

Commentary

The Special Case of Human Astrocytes

Alexei Verkhratsky ^{1,2,3,*} , Nancy Ann Oberheim Bush ⁴, Maiken Nedergaard ^{3,5}
and Arthur Butt ^{6,*} 

¹ Faculty of Biology, Medicine and Health, University of Manchester, Manchester, M13 9PT, UK

² Center for Basic and Translational Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

³ Achúcarro Basque Center for Neuroscience, 48940 Leioa, Spain; Maiken_Nedergaard@URMC.Rochester.edu

⁴ Division of Neuro-Oncology, Department of Neurological Surgery, University of California, San Francisco, CA 92093, USA; NancyAnn.OberheimBush@ucsf.edu

⁵ Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY 14642, USA

⁶ Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Science, University of Portsmouth, Portsmouth PO1 2UP, UK

* Correspondence: Alexei.Verkhatsky@manchester.ac.uk (A.V.); Arthur.butt@port.ac.uk (A.B.)

Received: 19 February 2018; Accepted: 19 February 2018; Published: 1 March 2018



Abstract: In this first issue of *Neuroglia*, it is highly appropriate that Professor Jorge A. Colombo at the Unit of Applied Neurobiology (UNA, CEMIC-CONICET) in Buenos Aires, Argentina, writes a perspective of idiosyncrasies of astrocytes in the human brain. Much of his work has been focused on the special case of *interlaminar astrocytes*, so-named because of their long straight processes that traverse the layers of the human cerebral cortex. Notably, interlaminar astrocytes are primate-specific and their evolutionary development is directly related to that of the columnar organization of the cerebral cortex in higher primates. The human brain also contains *varicose projection astrocytes* or *polarized astrocytes* which are absent in lower animals. In addition, classical protoplasmic astrocytes dwelling in the brains of humans are ≈ 15 -times larger and immensely more complex than their rodent counterparts. Human astrocytes retain their peculiar morphology even after grafting into rodent brains; that is, they replace the host astrocytes and confer certain cognitive advantages into so-called ‘humanised’ chimeric mice. Recently, a number of innovative studies have highlighted the major differences between human and rodent astrocytes. Nonetheless, these differences are not widely recognized, and we hope that Jorge Colombo’s *Perspective* and our associated *Commentary* will help stimulate appreciation of human astrocytes by neuroscientists and glial cell biologists alike.

Keywords: astroglia; protoplasmic astrocytes; interlaminar astrocytes; varicose projection astrocytes; human brain; astroglial domains

The widespread notion of neuroglia as the “neglected cells of neuroscience” is far from reality. The introduction of the concept of neuroglia as the connective tissue of the brain by Virchow [1] was followed by a steady flow of discoveries on glial cells and their roles in health and disease. All of the prominent neuroanatomists and neuropathologists of the second half of the 19th and early 20th century studied neuroglia (see for example [2–14]). These great minds laid the foundation of our knowledge of glial cells, mainly based on observation of human tissue.

It was the invention of “*La reazione nera*” by Golgi [15,16] and the many other staining techniques that it instigated which widened the visualisation and characterisation of diverse types of glia in the human brain. Prior to this, several types of glial cells had been described, most notably specialised types of radial astrocytes called the retinal Müller cells [17,18] and the cerebellar Bergmann glia [19]. In the following 50 years, the profuse use of Golgi staining resulted in the description of multiple

morphological phenotypes of parenchymal glia (Figures 1 and 2), which were named “astroglia” in 1895 [20] (for further details on glial history, see [21–23]). The absolute majority of the original histological characterisation of glial cells was performed on human tissue, and there was little attempt to compare with smaller mammals, which were of little interest to classical neuroanatomists. This is diametrically opposed to the heavy reliance of modern neuroscience on rodent models.

