

Article

Protoplasmic Astrocytes Are Poorly Understood Cells in Adult Human Brain Tissue

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Abstract

Background/Objectives: The traditional classification of astrocytes was based on morphological differences between astrocytes and their location in brain tissue. Astrocytes stained by impregnation techniques were divided into protoplasmic and fibrous astrocytes. We still use this classification, often supplemented by GFAP immunostaining. However, protoplasmic astrocytes have been found in the human cerebral cortex as GFAP-negative cells. **Methods:** In this study, astrocytes were identified using Cajal's gold sublimation method and GFAP immunostaining. Biopsy samples of normal brain tissue ($n = 25$) were obtained from adult patients diagnosed with traumatic brain injury, stroke, gliomas and brain metastases. **Results:** In all samples, GFAP-positive fibrous astrocytes were found in the subpial region (layer I–II) and in the white matter. GFAP-positive protoplasmic astrocytes were absent or occurred only rarely in the cortical gray matter (layer III–VI) in samples from patients diagnosed with a tumor. Similar staining was also observed using the Cajal method. However, in samples from patients with traumatic brain injury accompanied by high intracranial pressure, GFAP-positive areas with numerous astrocytic processes and cells with a morphology similar to protoplasmic astrocytes were found. **Conclusions:** We can conclude that protoplasmic astrocytes are GFAP-negative cells that respond to brain injury by GFAP expression. We consider this finding to be a sign of protoplasmic astrocyte differentiation. On the other hand, fibrous astrocytes are GFAP-positive and respond to brain injury with increased GFAP expression. These results raise questions regarding the classification of astrocytes and, in particular, the histological visualization of the neuro-glial-vascular unit.

Keywords: astrocytes; GFAP; vimentin; cajal staining; neuron–glia interaction

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1. Introduction

Since their discovery at the end of the 19th century, astrocytes have been classified as protoplasmic and fibrous. Recent publications containing original drawings show protoplasmic astrocytes as cells with highly branched processes distributed in the gray matter, while fibrous astrocytes are characterized by straight, long, less branched processes located mainly in the white matter [1]. The first morphological classification of astrocytes in adult human brain sections was obtained from preparations using the Golgi method [2].

Initially gliofibrils appeared to be absent in protoplasmic astrocytes. Later, Cajal, using his gold chloride sublimate method, recognized fibrils in both gray and white matter astrocytes [3]. However, in Cajal's preparations, fibrous astrocytes in the white matter

were stained with his gold chloride sublimate, as well as with the Golgi method, and protoplasmic astrocytes were stained using the silver carbonate method (del Rio), and published pictures originate from the human hippocampus [1,4,5]

Later, the structure of gliofibrils, which are formed by two intermediate filaments, GFAP and vimentin, was described. GFAP was isolated from glial plaques of a patient with multiple sclerosis and became widely used as specific marker for cells of astroglial origin [6]. Vimentin has long been described as specific to cells of mesenchymal origin, present transiently only in immature astrocytes [7]. Currently, numerous studies confirm the co-expression of vimentin with GFAP or other intermediate filaments [8,9].

Problematic imaging of protoplasmic astrocytes is evident in some studies or books where authors circumvent this problem. In the book *Human histology* (2010), astrocytes are distinguished on the basis of GFAP expression [10]. Fibrous astrocytes are described as rich in GFAP bundles, while protoplasmic astrocytes contain only a few GFAP bundles. They demonstrate only immunoperoxidase staining of fibrous astrocytes, not of protoplasmic ones.

The book *Neuroglia* (1995) states that numerous astrocytes in the normal brain are GFAP-negative in immunocytochemistry and that GFAP-negative astrocytes predominate in the gray matter, without any explanation of how these results were obtained, and how we can distinguish GFAP-negative astrocytes [3]. In contrast to this statement the authors declare that cultures of fibrous and protoplasmic astrocytes are strongly immunoreactive. However, they describe astrocyte classification of cultures derived from 18-day-old rat fetuses. Both types of astrocytes are demonstrated from cortical gray matter but the cultures clearly contain only astrocytes of fibrous morphologies.

We previously published the characteristics of 70 adult human brain cultures, prepared from biopsy of patients with non-tumor diagnoses [9]. The cultures were prepared separately from cortical gray matter and subcortical white matter. We expected to find morphologically distinct fibrous and protoplasmic astrocytes. However, both cultures differed only in the number of GFAP-positive cells, which were absent or rare in cultures from cortical gray matter. On the other hand, confluent cultures (3 to 6 weeks after explants seeding) from white matter contained 0.1% of GFAP-positive astrocytes which showed three main morphologies of astrocytes: stellate, bipolar and large plasmatic cells [11]. Stellate astrocytes may correspond to fibrous astrocytes, but no protoplasmic morphologies were found in all cultures. GFAP-positive cells disappeared in early passages. The predominant cell population in adult human brain cultures was flat or spindle-shaped GFAP-negative but fibronectin-positive cells [9]. These cultures have been poorly defined until now and are usually referred to as “glia-like” cells. This term was introduced for GFAP-negative cells with some morphological similarities to astrocytes [12]. Some authors consider that GFAP-negative cultures of adult human brain are of non-glial origin [13,14]. Other studies showed that primary cultures derived from fetal human brain contain more than 90% of GFAP-positive cells [15]. Similarly, almost pure culture of GFAP-positive astrocytes can be obtained from the brain of newborn rodents [13].

Initially, glial cells were considered to be supporting tissue in the CNS. Currently, much attention is being paid to the interactions between neurons and glial cells, which are considered fundamental to understanding brain development and function [16,17]. Dysfunction of neuron–glia interactions may contribute to the pathogenesis of neurodevelopmental disorders or Parkinson’s disease [18–20]. In this study, we demonstrate protoplasmic astrocytes in normal and damaged adult human cerebral cortex using the Cajal impregnation method and immunohistochemical stains.

2. Materials and Methods

2.1. Brain Biopsy Materials

Brain samples were kindly provided by the Department of Neurosurgery, Derer's Hospital, Bratislava, Slovakia. This study provides an overview of the findings obtained from brain biopsy samples collected and examined between 1989 and 2011. Experiments with human brain biopsy samples were performed according to Slovak laws 272/1994 and 76/2004 and approved by the Ethical Committee of UNB Bratislava. For this study, we selected brain biopsy samples from the frontal or temporal lobe containing gray and white matter (Figure 1A). Biopsy samples were obtained from 25 adult patients who underwent neurosurgery for traumatic contusion with intraparenchymal hemorrhages (5), subdural hematoma (2), and aneurysm (2). These diagnoses usually cause high intracranial pressure accompanied by coma and require decompressive resection of brain tissue (Figure 1B). Further biopsy samples from conscious patients with various neurological symptoms were obtained from patients with primary brain gliomas ($n = 10$) and brain metastases ($n = 6$). Brain biopsy samples were obtained from access resection of brain tissue (Figure 1C,D) and were macroscopically normal tissue. Brain samples from laboratory animals can be clearly defined, but human brain biopsies are heterogeneous; it is only possible to collect and select samples from patients with a similar diagnosis and macroscopically normal or damaged brain tissue.

