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Review

Rosenfeld's Staining: A Valuable Tool for *In Vitro* Assessment of Astrocyte and Microglia Morphology

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Abstract: In homeostasis, the glial cells support pivotal functions, such as neuronal differentiation, neuroprotection, nutrition, drug metabolism, and immune response in the central nervous system (CNS). Among these cells, astrocytes and microglia have been highlighted due to their role in the pathogenesis of several diseases or due to their role in the defense against several insults (ex., chemicals, and pathogens). *In Vitro* cytological analysis of astrocytes and microglia has contributed to the understanding of the role of morphological changes in glial cells associated with a neuroprotective or neurotoxic phenotype. Currently, the main tools used for the investigation of glial cell morphology in culture are phase contrast microscopy or immunolabeling/fluorescence microscopy. However, generally, phase contrast microscopy does not generate images with high resolution and therefore does not contribute to visualizing a single cell morphology in confluent cell cultures. On the other hand, immunolabeling requires high-cost consumable antibodies, epifluorescence microscope or confocal microscope, and presents critical steps during the procedure. Therefore, identifying a fast, reproducible, low-cost alternative method that allows the evaluation of glial morphology is essential, especially for neuroscientists from low-income countries. This article aims to revise the use of Rosenfeld's staining, as an alternative low-cost and easy-to-reproduce method to analyze astrocytic and microglial morphology in culture. Additionally, it shows Rosenfeld's staining as a valuable tool to analyze changes in neural cell morphology in toxicological studies.

Keywords: Rosenfeld's staining; astrocytes; microglia; PC-12 cells; morphology

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1. Introduction

Glia, in the CNS, are composed of specialized cells, such as astrocytes, microglia, oligodendrocytes, and ependymal cells [1]. Microglia and oligodendrocytes were first described by Pio del Rio Hortega, in 1919 and 1921, respectively [2]. Initially, they were pointed to as static cells in the brain that performed a few functions, such as immune surveillance and phagocytosis [3]. However, with the advancement of techniques for analysis, it was observed that they can interact with each other or with other cellular components of the CNS, such as neurons, acting on their development, maintenance, immune system, and homeostasis [3,4].

The advent of primary cultures of glia has contributed to the study of specific molecular pathways and the different mechanisms involved in neuroinflammation. Among its applications, there are studies using *in vitro* models of neuroinflammation induced by lipopolysaccharide (LPS), models of mechanical injury or induced by glutamatergic excitotoxicity [5]. In the primary culture of glial cells, the analysis of morphological changes in microglia and astrocytes is essential to characterize the glial cell response to inflammatory stimuli. In the activation of an inflammatory state, both microglia and astrocytes acquire typical morphological changes that can be characterized by staining and immunostaining techniques [6].

Morphological analysis is a valuable tool for the pharmacological approach of natural anti-inflammatory compounds, such as flavonoids [7]. The study of microglia and astrocyte morphology brings important data on their biological state since these cells can change their shape as part of a complex response to different kinds of stimuli. On the other hand, neuronal cell lineages have been used in studies on the protective effects of compounds. In these approaches, the study of neuronal cell morphology is applicable as a damage-associated marker. Many techniques, such as immunocytochemistry, immunohistochemistry, and immunofluorescence have been used, and currently, the use of specific antibodies for proteins of the cytoskeleton or cell surface, are employed. They can guarantee high specificity. However, the cost of these consumables is expensive [8–10].

Rosenfeld's staining has been used as an easy and cheap alternative technique to evaluate astrocyte and microglia morphology in primary culture. Rosenfeld's stain is a solution prepared with Giemsa and May–Grünwald dye, which generates a strong contrast between the cell nuclei stained in purple and the cytoplasm stained in blue [11]. The method was developed for staining blood smears [11]. Nowadays, it has been used to analyze neural cell morphology, including astrocytes, microglia, and neurons in primary culture, as a tool to investigate glial reactivity and cell damage [7,12–16] (Table 1).

In this article, we highlighted Rosenfeld's staining as a useful low-cost method to analyze the morphology of astrocytes and microglia in primary culture from the rodent brain and its applications. Additionally, we highlighted its use for the analysis of morphological changes in neural cells as a complementary tool for cytotoxicity assay.

Table 1. Rosenfeld's staining applications for phenotypic analysis in different cultures of central nervous system cells.

Reference	Title	Aim of the Study	Tissue/Cell Culture
Dourado et al. [7]	Neuroimmunomodulatory and Neuroprotective Effects of the Flavonoid Apigenin in *in vitro* Models of Neuroinflammation Associated With Alzheimer's Disease.	To evaluate the neuroprotective and neuroimmunomodulatory potential of apigenin using *in vitro* models of neuroinflammation associated with AD.	Primary co-cultures of neurons and glial cells from brain neonates and embryos of Wistar rats.
Cholich et al. [12]	Cytotoxic activity induced by the alkaloid extract from Ipomoea carnea on primary murine mixed glial cultures.	To verify the cytotoxicity of the alkaloids swainsonine and calistheins B1, B2, B3 and C1 from *Ipomoea carnea*.	Mixed glial primary cultures from neonatal mice (CF-1).
Nascimento et al. [13]	Involvement of astrocytic CYP1A1 isoform in the metabolism and toxicity of the alkaloid pyrrolizidine monocrotaline.	To elucidate the metabolism and the toxicity of monocrotaline in C6 astrocyte cell line and primary cultures of astrocytes by investigating metabolic enzymatic mechanisms of the cytochrome P450 (CYP) system and conjugation with glutathione.	C6 cell line derived from rat glioma and primary cultures of astrocytes from brain Wistar rat.