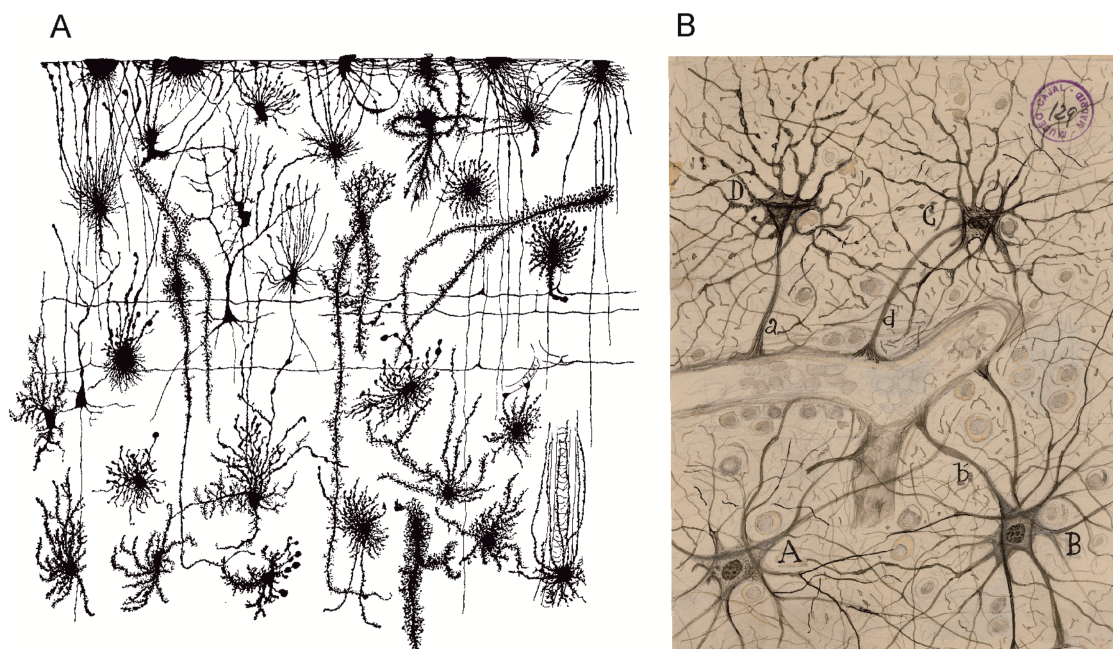


Figure 1. Early images of human astroglia. (A) Glial polymorphism in human foetal cortex as seen by Gustav Retsius [24]. (B) Perivascular astrocytes drawn by Santiago Ramón y Cajal; the image is from the collection of the Cajal Legacy at the Cajal Institute of the Spanish Research Council (CSIC). “©CAJAL INSTITUTE, CSIC”, Madrid, Spain.

The early advances in glial morphology inspired abundant speculation on glial function. Some argued that glia existed merely to fill the otherwise empty spaces and provide a structural matrix, within which neurones are embedded [9], whereas some, however, went much further and assigned glia fundamental homeostatic functions [25], whilst still others, most notably Carl Ludwig Schleich and Santiago Ramón y Cajal, suggested that glial cells control local blood flow, initiate sleep, and regulate information transfer in neuronal networks [8,14,26]. It is now apparent that astrocytes fulfil all of these operations and more, including the most fundamental neuronal attribute of synaptogenesis [27–30]. Similarly, the fundamental role of neuroglia in neurological diseases was highlighted by the most prominent neuropathologists, such as Franz Nissl, Carl Frommann, Ludwig Merzbacher, Alois Alzheimer, and Nicolas Achucarro [13,31–34]. At the turn of the 20th century, William Ford Robertson identified what he called “mesoglia” and proposed that they underwent pathological transformation in the diseased brain [35,36]. These mesoglia were Cajal’s “third element”, and they were characterised in detail by Pio del Rio Hortega, who identified them as oligodendrocytes and microglia. Del Rio Hortega clearly perceived microglia as having a defensive function [37–39] and that of oligodendrocytes in axonal myelination [40], which are indispensable in moulding the human brain connectome. Robertson’s mesoglia almost certainly also incorporated the last addition to the glial family, namely NG2-glia, which were clearly identified by William Stallcup and his colleagues [41] and are also known as polydendrocytes or synantocytes [42].

