**Name of Journal:** *World Journal of Clinical Cases*

**Manuscript NO:** 82421

**Manuscript Type:** MINIREVIEWS

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

Gradisnik L *et al*. Astrocytes and their functions in health and disease

Lidija Gradisnik, Tomaz Velnar

**Lidija Gradisnik,** Institute of Biomedical Sciences, Medical Faculty Maribor, Maribor 2000, Slovenia

**Tomaz Velnar,** Department of Neurosurgery, University Medical Centre Ljubljana, Ljubljana 1000, Slovenia

**Tomaz Velnar,** AMEU ECM Maribor, Maribor 2000, Slovenia

**Author contributions:** Velnar T and Gradisnik L drafted the manuscript, participated in the design of the study and were involved with data collection; Velnar T participated in design and oversight of the study; all authors read and approved the final manuscript.

**Corresponding author: Tomaz Velnar, PhD, Doctor,** Department of Neurosurgery, University Medical Centre Ljubljana, Zaloska 7, Ljubljana 1000, Slovenia. tvelnar@hotmail.com

**Received:** December 17, 2022

**Revised:** February 19, 2023

**Accepted:** April 14, 2023

**Published online:**

**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

Gradisnik L, Velnar T. Astrocytes in the central nervous system and their functions in health and disease: A review. *World J Clin Cases* 2023; In press

**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 (Figure 1). 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[3,5]. 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 wk 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+, C1-, and Ca2+, 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-γ). 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-γ 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, 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 d *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, glutamate transporter-1 (EAAT2 in humans), a glutamate transporter, glutathione peroxidase, glutamine synthetase, and aquaporin 4, an astrocyte-specific water channel[5,7,8,74,76].

**CONCLUSION**

Astrocytes are the central nervous system (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.

**REFERENCES**

1 **Montgomery DL**. Astrocytes: form, functions, and roles in disease. *Vet Pathol* 1994; **31**: 145-167 [PMID: 8203078 DOI: 10.1177/030098589403100201]

2 **Sofroniew MV**, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol* 2010; **119**: 7-35 [PMID: 20012068 DOI: 10.1007/s00401-009-0619-8]

3 **Kettenmann H**, Verkhratsky A. [Neuroglia--living nerve glue]. *Fortschr Neurol Psychiatr* 2011; **79**: 588-597 [PMID: 21989511 DOI: 10.1055/s-0031-1281704]

4 **Nimmerjahn A**. Astrocytes going live: advances and challenges. *J Physiol* 2009; **587**: 1639-1647 [PMID: 19204050 DOI: 10.1113/jphysiol.2008.167171]

5 **Bedner P**, Jabs R, Steinhäuser C. Properties of human astrocytes and NG2 glia. *Glia* 2020; **68**: 756-767 [PMID: 31596522 DOI: 10.1002/glia.23725]

6 **de Majo M**, Koontz M, Rowitch D, Ullian EM. An update on human astrocytes and their role in development and disease. *Glia* 2020; **68**: 685-704 [PMID: 31926040 DOI: 10.1002/glia.23771]

7 **Hamby ME**, Sofroniew MV. Reactive astrocytes as therapeutic targets for CNS disorders. *Neurotherapeutics* 2010; **7**: 494-506 [PMID: 20880511 DOI: 10.1016/j.nurt.2010.07.003]

8 **Khakh BS**, Sofroniew MV. Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci* 2015; **18**: 942-952 [PMID: 26108722 DOI: 10.1038/nn.4043]

9 **Denis-Donini S**, Glowinski J, Prochiantz A. Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurones. *Nature* 1984; **307**: 641-643 [PMID: 6694754 DOI: 10.1038/307641a0]

10 **Giffard RG,** Ouyang YB. Cell Culture: Primary neural cells, in: Squire RL (ed), Encyclopaedia of neuroscience. Elsevier: London, 2009: 633-637