Table 1. *Cont.*

Reference	Title	Aim of the Study	Tissue/Cell Culture
Zuo et al. [14]	Baicalin Attenuates Ketamine-Induced Neurotoxicity in the Developing Rats: Involvement of PI3K/Akt and CREB/BDNF/ Bcl-2 Pathways.	Investigate the neuroprotective effects of baicalin against ketamine-induced apoptotic neurotoxicity.	Neurons and glia in the mixed cultures from the cerebral cortex of neonatal Sprague–Dawley rats.
Coelho et al. [17]	Flavonoids from the Brazilian plant *Croton betulaster* inhibit the growth of human glioblastoma cells and induce apoptosis.	To investigate the effects of the flavonoids 5-hydroxy-7,4′-dimethoxyflavone, casticin, and pendulletin on the growth and viability of the human glioblastoma cell line GL-15.	GL-15 cell line derived from human glioblastoma.
Zuo et al. [15]	Existence of glia mitigated ketamine-induced neurotoxicity in neuron–glia mixed cultures of neonatal rat cortex and the glia-mediated protective effect of 2-PMPA.	Compare ketamine-induced neurotoxicity and explored the neuroprotective effect of the NAAG peptidase inhibitor 2-(phosphonomethyl) pentanedioic acid (2-PMPA).	Neuron–glia mixed cultures and neuronal cultures of neonatal Sprague Dawley rats.
Wang et al. [16]	Primary co-culture of cortical neurons and astrocytes of new-born SD rats.	Establish a simple and practical co-culture method.	Co-culture of cortical neurons and astrocytes from new-born SD rats.

2. How Important Is Rosenfeld's Staining to Study the Microglia Morphology in Cultures?

Microglia are innate mononuclear phagocytes of mesodermal origin that arise from embryonic yolk sac precursors and constitute 10–20% of all brain cells [3]. Microglia is a cell with great morphological plasticity in CNS and in primary culture (Figure 1). During its development, the microglia present an "amoeboid" morphology characterized by large and round cell bodies with short and thick cell extensions [18]. In a healthy CNS, the mature microglia survey the brain when they present a branched morphology characterized by a small body and the emission of long and slender cell processes that actively monitor the CNS parenchyma [3,19] (Figure 1A).

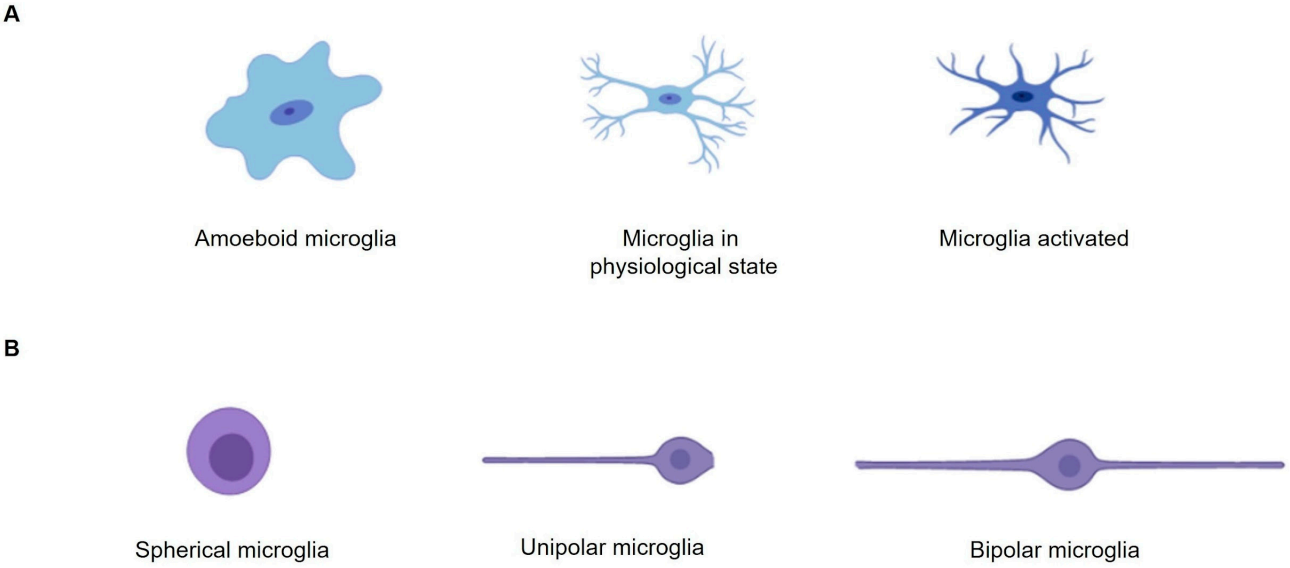


Figure 1. Representation of microglia morphologies. (**A**) In situ amoeboid, physiological/surveillance, and activated microglia, respectively. (**B**) *In vitro* spherical, unipolar, and bipolar microglia. Images were generated using BioRendeio.

The microglia express numerous receptors that recognize exogenous or endogenous molecular signals in the CNS microenvironment [19,20]. The activation of these receptors by signals of CNS injury is present in trauma, ischemia, infection, or neurodegenerative diseases [21,22] and can lead to changes in microglia morphology/phenotype and changes in secretory profile resulting in migration to the lesioned microenvironment, proliferation, and neuroinflammation. The morphological changes are characterized by shortening, retraction of processes, and increase in cell body, resulting in an ameboid morphology, similar to that observed at the beginning of development [19].