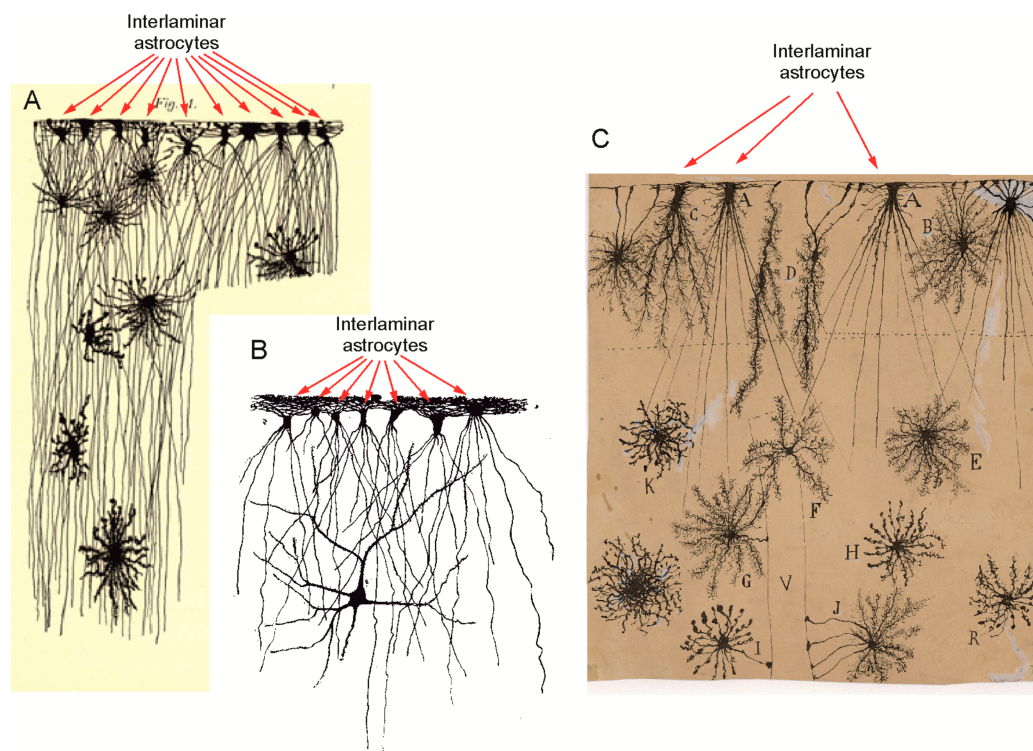


Figure 2. Early images of interlaminar astrocytes. (A) Glial cells from cerebral cortex of a one-year-old child drawn by Gustav Retzius [24]; numerous interlaminar astrocytes are clearly seen. (B) Interlaminar astrocytes as observed by William Lloyd Andriezen in 1893 [43]. (C) Golgi impregnated glia from human cortex (two-month-old child) in the plexiform layer (A–D), second and third layers (E–H and K, R, respectively) and perivascular glia (I, J). V, blood vessel. Cells labelled with A are interlaminar astrocytes. The image is from the collection of the Cajal Legacy at the Cajal Institute of the Spanish Research Council (CSIC). “©CAJAL INSTITUTE, CSIC”, Madrid, Spain.

The golden age of neuroglial research resulted in the detailed characterisation of human glial types. Much less was known about glial cell physiology until the 1950s, when the first microelectrode studies were performed on the brains of cats, dogs, and subsequently amphibians and rodents [44–48]. The latter soon became the experimental paradigm of choice, and human neuroglia were largely neglected for a long time. However, the comprehensive analyses performed during the last decade have revealed extraordinary differences between human and rodent glia, in particular, differences in their astrocytes [49–53].