11 **Zhang Y**, Barres BA. Astrocyte heterogeneity: an underappreciated topic in neurobiology. *Curr Opin Neurobiol* 2010; **20**: 588-594 [PMID: 20655735 DOI: 10.1016/j.conb.2010.06.005]

12 **Markiewicz I**, Lukomska B. The role of astrocytes in the physiology and pathology of the central nervous system. *Acta Neurobiol Exp (Wars)* 2006; **66**: 343-358 [PMID: 17265695]

13 **Chew LJ**, DeBoy CA, Senatorov VV Jr. Finding degrees of separation: experimental approaches for astroglial and oligodendroglial cell isolation and genetic targeting. *J Neurosci Methods* 2014; **236**: 125-147 [PMID: 25169049 DOI: 10.1016/j.jneumeth.2014.08.017]

14 **Anderson MA**, Ao Y, Sofroniew MV. Heterogeneity of reactive astrocytes. *Neurosci Lett* 2014; **565**: 23-29 [PMID: 24361547 DOI: 10.1016/j.neulet.2013.12.030]

15 **Garcia-Abreu J**, Moura Neto V, Carvalho SL, Cavalcante LA. Regionally specific properties of midbrain glia: I. Interactions with midbrain neurons. *J Neurosci Res* 1995; **40**: 471-477 [PMID: 7616607 DOI: 10.1002/jnr.490400406]

16 **Lee SG**, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J Biol Chem* 2008; **283**: 13116-13123 [PMID: 18326497 DOI: 10.1074/jbc.M707697200]

17 **Nakagawa T**, Schwartz JP. Gene expression patterns in *in vivo* normal adult astrocytes compared with cultured neonatal and normal adult astrocytes. *Neurochem Int* 2004; **45**: 203-242 [PMID: 15145538 DOI: 10.1016/j.neuint.2003.09.007]

18 **Kimelberg HK**. The problem of astrocyte identity. *Neurochem Int* 2004; **45**: 191-202 [PMID: 15145537 DOI: 10.1016/j.neuint.2003.08.015]

19 **Sharif A**, Prevot V. Isolation and culture of human astrocytes. *Methods Mol Biol* 2012; **814**: 137-151 [PMID: 22144306 DOI: 10.1007/978-1-61779-452-0\_11]

20 **Oberheim NA**, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M. Uniquely hominid features of adult human astrocytes. *J Neurosci* 2009; **29**: 3276-3287 [PMID: 19279265 DOI: 10.1523/JNEUROSCI.4707-08.2009]

21 **Lange SC**, Bak LK, Waagepetersen HS, Schousboe A, Norenberg MD. Primary cultures of astrocytes: their value in understanding astrocytes in health and disease. *Neurochem Res* 2012; **37**: 2569-2588 [PMID: 22926576 DOI: 10.1007/s11064-012-0868-0]

22 **John GR**. Investigation of astrocyte - oligodendrocyte interactions in human cultures. *Methods Mol Biol* 2012; **814**: 401-414 [PMID: 22144322 DOI: 10.1007/978-1-61779-452-0\_27]

23 **Sharif A**, Prévot V, Renault-Mihara F, Allet C, Studler JM, Canton B, Chneiweiss H, Junier MP. Transforming growth factor alpha acts as a gliatrophin for mouse and human astrocytes. *Oncogene* 2006; **25**: 4076-4085 [PMID: 16532035 DOI: 10.1038/sj.onc.1209443]

24 **Minchev G**, Kronreif G, Ptacek W, Dorfer C, Micko A, Maschke S, Legnani FG, Widhalm G, Knosp E, Wolfsberger S. A novel robot-guided minimally invasive technique for brain tumor biopsies. *J Neurosurg* 2019: 1-9 [PMID: 30660122 DOI: 10.3171/2018.8.JNS182096]

25 **Dammers R**, Haitsma IK, Schouten JW, Kros JM, Avezaat CJ, Vincent AJ. Safety and efficacy of frameless and frame-based intracranial biopsy techniques. *Acta Neurochir (Wien)* 2008; **150**: 23-29 [PMID: 18172567 DOI: 10.1007/s00701-007-1473-x]