Microglia morphology *in vitro* does not have the structure typically seen *in vivo* CNS. Generally, in *in vivo* study models, the ramified or amoeboid microglia are observed (Figure 1A). Meanwhile, round (spherical), unipolar, or bipolar/rod-shaped microglia are most commonly observed in *in vitro* experimental models [23,24] (Figure 1B). However, branches also can be observed *in vitro*. Szabo and Gulya [25] showed the morphology of microglia in primary culture as an amoeboid stage on day 1, with rounded or slightly ovoid cell shapes, and during their maturation on day 4, some of them presented asymmetrical shapes, and in stages later, from the day 7 up to 21, the microglia became more ramified (Figure 2) [25].

In addition to the morphological changes, molecular changes in the pattern of cytokines production and release, as well as in the chemokines and/or trophic factors, are present in microglial response [3]. Different types of stimuli *in vitro* can lead to two main distinct phenotypes in microglia. Based on the classification of peripheral macrophage polarization, it was considered that microglia stimulated by Lipopolysaccharide (LPS) or interferon-gamma (IFN-γ) presented an M1 phenotype for the expression of pro-inflammatory cytokines. In the interim, microglia stimulated by IL-4/IL-13 presented an M2 phenotype for the resolution of inflammation and tissue repair [26,27]. However, this classification has been discussed, since it emerges from the *in vitro* microglia response; meanwhile, M1 and M2 states fail as isolated pure phenomena *in vivo* [27].

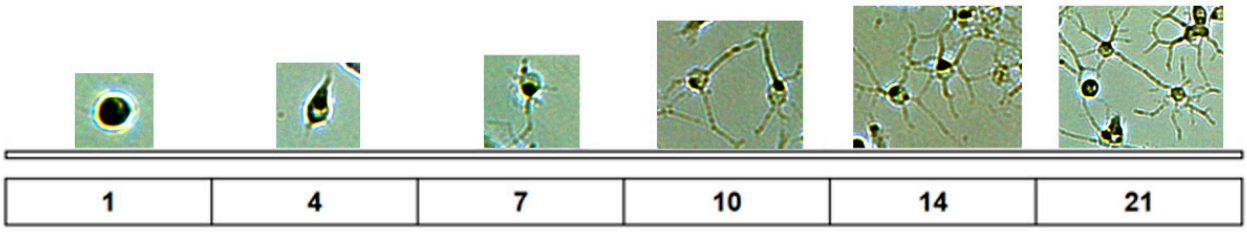


Figure 2. The evolution of microglial cell morphologies during 21 days *in vitro* stained with Rosenfeld's staining. On day 1: microglia present ameboid shape, with round or slightly ovoid cell forms. On day 4: microglial cells had become more asymmetric or rod-shaped, and some of these cells possessed more pronounced lamellipodae. From day 7 to 28, the microglia became more ramified. The primary culture of isolated microglia was prepared as described by Mecha et al. [28]. The cells were fixed with −20 °C methanol for 10 min and analyzed in a Leica DMIL Led Fluo microscopy, and the image was acquired on the Leica DFC7000 T camera with software.

The M1 phenotype or classically activated via toll-like receptors or interferon γ receptors has been characterized by the production of cytokines and chemokines, such as (IL-1β, IL-6, IL-12, TNF, CCL2), nitric oxide (NO) via suppression of induced NO synthase 2 (iNOS/NOS2) and expression of NADPH oxidase generating other reactive oxygen and nitrogen species. Therefore, it induces the expression of MHC-II, integrins (CD11b, CD11c), costimulatory molecules (CD36, CD45, CD47), and Fc receptors. This phenotype can be induced by components of the bacterial cell wall, such as LPS or peptidoglycan [23,29,30]. On the other hand, the M2 phenotype or alternatively activated by IL-4 and IL-13, both anti-inflammatory cytokines, has been characterized by its ability to reduce, protect or

repair the response to inflammation, producing anti-inflammatory cytokines (IL-10, TGF-β, IL-6), growth factors (IGF-1, FGF, CSF1), nerve-derived growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins and glial cell line-derived neurotrophic factor (GDNF) [19,31,32]. Each of these phenotypes synthesizes a different spectrum of cytokines and expresses different receptors on their cell surface. However, these phenotypes are not stable. *In vitro* experiments have already shown that there are changes from one phenotype to another in response to biochemical stimuli. Thus, microglia can change its behavior over the course of a disease, depending on the transcriptional profiles and on the region of the brain in which it is found [33].

Primary microglia cultures are useful *in vitro* tools to investigate inflammatory mechanisms and molecular pathways in CNS diseases [34]. These models may also be helpful in understanding mechanisms associated with neuroprotective or neurotoxic functions of different phenotypes of microglia. In cultures, the M1 type phenotype can be induced by treatment with LPS and interferon-γ (IFNγ), while the M2 type phenotype can be induced by treatment with interleukin (IL)-4 and IL-13 [34]. In addition, therapeutic strategies that may target microglia can be evaluated, for example, in a study where transplantation of rat-derived microglial cells promotes functional recovery in a spinal cord injury model [35].

The main techniques used for the analysis of microglia morphology are phase contrast microscopy [34,35], immunocytochemical staining for CD68, OX42 [36], CD45, and PU.1 [37]. Immunocytochemical staining using the ABC method of the avidin-biotin-peroxidase complex can be useful [35].

It is totally clear that microglia can present the same morphology for different molecular identities. Moreover, the *in vitro* morphology is not the same in *in vivo*. The amoeboid and ramified microglia morphologies *in vitro*, can also be observed in the primary culture of isolated microglia from newborn Wistar rats (Figure 2). On the other hand, despite the amoeboid and ramified, other morphologies have been described *in vivo*, such as rod microglia, hyper-ramified, honeycomb, jellyfish, bulbous endings of microglial processes, and ball-and-chain structures [25,38].