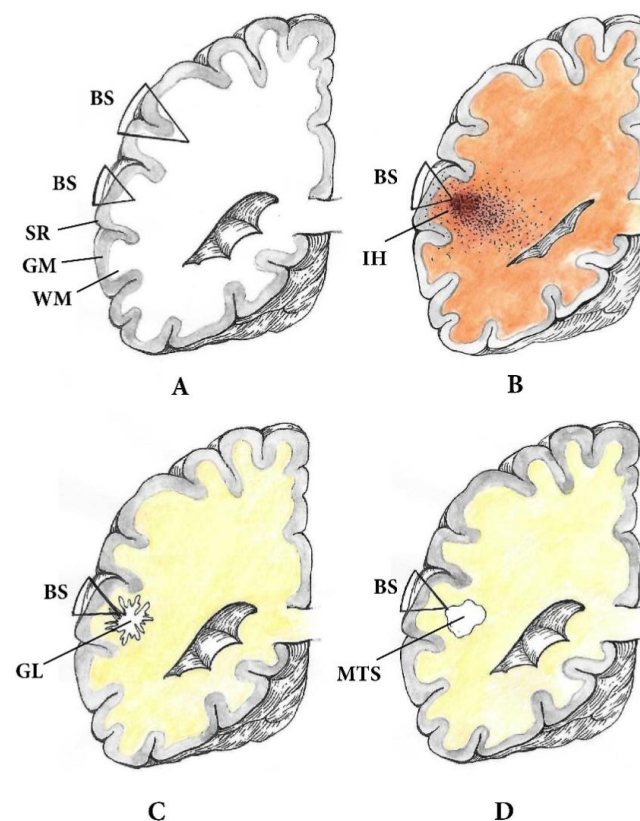


Figure 1. Schema of biopsy samples. Cross-section of forebrain and frontal lobe. Biopsy samples (BS), subpial region (SR), gray matter (GM), and white matter (WM), described as subcortical gray matter (A). Traumatic brain contusion, orange color indicates high intracranial pressure (B), yellow color indicates chronic intracranial pressure in patients with glioma (C) and brain metastasis (D).

Brain biopsy samples were fixed in 4% neutral formalin and embedded in paraffin for immunohistochemistry or fixed in formalin and ammonium bromide. Parts of brain biopsy samples were covered with cryo-embedding media (OCT) and used for cryosections.

2.2. Immunofluorescence Staining

To identify astroglial cell types, we used antibodies against GFAP, clone GF-01 (1:100, Exbio, Prague, Czech Republic, Product No. 11-255-C100), clone G-A-5 (1:100, Sigma, Darmstadt, Germany, No. G3893), polyclonal antibodies to GFAP (1:100, Dako, Glostrup, Denmark, No. Z0334), and polyclonal antibodies to S-100B (1:100, DAKO Z0311). The sections were also stained for vimentin with monoclonal antibodies, clone V9, (1:100, Sigma, No. V6630), and clone Vi-01 (1:100, Exbio, Prague No. 11-254-C100).

Immunofluorescence staining was performed on brain cryosections (10 µm thick) fixed in methanol-acetone (1:1) solution for 15 min. (at −15 °C). Nonspecific background staining was reduced by 3% normal goat serum. Cryosections for indirect immunofluorescence were incubated 1 h with primary antibodies and 30 min with 1:50 diluted fluorescein- or rhodamine-conjugated antibodies (F-9137, A21202 T5393 Sigma). Fluorescence examination was performed using Olympus IMT2 and Olympus BX-51 microscopes, Hamburg Germany.

2.3. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections of brain samples were used for indirect immunoperoxidase and PAP staining. The sections were dewaxed and rehydrated; endogenous peroxidase was blocked in a solution of 0.3% H₂O₂ in methanol for 15 min.

Nonspecific background staining was reduced by 3% BSA solution. The same primary antibodies were used as in the immunofluorescence staining described above, but at a dilution of 1:50. Incubation with primary antibodies was 1 h. Slides were washed and incubated for 30 min with peroxidase-conjugated secondary antibodies purchased from DAKO (No. P217, P161). For PAP staining secondary antibodies were applied for 30 min (No. Z0259, Z0196, DAKO) following with PAP complex in dilution 1:50 for 30 min (No. Z0113, B650, DAKO). The diaminobenzidine reagent was used as chromogen. The sections were counterstained with hematoxylin for 30 s and mounted with Solacryl BMX under coverslips.

2.4. Cajal Gold Chloride Sublimate Impregnation Technique

Small pieces of brain tissue were fixed in a solution of formalin and ammonium bromide for 5 days. Prepared 20 µm sections of fixed tissue, washed with distilled water, were placed in a gold bath containing 0.5 g mercuric chloride dissolved in 50 mL warm distilled water and 10 mL 1% brown gold chloride solution; incubation lasted 4 h. This solution must be filtered and always fresh. The sections were washed with distilled water and fixed in 5% Cajal's solution (sodium thiosulphate) for 5 min and washed in 50% alcohol and then in distilled water, mounted on gelatinated slides, and mounted with Solacryl BMX.