26 **Jakovcevski I**, Filipovic R, Mo Z, Rakic S, Zecevic N. Oligodendrocyte development and the onset of myelination in the human fetal brain. *Front Neuroanat* 2009; **3**: 5 [PMID: 19521542 DOI: 10.3389/neuro.05.005.2009]

27 **Rustenhoven J**, Park TI, Schweder P, Scotter J, Correia J, Smith AM, Gibbons HM, Oldfield RL, Bergin PS, Mee EW, Faull RL, Curtis MA, Scott Graham E, Dragunow M. Isolation of highly enriched primary human microglia for functional studies. *Sci Rep* 2016; **6**: 19371 [PMID: 26778406 DOI: 10.1038/srep19371]

28 **Wu VW**, Nishiyama N, Schwartz JP. A culture model of reactive astrocytes: increased nerve growth factor synthesis and reexpression of cytokine responsiveness. *J Neurochem* 1998; **71**: 749-756 [PMID: 9681466 DOI: 10.1046/j.1471-4159.1998.71020749.x]

29 **Nedergaard M**, Ransom B, Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci* 2003; **26**: 523-530 [PMID: 14522144 DOI: 10.1016/j.tins.2003.08.008]

30 **Temple S**, Alvarez-Buylla A. Stem cells in the adult mammalian central nervous system. *Curr Opin Neurobiol* 1999; **9**: 135-141 [PMID: 10072370 DOI: 10.1016/s0959-4388(99)80017-8]

31 **Bellaver B**, Souza DG, Souza DO, Quincozes-Santos A. Hippocampal Astrocyte Cultures from Adult and Aged Rats Reproduce Changes in Glial Functionality Observed in the Aging Brain. *Mol Neurobiol* 2017; **54**: 2969-2985 [PMID: 27026184 DOI: 10.1007/s12035-016-9880-8]

32 **Ballarin C**, Peruffo A. Primary cultures of astrocytes from fetal bovine brain. *Methods Mol Biol* 2012; **814**: 117-126 [PMID: 22144304 DOI: 10.1007/978-1-61779-452-0\_9]

33 **Mizee MR**, Miedema SS, van der Poel M, Adelia, Schuurman KG, van Strien ME, Melief J, Smolders J, Hendrickx DA, Heutinck KM, Hamann J, Huitinga I. Isolation of primary microglia from the human post-mortem brain: effects of ante- and post-mortem variables. *Acta Neuropathol Commun* 2017; **5**: 16 [PMID: 28212663 DOI: 10.1186/s40478-017-0418-8]

34 **Gradisnik L**, Maver U, Bosnjak R, Velnar T. Optimised isolation and characterisation of adult human astrocytes from neurotrauma patients. *J Neurosci Methods* 2020; **341**: 108796 [PMID: 32450111 DOI: 10.1016/j.jneumeth.2020.108796]

35 **Allen NJ**. Astrocyte regulation of synaptic behavior. *Annu Rev Cell Dev Biol* 2014; **30**: 439-463 [PMID: 25288116 DOI: 10.1146/annurev-cellbio-100913-013053]

36 **Wolf F**, Kirchhoff F. Neuroscience. Imaging astrocyte activity. *Science* 2008; **320**: 1597-1599 [PMID: 18566273 DOI: 10.1126/science.1160122]

37 **Tanti GK**, Srivastava R, Kalluri SR, Nowak C, Hemmer B. Isolation, Culture and Functional Characterization of Glia and Endothelial Cells From Adult Pig Brain. *Front Cell Neurosci* 2019; **13**: 333 [PMID: 31474831 DOI: 10.3389/fncel.2019.00333]

38 **Slanzi A**, Iannoto G, Rossi B, Zenaro E, Constantin G. In vitro Models of Neurodegenerative Diseases. *Front Cell Dev Biol* 2020; **8**: 328 [PMID: 32528949 DOI: 10.3389/fcell.2020.00328]