Among the hypotheses for this difference is the idea that it is due to the inherent limitations of the technique that *in vitro* models do not reproduce the wide range of conditions found in the cerebral microenvironment *in vivo*, which the continuous exposure of the microglia to numerous structures surrounding the CNS, resulting in a complex interaction between them [19,39]. Despite this, the *in vitro* study of microglia morphology can contribute to general information on microglia activation as a response to different types of stimuli.

3. How Important Is Studying Astrocyte Morphology in Cultures?

Unlike microglia, macroglia cells (ependymal cells, oligodendrocytes, and astrocytes) have the same embryonic origin as neurons, the neural tube that arises from the ectoderm [40,41].

Astrocytes constitute a highly heterogeneous population that represents 19 to 40% of the total brain cells. They have numerous functions, including nutritional and structural support for neurons, homeostasis, regulation of glutamate metabolism, glial transmission, cell signaling via Ca^{2+} release and absorption, and maintenance of the blood–brain barrier integrity [42].

In response to damage in the CNS, astrocytes react with reactivity, a process known as astrocyte reactivity [43]. Astrocyte reactivity involves changes in morphology, increased expression of GFAP and vimentin, increased proliferation, and secretion of inflammatory mediators and/or growth factors [44].

Like microglia, astrocytes are able to rapidly and reversibly remodel their structure in the CNS and also have a recognized morphological diversity in culture (Figure 3). Among the morphological changes related to astrogliosis the hypertrophy of the cell body and astrocytic processes and extension of cell processes are remarkable (Figure 3A). These changes result in the formation of a glial scar, which acts as a physical barrier to restrict the damage and separate the healthy tissue from the damaged tissue [45].

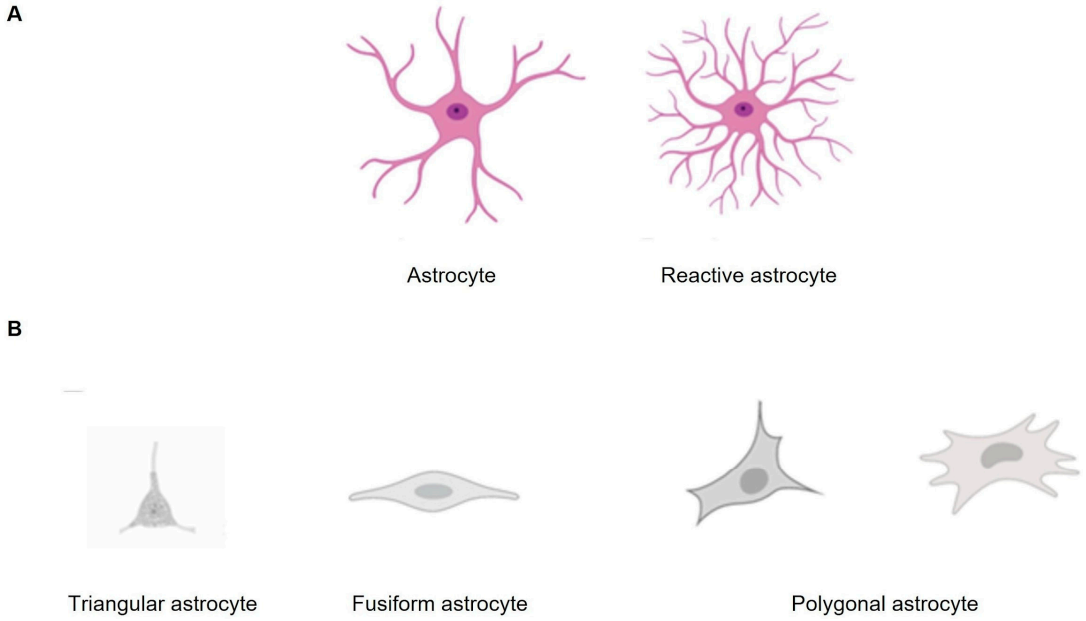


Figure 3. Representation of astrocyte morphologies. (**A**) Astrocytes in a physiological and reactive state, respectively. (**B**) Astrocytes in different morphologies found in *in vitro* culture: triangular, fusiform, and polygonal. Images were generated using BioRender.

In vivo, there are several morphological types of astrocytes present in different anatomical structures of the CNS. Among them, the largest group are protoplasmic astrocytes of the gray matter and fibrous astrocytes of the white matter. In contrast, fibrous astrocytes are found in white matter and have long, linear processes, in addition to greater expression of GFAP, compared to protoplasmic astrocytes [46]. Like protoplasmic astrocytes, they also make extensive contact with blood vessels through their processes and form gap junctions between distal processes of neighboring astrocytes. However, while protoplasmic astrocyte processes involve synapses, fibrous astrocyte processes involve Ranvier nodes [47,48]. Other minor populations are interlaminar astrocytes (found in the primate cortex), tanycytes (found in the periventricular organs, the hypophysis and the raphe part of the spinal cord), pituicytes in the neurohypophysis, perivascular and marginal astrocyte, ependymocytes, choroid plexus cells in the ventricles, and retinal pigment epithelial cells in the subretinal space [49]. Especially in the cerebellum, we can find Bergmann glial cells and Velate astrocytes [49,50].