3. Results

3.1. Immunofluorescence

To avoid cross-reactivity of antibodies to intermediate filaments such as GFAP and vimentin, we decided to use indirect immunofluorescence staining. Cryosections were examined using two monoclonal antibodies and polyclonal antibodies against GFAP. Staining was similar for all antibodies, with the exception of polyclonal antibodies, for which we observed higher intensity of nonspecific background. GFAP was detected in all examined brain samples in the subpial region (layers I–II), and in the white matter. The positively stained cells had the morphology of fibrous astrocytes. However, GFAP-positive protoplasmic astrocytes were observed in association with damaged brain tissue. GFAP-staining in samples from patients with traumatic brain injury revealed positive staining of astrocyte processes in the subpial region (Figure 2A). Protoplasmic astrocytes occurred

in the cortical gray matter (Figure 2B,C). However, areas containing only short astrocytic processes (Figure 2D) or areas with longer fibers (Figure 2E) were predominant. Cerebral vessels surrounded exclusively by GFAP-positive processes were rare (Figure 2F). In these biopsy samples, which were primarily damaged by traumatic contusion, GFAP-negative astrocytes began to express GFAP in their processes. We observed areas where protoplasmic astrocytes exhibited numerous processes (Figure 2G–I). Samples from patients with gliomas or brain metastases who presented with long-term chronic intracranial pressure and neurological symptoms without a comatose stage showed a rare occurrence of protoplasmic astrocytes (Figure 2J,K). Numerous fibrous astrocytes present in the white matter were GFAP-positive (Figure 2L). Indirect immunofluorescence to vimentin was performed on cryosections using two monoclonal antibodies: clone V9, fluorescein-conjugated secondary antibodies (Figure 3A–F), and clone Vi-01, rhodamine-conjugated secondary antibodies (Figure 3G–I). The intensity of staining was similar for both antibodies. Vimentin-positive staining in a biopsy sample from a patient with traumatic brain contusion showed a network of positive processes in the subpial region (Figure 3A) and areas of processes of varying length in the gray matter (Figure 3B). Vimentin was also present in fibrous astrocytes in the white matter (Figure 3C). In a sample from a patient with glioma, positive staining was present in the subpial region (Figure 3D) and in the white matter (Figure 3F). Vimentin-positive protoplasmic astrocytes occurred rarely in the gray matter (Figure 3E). On the other hand, positively stained protoplasmic astrocytes were absent in the sample from the patient with brain metastasis (Figure 3G–I). Vimentin-positive staining was present in the subpial region, and the arrow indicates positively stained meninges (Figure 3G). Negatively stained protoplasmic astrocytes and positively stained cerebral vessels were present in the cortical gray matter (Figure 3H). Vimentin was present in fibrous astrocytes (Figure 3I).

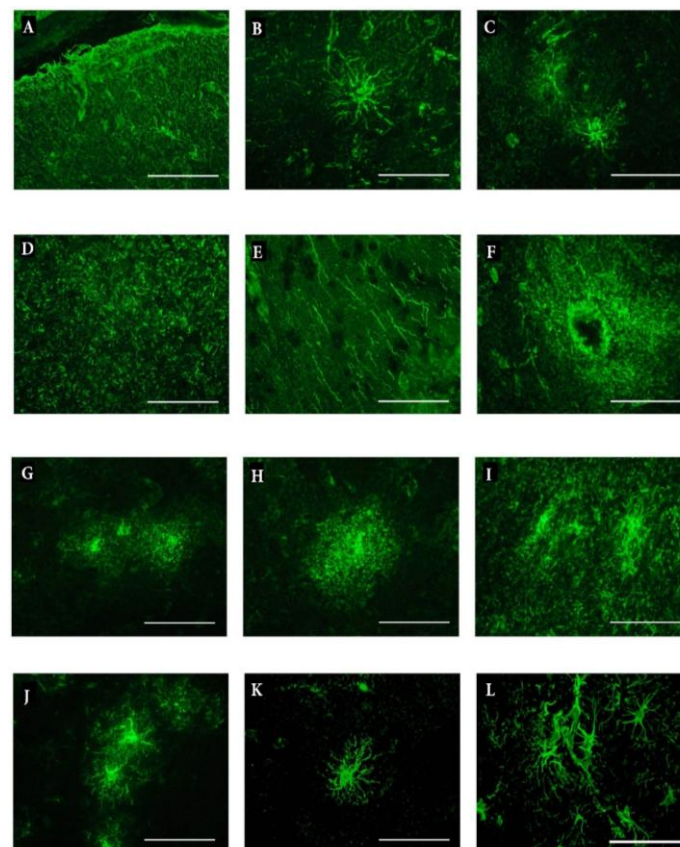


Figure 2. Indirect immunofluorescence staining with monoclonal antibodies to GFAP, clone G-5-A (A–E) and clone GF-01 (J–L). (A–E) 18-year-old patient with traumatic brain contusion: (A) GFAP+ cells

and fibers in subpial region. (B,C) Protoplasmic astrocytes in gray matter. (D,E) Astrocytic processes of varying lengths in gray matter. (F–I) 64-year-old patient with contusion: (F) GFAP+ astrocytic processes surrounding brain vessel. (G–I) Differentiation of protoplasmic astrocytes. (J–L) 38-year-old patient with glioma: (J,K) Protoplasmic astrocytes in gray matter and (K) fibrous astrocytes in white matter. Scale bars = 50 μ m.