39 **Sofroniew MV**. Reactive astrocytes in neural repair and protection. *Neuroscientist* 2005; **11**: 400-407 [PMID: 16151042 DOI: 10.1177/1073858405278321]

40 **Araque A**, Sanzgiri RP, Parpura V, Haydon PG. Astrocyte-induced modulation of synaptic transmission. *Can J Physiol Pharmacol* 1999; **77**: 699-706 [PMID: 10566947]

41 **Horner PJ**, Palmer TD. New roles for astrocytes: the nightlife of an 'astrocyte'. La vida loca!. *Trends Neurosci* 2003; **26**: 597-603 [PMID: 14585599 DOI: 10.1016/j.tins.2003.09.010]

42 **Verkhratsky A**. Physiology of neuronal-glial networking. *Neurochem Int* 2010; **57**: 332-343 [PMID: 20144673 DOI: 10.1016/j.neuint.2010.02.002]

43 **Shandra O**, Robel S. Imaging and Manipulating Astrocyte Function In Vivo in the Context of CNS Injury. *Methods Mol Biol* 2019; **1938**: 233-246 [PMID: 30617984 DOI: 10.1007/978-1-4939-9068-9\_16]

44 **Azevedo FA**, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol* 2009; **513**: 532-541 [PMID: 19226510 DOI: 10.1002/cne.21974]

45 **Pekny M**, Pekna M, Messing A, Steinhäuser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A. Astrocytes: a central element in neurological diseases. *Acta Neuropathol* 2016; **131**: 323-345 [PMID: 26671410 DOI: 10.1007/s00401-015-1513-1]

46 **Carmen J**, Magnus T, Cassiani-Ingoni R, Sherman L, Rao MS, Mattson MP. Revisiting the astrocyte-oligodendrocyte relationship in the adult CNS. *Prog Neurobiol* 2007; **82**: 151-162 [PMID: 17448587 DOI: 10.1016/j.pneurobio.2007.03.001]

47 **Foo LC**, Allen NJ, Bushong EA, Ventura PB, Chung WS, Zhou L, Cahoy JD, Daneman R, Zong H, Ellisman MH, Barres BA. Development of a method for the purification and culture of rodent astrocytes. *Neuron* 2011; **71**: 799-811 [PMID: 21903074 DOI: 10.1016/j.neuron.2011.07.022]

48 **Venkadesh S**, Van Horn JD. Integrative Models of Brain Structure and Dynamics: Concepts, Challenges, and Methods. *Front Neurosci* 2021; **15**: 752332 [PMID: 34776853 DOI: 10.3389/fnins.2021.752332]

49 **López-Hidalgo M**, Schummers J. Cortical maps: a role for astrocytes? *Curr Opin Neurobiol* 2014; **24**: 176-189 [PMID: 24419141 DOI: 10.1016/j.conb.2013.11.001]

50 **Floden AM**, Combs CK. Microglia repetitively isolated from *in vitro* mixed glial cultures retain their initial phenotype. *J Neurosci Methods* 2007; **164**: 218-224 [PMID: 17553568 DOI: 10.1016/j.jneumeth.2007.04.018]

51 **Fellin T**, Ellenbogen JM, De Pittà M, Ben-Jacob E, Halassa MM. Astrocyte regulation of sleep circuits: experimental and modeling perspectives. *Front Comput Neurosci* 2012; **6**: 65 [PMID: 22973222 DOI: 10.3389/fncom.2012.00065]

52 **Bazargani N**, Attwell D. Astrocyte calcium signaling: the third wave. *Nat Neurosci* 2016; **19**: 182-189 [PMID: 26814587 DOI: 10.1038/nn.4201]

53 **Elmariah SB**, Oh EJ, Hughes EG, Balice-Gordon RJ. Astrocytes regulate inhibitory synapse formation *via* Trk-mediated modulation of postsynaptic GABAA receptors. *J Neurosci* 2005; **25**: 3638-3650 [PMID: 15814795 DOI: 10.1523/JNEUROSCI.3980-04.2005]

