular markers and are indispensable tools for their identification and characterization in cell culture. GFAP therefore serves as a classical marker for astrocytes, as it is a sensitive and reliable marker for immunocytochemical identification [1, 7]. GFAP as one of the intermediate filaments, which also include actin, nestin, vimentin and others that are important for cytoarchitectonic functions. It plays an important role in the formation of glial scars and reactive astrogliosis, and different isoforms of GFAP can be expressed in a heterogeneous manner under both normal and pathological conditions [9, 10]. GFAP labels only reactive astrocytes and may sometimes not be detectable in astrocytes of healthy tissue. In addition, human oligodendrocyte progenitor cells (OPCs), which can produce astrocytes and oligodendrocytes until their final division, may be GFAP-positive [58]. Moreover, the use of GFAP expression as a marker for astrocytes is justified by the finding that only astrocytes in the postnatal brain express the GFAP gene and GFAP expression increases between 7 and 15 days *in*

vitro [48, 49]. The content of intracellular actin also differs depending on the staining intensity. Stellate forms are deficient of actin fibres. On the other hand, astrocytes that adopt a polygonal shape *in vitro* contain distinct actin fibres [74-76].

In addition, astrocytes express other important markers used for immunocytochemical identification. These include proteins such as GLAST, a glutamate-aspartate transporter, which is the most highly expressed among markers in astrocyte cells. Other common markers for astrocytes include S100B, which belongs to the calcium-binding protein family, GLT -1 (EAAT2 in humans), a glutamate transporter, glutathione peroxidase, glutamine synthetase, and aquaporin 4 (AQP4), an astrocyte-specific water channel [5, 7, 8, 74, 76].

CONCLUSION

Astrocytes are the CNS cells with versatile and important functions. There is still much to be discovered, and thus more research is needed to elucidate their metabolism, their connections to other cells, their role in disease and CNS stress, neurodegeneration, and synaptic function, among others.

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