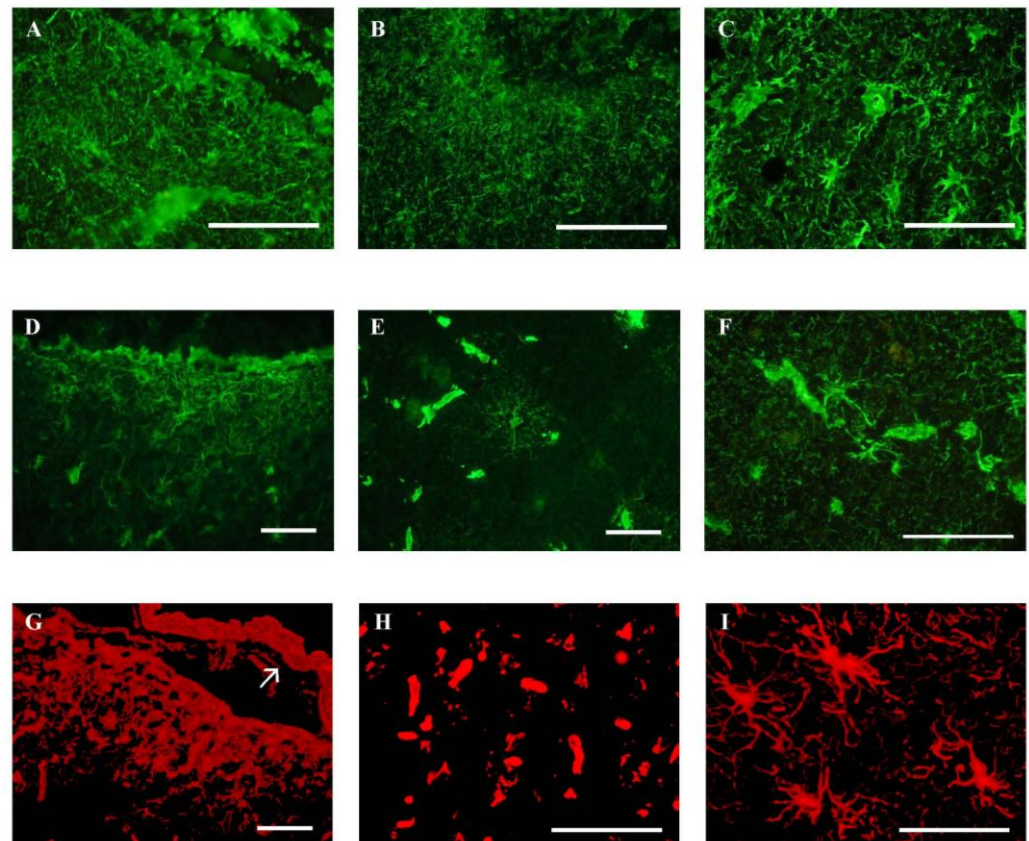


Figure 3. Indirect immunofluorescence with monoclonal antibodies to vimentin, clone V9 (A–F) and clone Vi-01 (G–I). (A–C) 42-year-old patient with traumatic brain contusion: (A) Vimentin+ astrocytic processes in subpial region, and (B) in gray matter. (C) Vimentin+ fibrous astrocytes in white matter. (D–F) 60-year-old patient with glioma: (D) Vimentin+ cells in subpial region. (E) Vimentin+ brain vessels (bvs) and one weakly stained protoplasmic astrocyte in gray matter. (F) bvs and fibrous astrocytes in white matter. (G–I) 47-year-old patient with brain metastasis: (G) Vimentin+ astrocytes and meninges (white arrow) in subpial region. (H) Positively stained bvs and negative astrocytes in gray matter. (I) Vimentin+ fibrous astrocytes in white matter. Scale bars = 50 μ m.

3.2. Immunohistochemistry

Astrocytes were also identified immunohistochemically for GFAP, vimentin, and S100B. GFAP staining was performed using two monoclonal and polyclonal antibodies. Staining with all antibodies showed similar results. GFAP-positive protoplasmic astrocytes were found in association with brain injury causing high intracranial pressure. In brain samples with the same diagnosis, bleeding from a ruptured aneurysm, different staining of protoplasmic astrocytes was observed. However, the different intensity of bleeding was caused by different GFAP immunoreactivity. In a brain sample from a patient with high intracranial pressure, protoplasmic astrocytes stained positively for GFAP (Figure 4A–C), whereas in less damaged brain tissue with mild bleeding, GFAP-positive protoplasmic astrocytes were not present (Figure 4E). Positive staining was observed in the subpial area (Figure 4D) and in the white matter (Figure 4F).

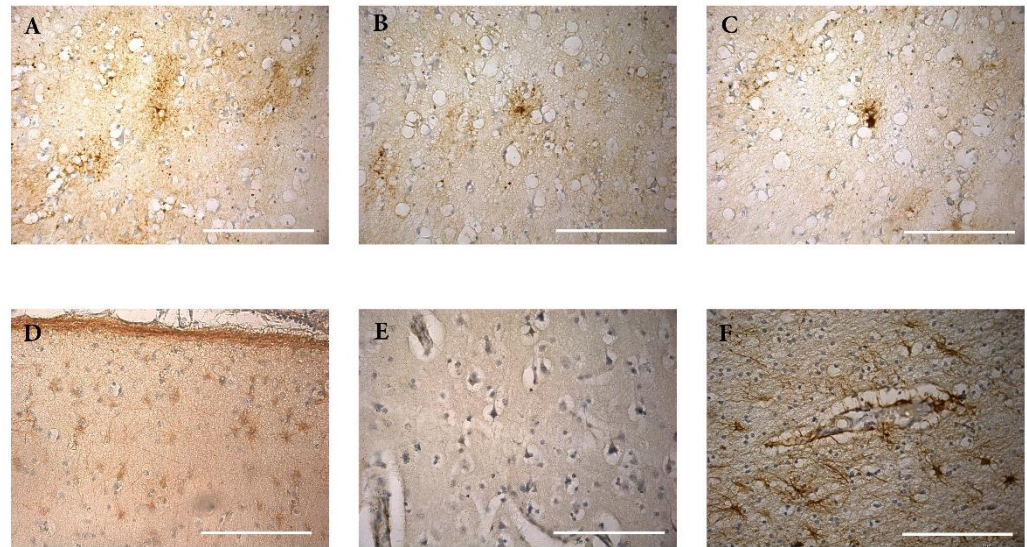


Figure 4. Immunohistochemical staining with monoclonal antibodies to GFAP, clone GF-01. A 42-year-old patient with intense bleeding from an aneurysm: (A–C) Differentiation of protoplasmic astrocytes, GFAP+ cells in gray matter. A 34-year-old patient with less intense bleeding from an aneurysm: (D) GFAP+ cells in subpial region. (E) GFAP- astrocytes in gray matter. (F) Brain vessel surrounded by fibrous astrocytes in white matter. Scale bars = 50 μ m.

GFAP-positive protoplasmic astrocytes were rarely observed in samples from patients with gliomas (Figure 5A–C) and were almost entirely absent in samples from patients with brain metastases (Figure 5D–I). GFAP-positive astrocytes with a fibrous morphology were found in the subpial region (Figure 5A), while the gray matter contained faintly stained cells (Figure 5B) or a group of GFAP-positive cells with the nonspecific morphology of protoplasmic astrocytes (Figure 5C). In contrast, GFAP staining of brain sections from a patient with brain metastases was negative in the gray matter and positive in the white matter (Figure 5D), and morphological features of fibrous astrocytes (Figure 5E) and areas of reactive astrocytes (Figure 5F) were present in the white matter near the brain metastasis. However, protoplasmic astrocytes in the gray matter remained GFAP-negative. It is noteworthy to observe the blood vessels in the cerebral cortex and any stained astrocytes (Figure 5G) or a weak staining of a single astrocyte without endfeet on the vessels (Figure 5H). In contrast, GFAP-positive endfeet of fibrous astrocytes surrounded the vessel in the white matter (Figure 5I). Staining for vimentin using two monoclonal antibodies was negative, with rare presence of weakly stained fibrous astrocytes.

Two biopsy samples from a single patient with a glioma were examined by immunohistochemical staining using polyclonal antibodies against S100B. The first sample was an accessive brain biopsy from a patient with glioma (Figure 6A–C). S100B-positive staining was present in the subpial region (Figure 6A) and in white matter (Figure 6C), while S100B-negative staining was present in the gray matter (Figure 6B). The second sample was decompressive biopsy; a week later, the same patient underwent repeat surgery due to post-operative bleeding accompanied by increased intracranial pressure (Figure 6D–F). The number of S100B-positive cells increased in the subpial region (Figure 6D) and in the white matter (Figure 6F). S100B-positive cells were also found in the gray matter (Figure 6E). Similar to GFAP, high intracranial pressure induced the expression of S100B in protoplasmic astrocytes. However, S100B antibodies stained evidently fewer astrocyte processes.