In vitro, the main astrocyte morphologies are triangular, fusiform, or polygonal (Figure 3B), which can change for a reactive type in the presence of an inflammatory stimulus (Figure 3A). Among the techniques most commonly used to evaluate *in vitro* astrogliosis, immunofluorescence for GFAP stands out, because it can associate the analysis of the morphological changes with analysis of an important molecular marker. Aldehyde dehydrogenase 1 family member L1 (ALDH1L1) are specific enzymes that are also important as molecular markers of astrogliosis [51]. However, it is less related to the integral morphology of astrocytes when compared with GFAP, which is a constituent of their cytoskeleton. We have provided *in vitro* studies of astroglial morphology to evaluate the

effect of natural bioactive compounds, such as neurotoxic alkaloids [17,52], or neuroprotective terpenoids, or flavonoids on LPS-stimulated cells [7,50,53]. In this sense, Rosenfeld's staining is a valuable tool for qualitative analysis [17,52,54] and is useful to determine vacuolated cells [13,55].

4. Rosenfeld's Staining: An Alternative Technique to Evaluate Cell Morphology in Glial Cell Culture

Rosenfeld's staining is a modified technique from the original Romanowisky staining [11]. Romanowisky dye, developed in 1891 by the Russian Dmitri Romanowsky, is prepared by combining the aqueous solutions of the cationic methylene blue dye and the anionic eosin dye. As a result, there is a strong contrast between the cell nuclei stained in purple (eosin) and the RNA-enriched cytoplasm stained in blue (methylene blue) [56]. Besides Rosenfeld's staining, the Romanowisky technique was a precursor to several other staining methods, such as those of Leishman, Wright, May–Grünwald, and Giemsa. The distinction between these methods lies in the different proportions of methylene blue and eosin used in their composition [56]. Rosenfeld's stain is a solution of Giemsa and May–Grünwald dye in methanol [11], useful for dying hematological cells [57,58], which has been adopted to dye neural cells, such as astrocytes and microglia [7,59] (Figure 4; Table 1).

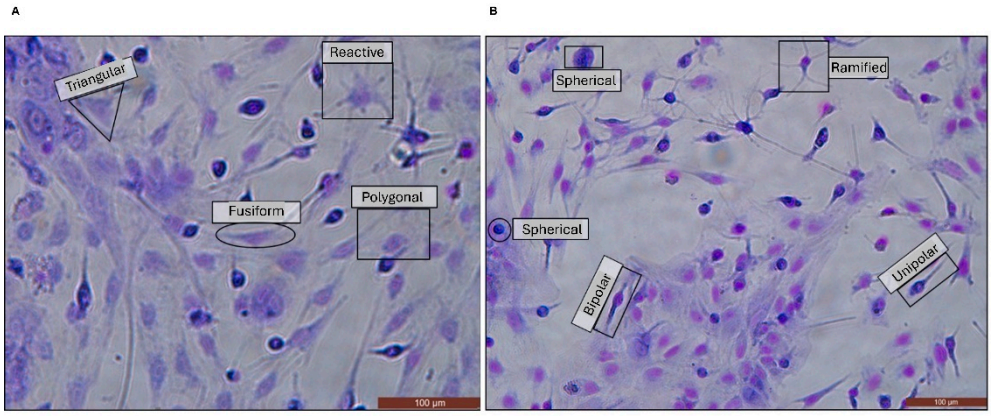


Figure 4. Rosenfeld's staining in mixed primary culture of astrocytes and microglia from the brains of Wistar rats is useful for differential analysis of morphology. (**A**) The main astrocytic morphologies *in vitro*: triangular, fusiform, polygonal, and reactive. (**B**) The main microglial morphologies *in vitro*: vigilant, reactive, spherical, bipolar, and unipolar. However, it is not possible to assume that the cells are specifically astrocytes or microglia due to their morphology. An additional method for cell marker detection, for example, immunocytochemistry, must be performed for the characterization of cell type [52]. Primary culture of astrocytes and microglia was performed according to Silva et al. [52] and approved by the local Ethical Committee for Animal Experimentation CEUA-ICS-UFBA, protocol number (6,731,220,818). Scale bar = 100 µm.

Rosenfeld's staining technique in fixed neural cell culture is a fast and easy procedure (Supplementary Material S1). It is an alternative method to replace immunostaining on glial cell morphology analysis because astrocytic and microglial body and cell processes are easily visualized (Figure 4). On the other hand, it is not acceptable for differential characterization of astrocytes or microglia morphology in mixed culture (Figure 4) since it is necessary to use in a purified primary culture (Figure 5) or cell lineage, or immunofluorescence (Figure 6A,B; Table 2), or an associative use with immunocytochemistry for differential cell marker (Figure 6C; Table 2). In the primary culture of isolated microglia from newborn Wistar rats, the use of Rosenfeld's staining provides a clear observation of different microglia morphologies *in vitro*, such as ramified spherical (round) or bipolar morphology (Figure 5). However, a few cells with polygonal morphology, characteristic of

astrocyte morphology in culture, can be visualized (Figure 5). In this sense, the purity of the primary culture of isolated microglia needs to be evaluated with immunohistochemistry or immunofluorescence (Figure 6).