First and foremost, astrocytes are many times larger and much more complex in the human brain than their rodent counterparts. Human protoplasmic astrocytes have about 10 times more primary processes and a more complex secondary process arborisation, with an average volume that is about 16.5 times larger than the corresponding domain in a rat brain (Figure 3) [50]. The larger human protoplasmic astrocytes have also extended outreach onto neuronal structures, on average contacting and encompassing up to two million synapses residing in their territorial domains. This is significantly more than the integrating capacity of rodent protoplasmic astrocytes, which cover $\approx 20,000$ – $120,000$ synaptic contacts [50,54]. Similarly, human fibrous astrocytes have a domain 2.14-fold larger than that in rodents [50].

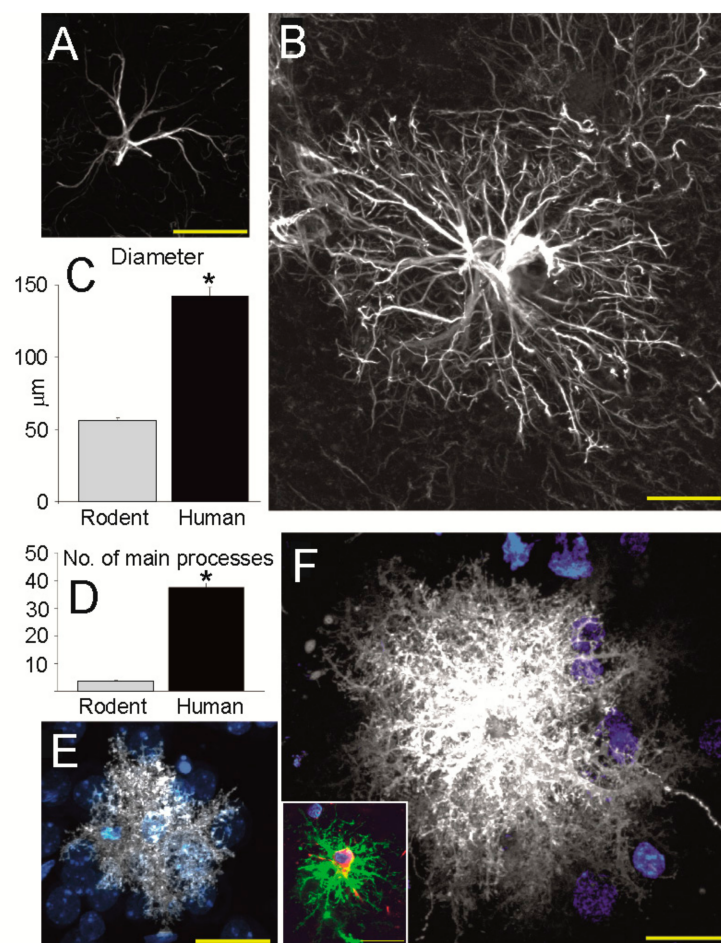


Figure 3. Comparison of rodent and human protoplasmic astrocytes. (A) Typical mouse protoplasmic astrocyte. Glial fibrillary acidic protein (GFAP) staining is shown in white. Scale bar: 20 μm . (B) Typical human protoplasmic astrocyte in the same scale. Scale bar: 20 μm . (C,D) Human protoplasmic astrocytes are 2.55-fold larger and have 10-fold more main GFAP processes than mouse astrocytes (human, $n = 50$ cells from seven patients; mouse, $n = 65$ cells from six mice; mean $\pm$ standard error mean (SEM); $*p < 0.005$, t -test). (E) Mouse protoplasmic astrocyte diolistically labelled with lipophilic dye DiI (white staining) and sytox (blue staining) revealing the full structure of the astrocyte, including its numerous fine processes. Scale bar: 20 μm . (F) Diolistically-labelled human astrocyte demonstrates the highly complicated network of fine process that defines the human protoplasmic astrocyte. Scale bar: 20 μm . Inset: Diolistically-labelled human protoplasmic astrocyte, also immunolabelled for GFAP (green staining), demonstrating colocalization. Scale bar: 20 μm . Reproduced with permission from [50].