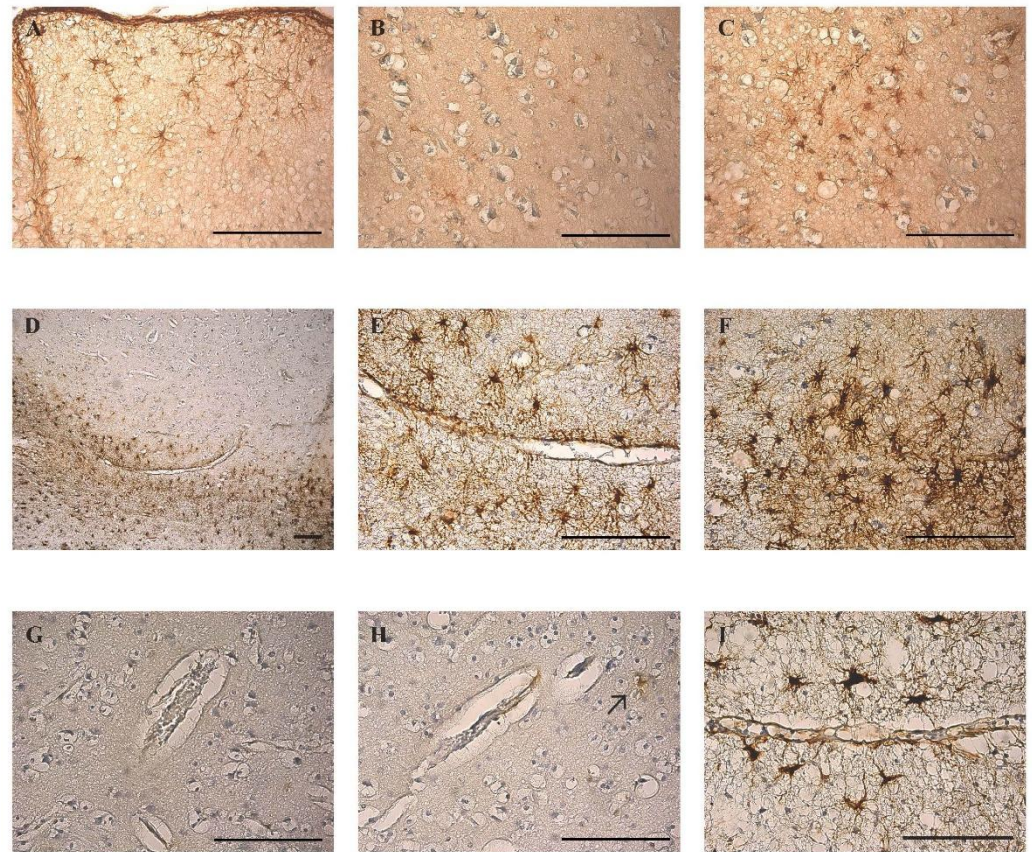


Figure 5. Immunohistochemical staining with polyclonal antibodies to GFAP. A 60-year-old patient with glioma: (A) GFAP+ cells in subpial region, similar to fibrous astrocytes. (B) GFAP weakly stained astrocytes in gray matter and (C) an area with several positive astrocytes of intermediate morphology in gray matter. A 59-year-old patient with brain metastasis: (D) Numerous GFAP+ fibrous astrocytes present in white matter and negative protoplasmic astrocytes in gray matter. (E,F) Increased GFAP staining in reactive fibrous astrocytes in white matter and (I) endfeet of fibrous astrocytes surrounding a brain vessel. (G) Note brain vessels in gray matter, GFAP- protoplasmic astrocytes or (H) only one weakly stained astrocyte (arrow). Scale bars = 50 μ m.

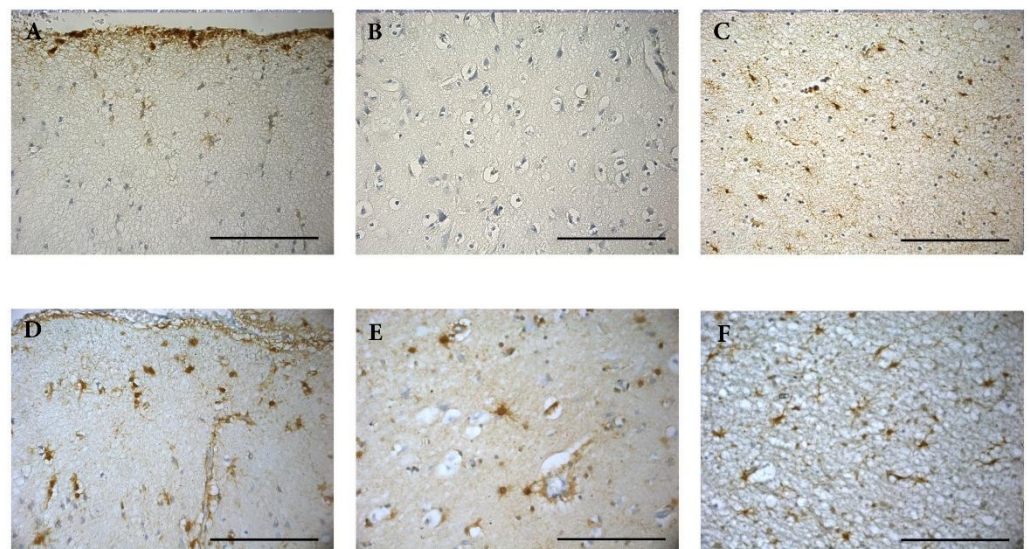


Figure 6. Immunohistochemical staining with polyclonal antibodies to S100B. A 44-year-old patient with glioma: (A) S100B+ astrocytes in subpial region and (C) in white matter. (B) S100B-negative stained protoplasmic astrocytes in gray matter. The same patient underwent repeat surgery a week

later for postoperative bleeding with increased intracranial pressure (D–F). The number of S100B+ cells increased in the subpial area (D) and in the white matter (F). Positive staining appeared in protoplasmic astrocytes (E). All S100+ cells showed fewer processes. Scale bars = 50 μ m.

We used Cajal's gold chloride sublimate staining which is usually demonstrated on cat spinal cord or brain tissue [3]. Further information about adequate animals for the impregnation method were described by Cajal: "It is very important to choose the proper animal to obtain good results. The cerebral cortex of an 8-day-old rabbit is better impregnated than the cortex of rat, guinea pig, white mouse, dog, etc. of the equivalent developmental age" [1].