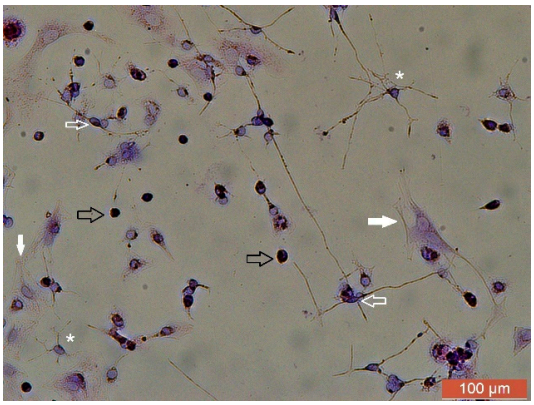


Figure 5. Rosenfeld's staining in the primary culture of isolated microglia from the brain is useful for specific analysis of microglial morphology. White asterisks show ramified morphology. Black outline arrows showing spherical (round) morphology. White outline arrows show bipolar morphology. Some cells with polygonal morphology, characteristic of astrocyte morphology, are visualized (White arrows). The primary culture of isolated microglia from newborn P0- P2 Wistar rats was performed according to Bispo da Silva et al. [60] and the procedure was approved by the local Ethical Committee for Animal Experimentation CEUA-ICS-UFBA, protocol number (6,731,220,818). Scale bar = 100 µm.

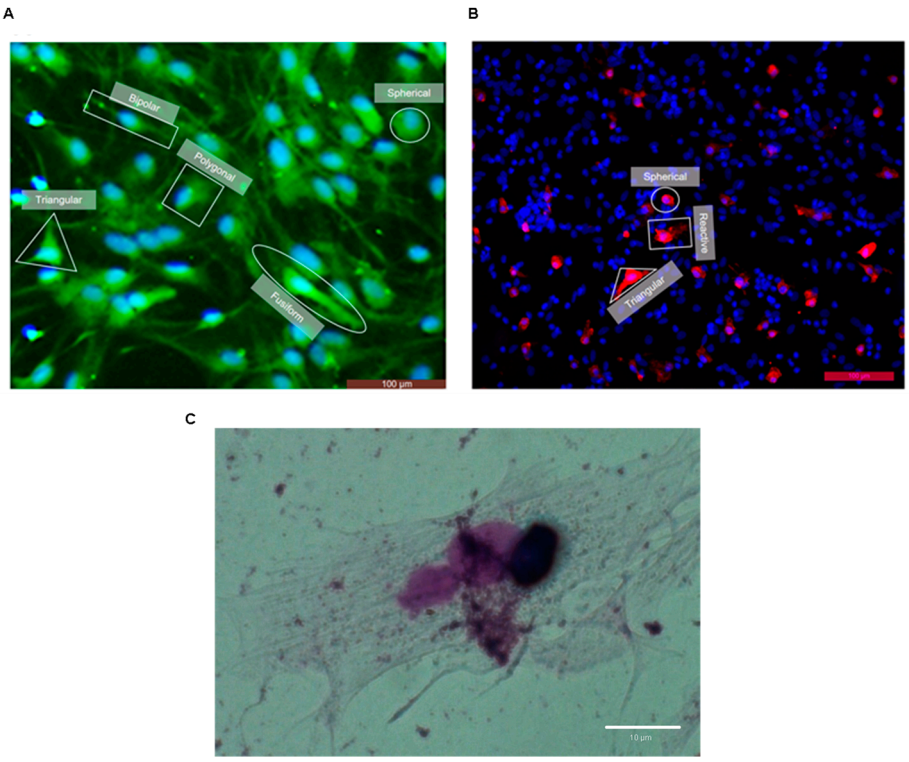
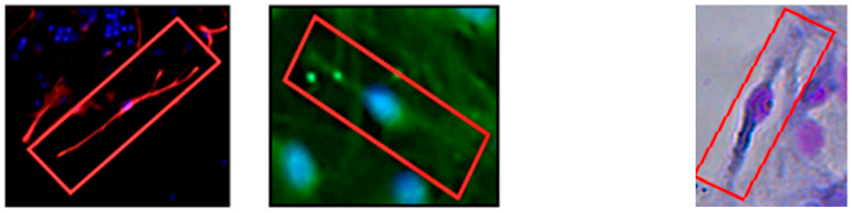
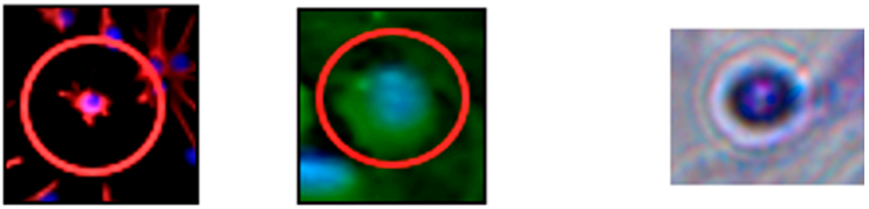
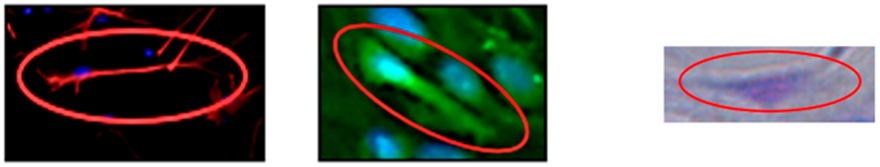
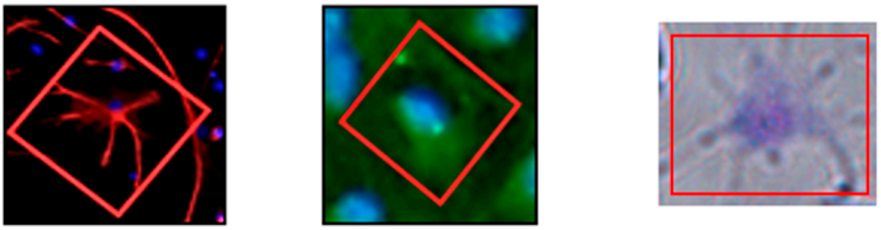
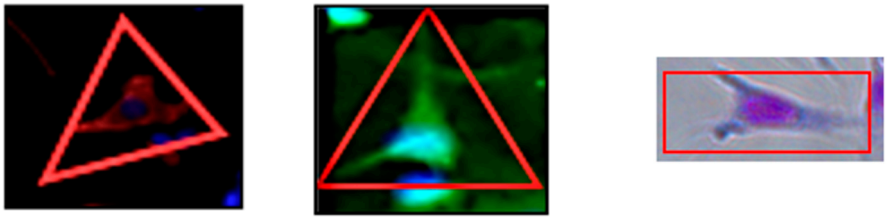