In addition to the principal types of astroglia being particularly large and complex, the brains of higher primates and humans also contain several types of glia that do not exist in lower animals. The first type of uniquely human astrocyte that came under scrutiny was the class of *interlaminar astrocytes*, so-named by Jorge Colombo [55], whose historic and personal perspective is published in *Neuroglia* [56]. Notably, these cells were also seen by early neuroanatomists (Figure 2). Interlaminar astrocytes account for a rather substantial population of all astrocytes in the human cortex, while their functional role still remains enigmatic. The small ($\approx 10 \mu\text{m}$ in diameter) spheroid cell bodies of interlaminar astrocytes dwell in cortical layer I (supragranular), and several short and one or two very long (up to 1 mm) processes emanate from these somata (Figure 4A,B). The processes of interlaminar astrocytes penetrate through the cortex, ending in deeper layers, from layer II to IV. These processes sometimes contact with blood vessels, while their terminal portions end with peculiar

bouton-like structures, generally known as *terminal masses* or *end bulbs*. The processes of interlaminar astrocytes run parallel to each other, giving an appearance of a palisade.

The second type of astrocyte peculiar to the human brain are the *varicose projection astrocytes* or *polarized astrocytes*, which send several very long (up to 1 mm) unbranched processes with varicosities that extend in all directions through the deep cortical layers (Figure 4C–H) [51]. Apart from their long varicose processes, these cells otherwise look similar to classical protoplasmic astrocytes, but can be distinguished by their immunopositivity to CD44, also known as the homing cell adhesion molecule [52]. The density of these cells demonstrate remarkable individual variation, and their appearance is somehow related to age; that is, they have never been detected in the neonatal human brain, and it has been speculated that the appearance of these cells may reflect age-dependent adaptive changes and reflect individual life experiences [52].