Here, we demonstrate our best stained sections and the method that produces these results: the gold chlorite sublimation method (Figure 7A–D) and immunohistochemical staining for GFAP (Figure 7E,F). Both stains were performed on a sample from one patient with glioma. Cajal's method showed a weakly stained subpial region (Figure 7A), negative protoplasmic astrocytes in gray matter (Figure 7B,C) and positive astrocytes in white matter (Figure 7C,D). GFAP staining was positive in fibrous (Figure 7E) and negative in protoplasmic astrocytes (Figure 7F).

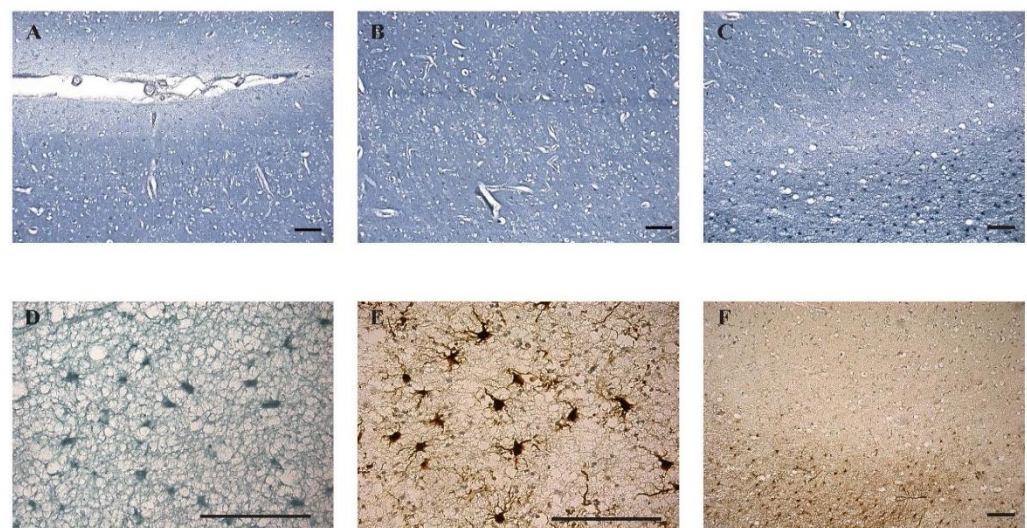


Figure 7. Cajal's gold chloride sublimate method (A–D) and immunohistochemical staining to GFAP, clone GF-01 (E,F). Biopsy sample from a 39-year-old patient with glioma was used for both staining methods. Cajal's technique showed a weakly stained subpial area (A), negatively stained protoplasmic astrocytes in gray matter (B,C), and positive stained fibrous astrocytes in white matter (C,D). Similar staining was achieved with GFAP antibodies: positively stained fibrous astrocytes (E), negatively stained protoplasmic astrocytes (F), a transitional area between the gray and white matter (C,F). Scale bar = 50 μ m.

4. Discussion

Previously, we demonstrated GFAP-negative protoplasmic astrocytes and GFAP-positive fibrous astrocytes in adult human brain tissue [21]. Recently, we showed that protoplasmic astrocytes can express GFAP in the damaged human brain cortex [22]. Sofroniew reported similar findings, demonstrating that reactive astrogliosis borders microinfarcts in the human cerebral cortex. He detected the presence of GFAP-positive fibrous astrocytes in subcortical white matter, whereas GFAP was not detectable in most protoplasmic astrocytes of the gray matter [23].

In this study, we summarize the findings obtained over the last three decades, which we can describe as problematic visualization of protoplasmic astrocytes in adult human brain tissue. S100B and GFAP have long been widely used as astroglial markers. We

examined S100B in human brain samples; however, this staining did not reveal protoplasmic astrocytes in normal gray matter; they were found only rarely in damaged cerebral gray matter. S100B is a less specific marker than GFAP, as it has been detected in some neuronal populations [24] and in oligodendrocytes [25,26]. Compared to GFAP staining, S100B-positive cells exhibited significantly fewer processes. In this study, GFAP was always present only in fibrous astrocytes located in the subpial region and in white matter. Protoplasmic astrocytes differentiated under pathological conditions, particularly those associated with high intracranial pressure. Areas were found in the gray matter containing only GFAP-positive short or longer fibers or areas of cells with numerous GFAP-positive processes. Based on our tissue culture studies, we consider this finding to be a differentiation of protoplasmic astrocytes which begins in the cell processes. Previously, we examined adult human brain cultures that contained only GFAP-negative “glia-like” cells. These cultures, with decreased growth during the terminal passages, showed morphological differentiation characterized by the formation of cell processes or the enlargement of cell bodies. GFAP-positive immunostaining first appeared in cell processes or at the cell periphery [21]. Under culture conditions, this finding clearly indicates astrocyte differentiation, but in tissue it could be interpreted as reactive astrogliosis. We also compared cultures from normal and damaged hemorrhagic brain tissue. Both cultures contained GFAP-negative cells, which were flat in normal cultures, but the cultures from severely damaged brain tissue may be entirely composed from spindle-shaped cells [21,27].

To the best of our knowledge, we have not found any studies that have clearly demonstrated, using immunohistochemical methods, the presence of a layer of or numerous cortical GFAP-positive protoplasmic astrocytes on paraffin sections of the human brain. The reduction and alteration of GFAP epitopes due to aldehyde fixation [28] cannot explain the absence of GFAP-positive astrocytes in the human brain cortex when fibrous astrocytes on the same sections are positively stained. However, numerous astrocytes of protoplasmic morphology visualized by immunofluorescence were demonstrated by Oberheim [29]. They examined brain tissue of 30 patients during surgical resections, including medically refractory epilepsy ($n = 30$), tumor ($n = 6$) and abscess ($n = 1$). However, we found no mention of whether protoplasmic astrocytes were present in all samples, nor of any differences between the stained cryosections from patients with tumors and those treated for refractory epilepsy. Despite the lack of data, we hypothesize that GFAP-positive protoplasmic astrocytes may represent differentiated cortical astroglial precursor cells that have been exposed to antiepileptic drugs over a long period of time.

Immunofluorescence staining using monoclonal antibodies against vimentin revealed positive staining in brain vessels, meninges, and astrocytes. Here we can confirm that aldehyde fixation is due to alteration of vimentin epitopes. On cryosections, we observed strong vimentin staining, whereas paraffin sections were negative or weakly stained with the same monoclonal antibodies. We assume that this difference in immunostaining led to the conclusion that vimentin is normally expressed only during certain early stages of astrocyte differentiation, but not in the normal mature brain [7].