54 **Souza DG**, Bellaver B, Souza DO, Quincozes-Santos A. Characterization of adult rat astrocyte cultures. *PLoS One* 2013; **8**: e60282 [PMID: 23555943 DOI: 10.1371/journal.pone.0060282]

55 **He Y**, Taylor N, Bhattacharya A. Isolation and Culture of Astrocytes from Postnatal and Adult Mouse Brains. *Methods Mol Biol* 2019; **1938**: 37-47 [PMID: 30617971 DOI: 10.1007/978-1-4939-9068-9\_3]

56 **Görg B**, Karababa A, Häussinger D. Hepatic Encephalopathy and Astrocyte Senescence. *J Clin Exp Hepatol* 2018; **8**: 294-300 [PMID: 30302047 DOI: 10.1016/j.jceh.2018.05.003]

57 **Allen NJ**, Barres BA. Neuroscience: Glia - more than just brain glue. *Nature* 2009; **457**: 675-677 [PMID: 19194443 DOI: 10.1038/457675a]

58 **Marignier R**, Nicolle A, Watrin C, Touret M, Cavagna S, Varrin-Doyer M, Cavillon G, Rogemond V, Confavreux C, Honnorat J, Giraudon P. Oligodendrocytes are damaged by neuromyelitis optica immunoglobulin G *via* astrocyte injury. *Brain* 2010; **133**: 2578-2591 [PMID: 20688809 DOI: 10.1093/brain/awq177]

59 **Rose CF**, Verkhratsky A, Parpura V. Astrocyte glutamine synthetase: pivotal in health and disease. *Biochem Soc Trans* 2013; **41**: 1518-1524 [PMID: 24256247 DOI: 10.1042/BST20130237]

60 **Nimmerjahn A**, Bergles DE. Large-scale recording of astrocyte activity. *Curr Opin Neurobiol* 2015; **32**: 95-106 [PMID: 25665733 DOI: 10.1016/j.conb.2015.01.015]

61 **Herculano-Houzel S**. The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia* 2014; **62**: 1377-1391 [PMID: 24807023 DOI: 10.1002/glia.22683]

62 **Oberheim NA**, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. *Methods Mol Biol* 2012; **814**: 23-45 [PMID: 22144298 DOI: 10.1007/978-1-61779-452-0\_3]

63 **Grupp L**, Wolburg H, Mack AF. Astroglial structures in the zebrafish brain. *J Comp Neurol* 2010; **518**: 4277-4287 [PMID: 20853506 DOI: 10.1002/cne.22481]

64 **Souto PA**, Marcotegui AR, Orbea L, Skerl J, Perazzo JC. Hepatic encephalopathy: Ever closer to its big bang. *World J Gastroenterol* 2016; **22**: 9251-9256 [PMID: 27895414 DOI: 10.3748/wjg.v22.i42.9251]

65 **Quero JC**, Hartmann IJ, Meulstee J, Hop WC, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological tests and automated electroencephalogram analysis. *Hepatology* 1996; **24**: 556-560 [PMID: 8781324 DOI: 10.1002/hep.510240316]

66 **Bosoi CR**, Rose CF. Identifying the direct effects of ammonia on the brain. *Metab Brain Dis* 2009; **24**: 95-102 [PMID: 19104924 DOI: 10.1007/s11011-008-9112-7]

67 **Nakagawa T**, Yabe T, Schwartz JP. Gene expression profiles of reactive astrocytes cultured from dopamine-depleted striatum. *Neurobiol Dis* 2005; **20**: 275-282 [PMID: 16242635 DOI: 10.1016/j.nbd.2005.03.009]

68 **Paixão S**, Klein R. Neuron-astrocyte communication and synaptic plasticity. *Curr Opin Neurobiol* 2010; **20**: 466-473 [PMID: 20471242 DOI: 10.1016/j.conb.2010.04.008]