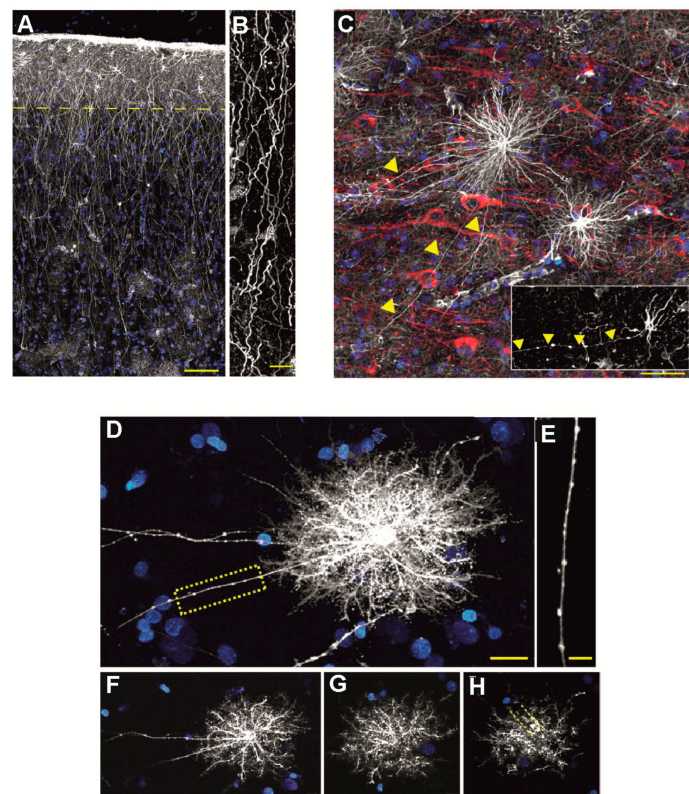


Figure 4. Interlaminar and varicose projection astrocytes in human cortex. **(A)** Pial surface and layers I–II of human cortex. GFAP staining is in white; 4',6-diamidino-2-phenylindole (DAPI) staining is in blue. Scale bar: 100 μ m. Yellow line indicates border between layer I and II. **(B)** Interlaminar astrocyte processes. Scale bar: 10 μ m. **(C)** Varicose projection astrocytes reside in layers V–VI and extend long processes characterised by evenly-spaced varicosities. Inset: Varicose projection astrocyte from chimpanzee cortex. GFAP staining is in white, microtubule-associated protein 2 (MAP2) staining is in red and DAPI staining is in blue. Yellow arrowheads indicate varicose projections. Scale bar: 50 μ m. **(D)** Diolistic labelling (in white) of a varicose projection astrocyte whose long process terminates in the neuropil, sytox staining is in blue. Scale bar: 20 μ m. **(E)** High-power image of the yellow box in **(B)** highlighting the varicosities seen along the processes. Scale bar: 10 μ m. **(F–H)** Individual z-sections of the astrocyte in **(E)** demonstrating long processes, straighter fine processes, and association with the vasculature. Reproduced with permission from [49,50].

The evolutionary development of human astrocytes, with processes that span multiple cortical layers, is directly related to the evolution of a highly complex columnar cortical organisation in higher primates. Moreover, developmental studies indicate that ontogeny recapitulates phylogeny;

for example, interlaminar astrocytes are first apparent at the end of the first month postnatal, and they reach their adult-like configuration by the second month of life [57]. It is tempting to speculate that the remarkably idiosyncratic morphology of human astrocytes translates into specific higher primate functions associated with information processing and intelligence. The theme of ‘intelligent astrocytes’ resurfaces sporadically; already in the 1960s, Robert Galambos proclaimed that “Glia is . . . conceived as genetically charged to organize and program neuron activity so that the best interests of the organism will be served; the essential product of glia action is visualized to be what we call innate and acquired behavioural responses. In this scheme, neurons in large part merely execute the instructions glia give them” [58]. This heretic and yet inspiring idea, which defines glia as a central element for information processing in the brain, has galvanized many followers [59–63], although it is still in need of credible experimental corroboration.

To attempt this corroboration, the chimeric model of ‘humanised’ mice was developed, in which the brains of neonatal animals were injected with human foetal glial cell progenitors. These human cells survived implantation and expanded to populate large areas of brain tissue and replace native rodent astrocytes. Moreover, mice bearing human astrocytes outperformed their wild type relatives in learning and memory tasks, while the threshold for long-term potentiation was reduced in these chimeras. Does this experiment indeed point out the intelligence potential of human astroglia? Or does the better performance of humanised mouse nervous tissue reflect a much higher capacity of human astrocytes to provide homeostatic and metabolic support? These points are not mutually exclusive, and the question remains open.

We hope that our *Commentary* has highlighted some key differences between astrocytes in the human brain and their simpler brethren in rodents. Currently, mice are the main animal model for studying brain function and pathology. Going forward, it is important to recognise the singularity of human astrocytes and interpret data from mice and other species appropriately. Jorge Colombo’s perspective of his career studying interlaminar astrocytes helps bring these cells to the attention of neurobiologists who might otherwise be unfamiliar with them, and it will hopefully help stimulate future studies on these remarkable cells.

Acknowledgments: We are grateful to Ricardo Martínez Murillo (Instituto Cajal, Spain) for providing images of Cajal’s drawings.

Author Contributions: All authors participated equally in writing this commentary.