Thanks to his microanatomical research and his study of the functions of the entire central nervous system, Santiago Ramón y Cajal is considered the father of modern neuroscience. His museum contains a huge number of histological slides from various animals, drawings, and many other items [1,30]. Despite preserved documentation, we find controversial data on astrocytes in the current literature. Virchow introduced the term neuroglia; Michael von Lenhossek introduced the term astrocytes for star-shaped neuroglial cells. Andriezen and Kolliker distinguished between gray matter protoplasmic and white matter fibrous glia. This classification was adopted by Ramón y Cajal, who replaced the designation of cells as glia with the term astrocytes [3,4,31]. Another study reports that Kolliker and Andriezen divided astrocytes into fibrous and protoplasmic, showing

drawings of astrocytes obtained only with Golgi-stained preparation [2]. Ramón y Cajal developed his own method of gold chloride sublimation, which enabled him to detect fibrils in astrocytes in the gray and white matter [3,4,31]. We attempted to perform this staining in the hope of finding both types of astrocytes in human brain tissue. Surprisingly, our staining of human brain tissue revealed only fibrous astrocytes in the subpial region and subcortical white matter. Similar to our GFAP staining, protoplasmic astrocytes were not visualized by the Cajal method. To find errors in our staining, we studied current articles dealing with his research. Here is an excerpt: “Each cell or fiber required a different impregnation time depending on the animal, the age, and the staining method. The suitable age of the animal to stain the cerebral cortex was 8–30 days in mice and 10–20 days in rabbits” [1].

Protoplasmic astrocytes are key cells in the neuron–glia interaction. They were discovered using the impregnation method developed by Golgi, who was the first to propose a nutritional role for glial cells [2]. We conclude that protoplasmic astrocytes are GFAP-negative cells in normal human brain cortical gray matter. However, no one has ever examined normal human brain biopsies, only macroscopically normal brain tissue or damaged tissue. It is debatable to what extent the brain tissue of patients treated for intractable epilepsy (patients undergoing long-term treatment with antiepileptic drugs) or necrotic samples (ischemic tissue) is normal or damaged. We found the biopsies from patients with brain metastases as less damaged human brain tissue with negative or, in some cases, a few weakly GFAP-positive stained protoplasmic astrocytes. Staining of brain samples from patients with gliomas revealed rarely GFAP-positively stained astrocytes, of which only a few cells showed morphology of protoplasmic astrocytes similar to Golgi’s drawings. We consider induced GFAP expression in damaged cortical gray matter to be a differentiation of protoplasmic astrocytes rather than reactive astrogliosis. In biopsy samples from patients with brain metastases, reactive astrogliosis was observed in the white matter, as hypertrophic strongly GFAP-positive fibrous astrocytes, while protoplasmic astrocytes remained GFAP-negative. However, following injury of the human brain, unknown processes may be triggered in the swollen brain that induce GFAP expression in protoplasmic astrocytes. In rodent brain tissue, an astroglial reaction was detected within one hour after focal mechanical trauma [28,32]. GFAP increased in ischemic areas of the rat brain within 2 days and was associated with the transformation of GFAP-negative cells into GFAP-positive cells [33].

Most experiments focusing on the neuron–glia interaction are performed on the rodent hippocampus [34,35], while basic questions regarding this topic in the human cerebral cortex remain unclear. The difficulty of visualizing protoplasmic astrocytes, combined with the crisis in neuronal immunodetection [36], may negatively affect progress in the field of neuron–glia interaction. Neuron–glia antigen 2 expressing glial cells (NG2) are commonly referred to as oligodendroglial progenitors during development and adulthood [37]. However, some studies suggest that NG2+ cells are precursors of oligodendrocytes and some protoplasmic astrocytes in the gray matter [38]. These findings together with newly developed astroglial markers, such as A1dh1L1, could potentially contribute to clarifying this issue [39–41].

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Faculty Hospital in Bratislava, Slovak Republic, code: ICH GCP 135/95, approval date: 3 July 2011.

Informed Consent Statement: Informed consent was signed by the patients or their legal guardians at the primary clinical site where the surgery and sample collection were performed.