Figure 6. Immunolabeling in mixed primary cultures of astrocytes and microglia can provide more evidence of differential cell morphology. (**A**) Using immunofluorescence for GFAP and nuclear staining with DAPI, the main astrocytic morphology can be observed in green: triangular, bipolar, polygonal, spherical, and fusiform. (**B**) Using immunofluorescence for Iba1 and nuclear staining with DAPI, microglial morphology can be observed in red: triangular, spherical, and reactive. (**C**) Using immunocytochemistry for OX-42 and Rosenfeld's staining, rounded and dark OX-42 positive microglia can be differentiated from astrocytes. In A and B, the nuclei were marked with DAPI. Primary culture of astrocytes and microglia was performed according to Silva et al. [52] and approved by the local Ethical Committee for Animal Experimentation CEUA-ICS-UFBA, protocol number (6,731,220,818).

Table 2. Comparison between glial cell morphology in immunostaining for GFAP and in Rosenfeld's staining. The red circle highlights single cells in different morphologies.

Morphology	GFAP+	Rosenfeld
Bipolar		
Spherical		
Fusiform		
Polygonal		
Triangular		

In a primary co-culture of neurons and glial cells from newborn Wistar rats, Rosenfeld's staining promotes a clear visualization of astrocytes under the neuronal network. Meanwhile, the images in phase-contrast microscopy without staining make astrocyte body visualization difficult (Figure 7A,B). Rosenfeld staining is also a valuable tool to analyze changes in neural cell morphology related to cytotoxicity. Silva et al. [55] used Rosenfeld's staining to evaluate the morphological changes associated with the cytotoxic effect of alkaloids from *Prosopis juliflora* in primary co-cultures of neurons and glial. Among the morphological changes observed using Rosenfeld's staining, vacuolization induced by alkaloids from *P. juliflora* or monocrotaline from *Crotalaria retusa* contributed to the cytological studies [13,55] (Table 2). In PC12 cells treated with 60 mM glutamic acid, the rounded/ameboid shape evidenced by stained with Rosenfeld's staining is associated with an increase in propidium iodide (IP) dye. Meanwhile, bipolar or ramified cells are less stained for IP (Figures 8 and 9).

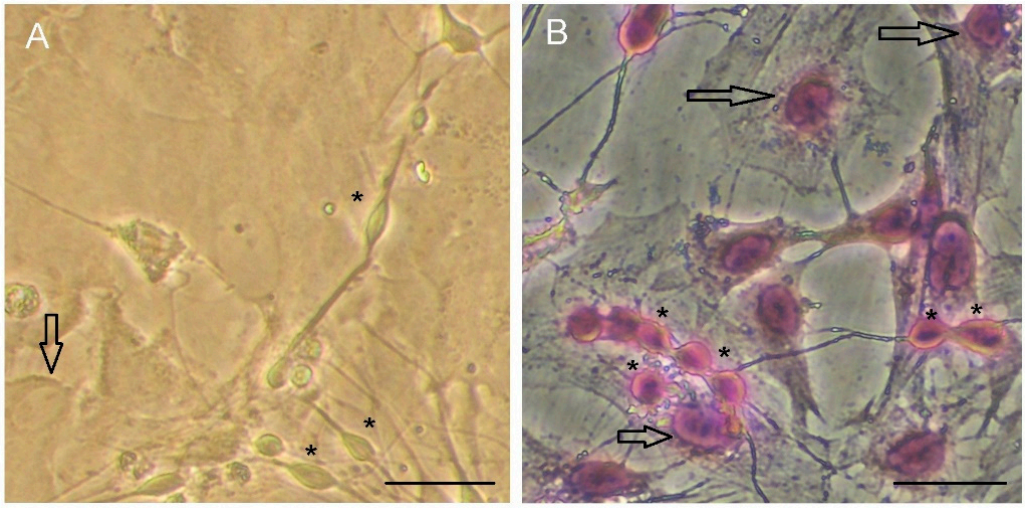


Figure 7. Rosenfeld's staining in the primary co-culture of neurons and glial cells from newborn rat brains. Arrows show astrocytes. Asterisks show neurons forming a network. The primary co-culture of neurons and glial cells was performed according to Silva et al. [55] and approved by the local Ethical Committee for Animal Experimentation CEUA- ICS-UFBA, protocol number (6,731,220,818). Scale bar = 50 µm. (**A**) Phase contrast microscopy of a non-stained primary co-culture of neurons and glial cells. (**B**) Phase contrast microscopy of a primary co-culture of neurons and glial cells stained with Rosenfeld's stain.