69 **Kimelberg HK**, Schools GP, Cai Z, Zhou M. Freshly isolated astrocyte (FIA) preparations: a useful single cell system for studying astrocyte properties. *J Neurosci Res* 2000; **61**: 577-587 [PMID: 10972954 DOI: 10.1002/1097-4547(20000915)61:6<577::AID-JNR1>3.0.CO;2-T]

70 **Lee SC**, Liu W, Brosnan CF, Dickson DW. Characterization of primary human fetal dissociated central nervous system cultures with an emphasis on microglia. *Lab Invest* 1992; **67**: 465-476 [PMID: 1359193]

71 **Schuenke K**, Gelman BB. Human microglial cell isolation from adult autopsy brain: brain pH, regional variation, and infection with human immunodeficiency virus type 1. *J Neurovirol* 2003; **9**: 346-357 [PMID: 12775418 DOI: 10.1080/13550280390201056]

72 **Ahrens MB**, Orger MB, Robson DN, Li JM, Keller PJ. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods* 2013; **10**: 413-420 [PMID: 23524393 DOI: 10.1038/nmeth.2434]

73 **Rotty JD**, Wu C, Haynes EM, Suarez C, Winkelman JD, Johnson HE, Haugh JM, Kovar DR, Bear JE. Profilin-1 serves as a gatekeeper for actin assembly by Arp2/3-dependent and -independent pathways. *Dev Cell* 2015; **32**: 54-67 [PMID: 25543281 DOI: 10.1016/j.devcel.2014.10.026]

74 **Ramakers GJ**, Moolenaar WH. Regulation of astrocyte morphology by RhoA and lysophosphatidic acid. *Exp Cell Res* 1998; **245**: 252-262 [PMID: 9851865 DOI: 10.1006/excr.1998.4224]

75 **Jakovcevski I**, Zecevic N. Sequence of oligodendrocyte development in the human fetal telencephalon. *Glia* 2005; **49**: 480-491 [PMID: 15578660 DOI: 10.1002/glia.20134]

76 **Burgos M**, Calvo S, Molina F, Vaquero CF, Samarel A, Llopis J, Tranque P. PKCepsilon induces astrocyte stellation by modulating multiple cytoskeletal proteins and interacting with Rho A signalling pathways: implications for neuroinflammation. *Eur J Neurosci* 2007; **25**: 1069-1078 [PMID: 17331203 DOI: 10.1111/j.1460-9568.2007.05364.x]

**Footnotes**

**Conflict-of-interest statement:** All theauthors report no relevant conflicts of interest for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** December 17, 2022