Data Availability Statement: No data were created or analyzed in this study.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Garcia-Lopez, P.; Garcia-Marin, V.; Freire, M. The histological slides and drawings of Cajal. *Front. Neuroanat.* **2010**, *4*, 1156.
2. Kettenmann, H.; Verkhratsky, A. Neuroglia: The 150 years after. *Trends Neurosci.* **2008**, *31*, 653–659.
3. Privat, A.; Gimenez-Ribotta, M.; Ridet, J.C. Morphology of astrocytes. In *Neuroglia*; Kettenmann, H., Ransom, B.R., Eds.; Oxford University Press: New York, NY, USA, 1995; pp. 3–22.
4. Garcia-Marin, V.; Garcia-Lopez, P.; Freire, M. Cajal's contributions to glia research. *Trends Neurosci.* **2007**, *30*, 479–487.
5. Navarette, M.; Araque, A. The Cajal school and the physiological role of astrocytes: A way of thinking. *Front. Neuroanat.* **2014**, *8*, 33.
6. Eng, L.F.; Dahl, D.; Uyeda, C.T. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Res.* **1972**, *43*, 429–435.
7. Montgomery, D.L. Astrocytes: Form, functions, and roles in disease. *Vet. Pathol.* **1994**, *31*, 145–167.
8. Schnitzer, J.; Franke, W.W.; Schachner, M. Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J. Cell Biol.* **1981**, *90*, 435–447.
9. Perzelova, A.; Macikova, I.; Tardy, M.; Mraz, P.; Bizik, I. Subpopulation of nestin positive glial precursor cells occur in primary adult human brain cultures. *Biologia* **2007**, *62*, 633–640.
10. Stevens, A.; Lowe, J. *Human Histology*, 2nd ed.; Mosby: Barcelona, Spain, 1997; pp. 77–98.
11. Macikova, I.; Perzelova, A.; Mraz, P.; Bizik, I.; Steno, J. GFAP-positive astrocytes are rare or absent in primary adult human brain tissue cultures. *Biologia* **2009**, *64*, 833–839.
12. Ponten, J.; Westermark, B. Properties of human malignant glioma cells in vitro. *Med. Biol.* **1978**, *56*, 184–193.
13. Rutka, J.T.; Kleppe-Hoifodt, H.; Emma, D.A.; Giblin, J.R.; Dougherty, D.V.; McCulloch, J.R.; DeArmond, S.J.; Rosenblum, M.L. Characterization of normal human brain cultures. *Lab. Investig. J. Tech. Methods Pathol.* **1986**, *55*, 71–85.
14. Gibbons, H.H.; Van Roon-Mom, W.; Greenwood, J.M.; Narayan, P.J.; Teoh, H.H.; Bergin, P.M.; Mee, E.W.; Wood, P.C.; Faull, R.L.M.; Dragunow, M. Cellular composition of human glial cultures from adult biopsy brain tissue. *J. Neurosci. Methods* **2007**, *166*, 89–98.
15. Rutka, J.T.; Giblin, J.R.; Balkissoon, R.; Wen, D.; Mayatt, C.A.; McCulloch, J.R.; Rosenblum, M.L. Characterization of fetal human brain cultures. *Dev. Neurosci.* **1987**, *9*, 154–173.
16. Perea, G.; Sur, M.; Araque, A. Neuron-glia networks: Integral gear of brain function. *Front. Cell. Neurosci.* **2014**, *8*, 378.
17. Demmings, M.D.; Chagas, L.S.; Traetta, M.; Rodrigues, R.S.; Acutain, M.F.; Barzkin, E.; Datusalia, A.K.; German-Castelan, L.; Mattered, V.S.; Mazenganya, P.; et al. (Re)building the nervous system: A review of neuron-glia interaction from development to disease. *J. Neurochem.* **2024**, *169*, e16258.
18. Kim, Y.S.; Choi, J.; Yoon, B. Neuron-glia interaction in neurodevelopmental disorders. *Cells* **2020**, *9*, 2176.
19. Miyazaki, I.; Asanuma, M. Neuron-astrocyte interactions in Parkinson's disease. *Cells* **2020**, *9*, 2623.
20. Dooves, S.; Velthoven, A.J.H.; Suciati, L.G.; Heine, V.M. Neuron-glia interactions in tuberous sclerosis complex affect the synaptic balance in 2D and organoid cultures. *Cells* **2021**, *10*, 134.
21. Perzelova, A.; Mares, V. Appearance of GFAP-positive cells in adult human brain cultures spontaneously decelerated in growth. *Glia* **1993**, *7*, 237–244.
22. Sivakova, I.; Polak, S.; Perzelova, A. Problematic visualization of human protoplasmic astrocytes Immunohistochemical stains. *Bratisl. Med. J.* **2024**, *125*, 780–784.
23. Sofroniew, M.V.; Vinters, H.V. Astrocytes: Biology and pathology. *Acta Neuropathol.* **2010**, *119*, 7–35.
24. Rickmann, M.; Wolf, J.R. S100 protein expression in subpopulation of neurons of rat brain. *Neuroscience* **1995**, *67*, 977–991.

25. Du, J.; Yi, M.; Zhou, F.; He, W.; Yang, A.; Qui, M.; Huang, H. S100B is selectively expressed by gray matter protoplasmic astrocytes and myelinating oligodendrocytes in the developing CNS. *Mol. Brain* **2021**, *14*, 154.
26. Hernandez-Ortega, K.; Canul-Euan, A.A.; Solis-Paredes, J.M.; Borboa-Olivares, H.; Reyes-Munos, E.; Estrada-Gutierrez, G.; Camacho-Arroyo, I. S100B action on glial and neuronal cells in the developing brain: An overview. *Front. Neurosci.* **2024**, *18*, 1425525.
27. Sivakova, I.; Polak, S.; Perzelova, A. “Glia-like” cells of fibroblast morphology are present in cultures from injured human brain tissue. *Bratisl. Med. J.* **2024**, *125*, 414–418.
28. Eng, L.F.; Ghirnikar, R.S. GFAP and astrogliosis. *Brain Pathol.* **1994**, *4*, 229–237.
29. Oberheim, N.A.; Takano, T.; Han, X.; He, W.; Lin, J.H.C.; Wang, F.; Xu, Q.; Wyatt, J.D.; Pilcher, W.; Ojemann, J.G.; et al. Uniquely hominid features of adult human astrocytes. *J. Neurosci.* **2009**, *29*, 3276–3287.
30. Castro, F. Cajal and the Spanish Neurological school: Neuroscience would have been a different story without them. *Front. Cell. Neurosci.* **2019**, *13*, 187.
31. Kimelberg, H.K. The problem of astrocyte identity. *Neurochem. Int.* **2004**, *45*, 191–202.
32. Mucke, L.; Oldstone, M.B.A.; Morris, J.C.; Nerenberg, M.I. Rapid activation of astrocyte-specific expression of GFA-LacZ transgene by focal injury. *New Biol.* **1991**, *3*, 465–474.
33. Petit, C.K.; Morgelo, S.; Felix, J.C.; Lesser, M.L. The two patterns of reactive astrogliosis in postischemic rat brain. *J. Cereb. Blood Flow Metab.* **1990**, *10*, 850–859.
34. Bushong, E.A.; Martone, M.E.; Jones, Y.Z.; Ellisman, M. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* **2002**, *22*, 183–192.
35. De Cicco, G.; Chiazza, F.; Borzoni, G.G.; Pessolano, E.; Bortolotto, V.; Grilli, M. Complex effects of short periods of high-fat diet on GFAP+ astrocytes and maturation of DCX+ cells in the dorsal hippocampus of adolescent mice. *Neuroglia* **2026**, *7*, 2.
36. Rakic, P. Adult neurogenesis in mammals: An identity crisis. *J. Neurosci.* **2002**, *22*, 614–618.
37. Parolisi, R.; Boda, E. NG2 Glia: Novel roles beyond re-/myelination. *Neuroglia* **2018**, *1*, 151–175.
38. Zhu, X.; Bergles, D.E.; Nishiyama, A. NG2 cells generate both oligodendrocytes and gray matter astrocytes. *Development* **2008**, *135*, 145–157.
39. Melzer, L.; Freiman, T.M.; Derouiche, A. Rab6A as a pan-astrocytic marker in mouse and human brain and comparison with other glial markers (GFAP, GS, Aldh1L1, SOX9). *Cells* **2021**, *10*, 72.
40. Mudannayake, J.M.; Mouravlev, A.; Fong, D.M.; Young, D. Transcriptional activity of novel ALDH1L1 promoters in the rat brain following AAV vector-mediated gene transfer. *Mol. Ther. Methods Clin. Dev.* **2016**, *3*, 16075.
41. Yang, Y.; Vidensky, S.; Jin, L.; Jie, C.; Lorenzini, I.; Frankl, M.; Rothstein, J.D. Molecular comparison of GLT1+ and ALDH1L1+ astrocytes in vivo in astroglial reporter mice. *Glia* **2011**, *59*, 200–207.

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