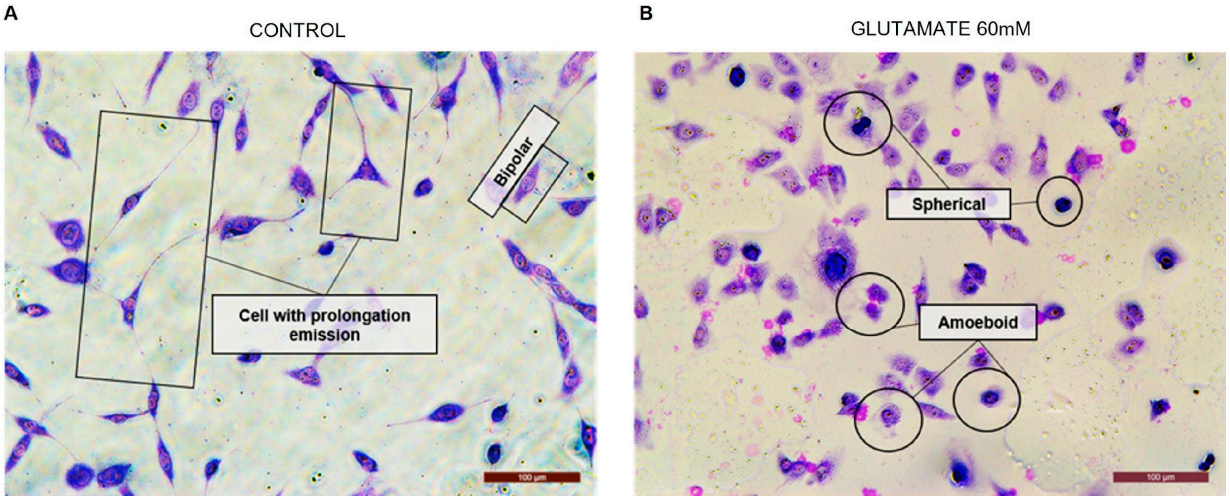


Figure 8. PC12Adh cell line culture purchased from the Cell Bank of the Federal University of Rio de Janeiro (BCRJ) was stained with Rosenfeld's staining. (**A**) PC12Adh cells in control conditions (untreated cells), fixed with −20 °C methanol and stained with Rosenfeld's staining. (**B**) PC12Adh cells were treated with 60 mM glutamate for 24 h, fixed with −20 °C methanol, and stained with Rosenfeld's staining.

The use of Rosenfeld's staining in neural cell cultures has been applied in several other investigations. It is a useful alternative in the evaluation of neuritic extension [61]. On the other hand, Dourado et al. [7] demonstrated the importance of this method to evaluate the protective effect of the flavonoid apigenin on the preservation of cell morphology in primary co-cultures of neurons and glial under inflammatory stimuli [7]. Moreover, looking at cell differentiation-like morphological changes, Coelho et al. [59] applied Rosenfeld's staining in glioma cells GL-15 lineage treated with antigliomatic flavonoids (Table 1).

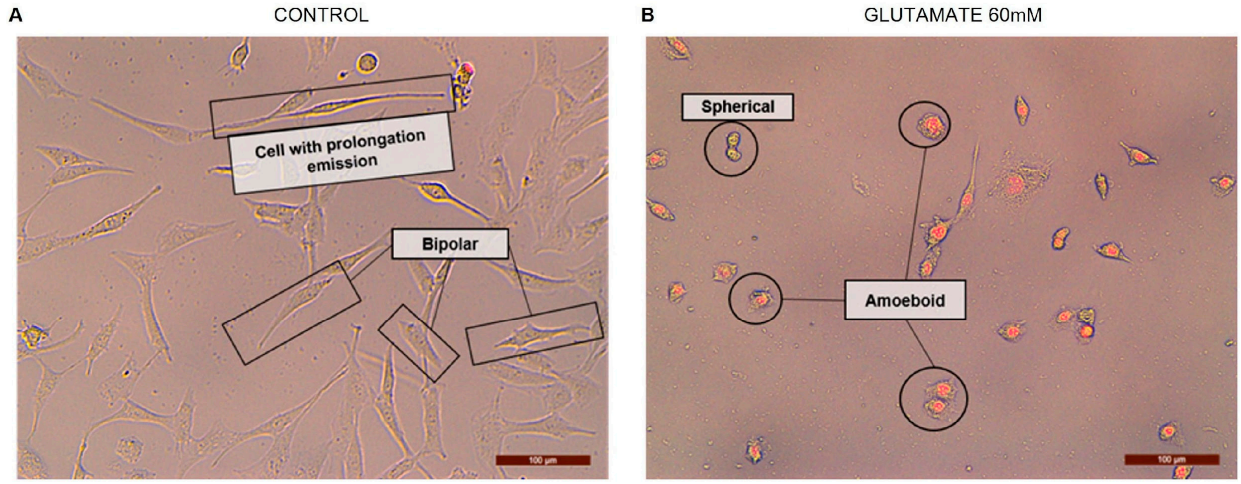


Figure 9. Cell viability assay in PC12Adh cell line culture performed the propidium iodide technique. Cells were considered dead based on the red fluorescence of their nuclei. (**A**) PC12Adh cells in control conditions (untreated cells); (**B**) PC12Adh cells treated with 60mM glutamate for 24 h. The spherical and amoeboid morphology are associated with cytotoxicity.

5. Conclusions

Rosenfeld's staining is a good technique for assessing the glial cell morphology in primary culture or cell lines. In addition to its effectiveness in relation to staining and applicability for analysis of cell morphology, this is an easy, fast, and low-cost technique, and therefore, it can be applied in research and also in practical classes to support students in identifying healthy cellular morphology, through the aspect of the cytoplasm and the nucleus. Its properties make it an alternative technique for research in low-income countries due to its low cost and easy protocol, so this can be an alternative to replace phase-contrast microscopy or immunostaining for morphological analyses. However, it is not applicable to the assessment of cell phenotypic identity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/neuroglia6020016/s1, Rosenfeld's stain protocol (ROSENFELD, 1947 adapted) [11].

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