**First decision:** January 3, 2023

**Article in press:**

**Specialty type:** Medicine, research and experimental

**Country/Territory of origin:** Slovenia

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

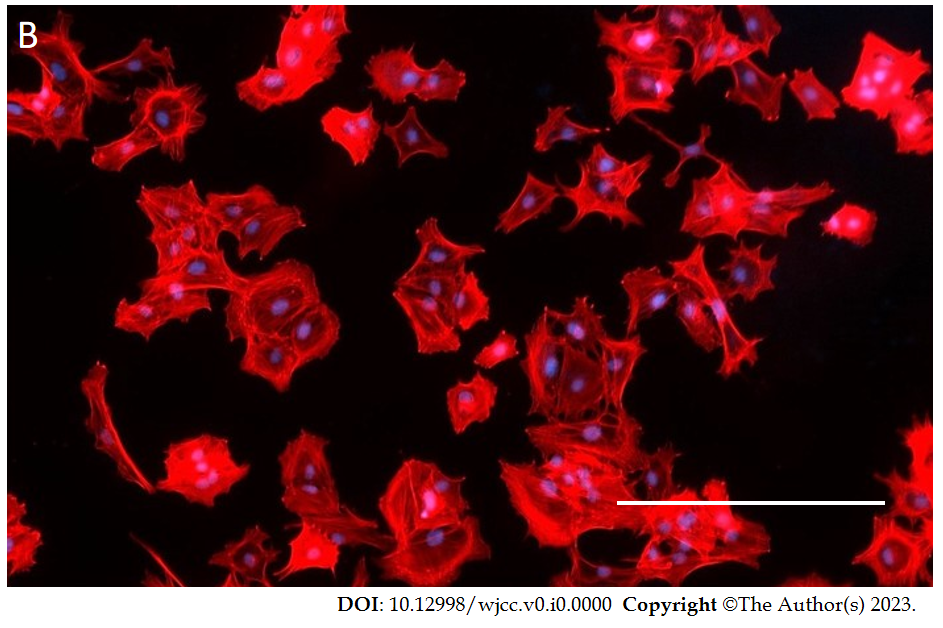
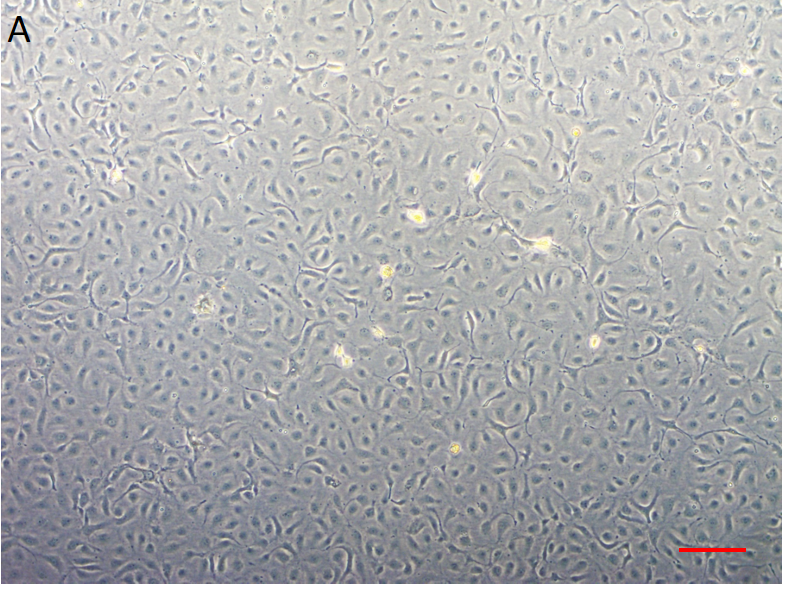
Grade C (Good): C, C

Grade D (Fair): D

Grade E (Poor): 0

**P-Reviewer:** Kotelevets SM, Russia; Perazzo JC, Argentina **S-Editor:** Fan JR **L-Editor:** A **P-Editor:**

**Figure Legends**



**Figure 1 Human astrocytes.** A: The primary culture of human astrocytes. Images: Zeiss Axiovert 40 inverted microscope, × 50 magnification. Scale bar = 200 μm. Original figure taken by the authors; B: The immunocytochemical characterization of adult human astrocytes. Morphology of the cells was determined with a fluorescent phalloidin conjugate, which binds selectively to actin filaments (red). In low-density cultures, adult astrocytes exhibit a polygonal form with actin filaments adjacent to the cell membrane. Nuclei were counter-stained with 4',6-diamidino-2-phenylindole (blue). Images: EVOS FL fluorescence microscope, × 10 magnification. Scale bar = 400 μm.

**Table 1 Main astrocyte functions**

|  |  |
| --- | --- |
|  | **Main function of astrocytes** |
| 1 | Role in neuronal migration |
| 2 | Formation of extracellular matrix proteins and adhesion molecules |
| 3 | Production of neurotrophic and neurite-promoting factors |
| 4 | Maintenance of blood-brain barrier |
| 5 | Participation in angiogenesis |
| 6 | Neurotransmission |
| 7 | Energy metabolism and regulation of central nervous system microenvironment |
| 8 | Role in detoxification, exocytosis of macromolecules, exocytosis of macromolecules, neuroendocrine functions |
| 9 | The response of astrocytes in various central nervous system insults |
| 10 | Role in immune response and phagocytosis |
| 11 | Brain protection from oxidative damage |