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

References

1. Virchow, R. *Die Cellularpathologie in ihrer Begründung auf Physiologische and Pathologische Gewebelehre. Zwanzig Vorlesungen Gehalten Während der Monate Februar, März und April 1858 im Pathologischen Institut zu Berlin*, First ed.; August Hirschwald: Berlin, Germany, 1858; 440p.
2. Deiters, O. *Untersuchungen über Gehirn und Rückenmark des Menschen und der Säugethiere*; Vieweg: Braunschweig, Germany, 1865.
3. Besser, L. Zur histogenese der nervösen elementarteile in den centralorganen der neugeborenen menschen. *Arch. Pathol. Anat. Physiol. Klin. Med.* **1866**, *36*, 305–333. [[CrossRef](#)]
4. Jastrowitz, M. Encephalitis und myelitis des ersten kindersalters. *Arch. Psychiatr.* **1870**, *2*, 389–414. [[CrossRef](#)]
5. Butzke, V. Studien über den feineren bau der grosshirnrinde. *Arch. Psych. Nervenkr.* **1871**, *3*, 575–601. [[CrossRef](#)]
6. Rindfleisch, E. *Handbuch der Pathologischen Gewebelehre. 3 aufl*; Engelmann: Leipzig, Germany, 1873.
7. Nansen, F. *The Structure and Combination of the Histological Elements of the Central Nervous System*; Bergens Museum Aarbs: Bergen, Norway, 1886.
8. Ramón y Cajal, S. *Algunas Conjeturas sobre el Mecanismo Anatómico de la Ideación, Asociación y Atención*. Imprenta y Libreria de Nicolas Moya: Madrid, Spain, 1895.
9. Weigert, C. *Kenntnis der Normalen Menschlichen Neuroglia*; Moritz Diesterweg: Frankfurt am Main, Germany, 1895; 213p.

10. Kölliker, A.v. *Handbuch der gewebelehre des menschen*. 6 aufl.; Engelmann: Leipzig, Germany, 1896.
11. Held, H. Über die neuroglia marginalis der menschlichen grosshirnrinde. *Monatschr. Psychol. Neurol* **1909**, 26, 360–416. [[CrossRef](#)]
12. Ramón y Cajal, S. *Histologie du Système Nerveux de l’Homme et des Vertébrés*. Maloine: Paris, France, 1909.
13. Achucarro, N. De l’évolutio[n] de la névroglie, et spécialement de ses relations avec l’appareil vasculaire. *Trab. Lab. Invest. Biol. (Madrid)* **1915**, 13, 169–212.
14. Ramón y Cajal, S. Contribution a la connaissance de la nevroglia cerebrale et cerebeleuse dans la paralysie generale progressive. *Trab. Lab. Invest. Biol. Univ. Madrid* **1925**, 23, 157–216.
15. Golgi, C. *Opera omnia*; Hoepli: Milan, Italy, 1903.
16. Golgi, C. Suella struttura della sostanza grigia del cervello (comunicazione preventiva). *Gazzetta Medica Italiana Lombardia* **1873**, 33, 244–246.
17. Müller, H. Zur histologie der netzhaut. *Z. Wissenschaft. Zool* **1851**, 3, 234–237.
18. Schulze, M. *Observationes de retinae structura penitiori*; Published lecture; University of Bonn: Bonn, Germany, 1859.
19. Bergmann, K. Notiz über einige strukturverhältnisse des cerebellums und rückenmarks. *Z. Med.* **1857**, 8, 360–363.
20. Lenhossék, M.v. *Der Feinere Bau des Nervensystems im Lichte Neuester Forschung*, 2nd ed.; Fischer’s Medicinische Buchhandlung H. Kornfeld: Berlin, Germany, 1895.
21. Verkhratsky, A.; Nedergaard, M. Physiology of astroglia. *Physiol. Rev.* **2018**, 98, 239–389. [[PubMed](#)]
22. Kettenmann, H.; Verkhratsky, A. Neuroglia: The 150 years after. *Trends Neurosci.* **2008**, 31, 653–659. [[CrossRef](#)] [[PubMed](#)]
23. Verkhratsky, A.; Butt, A.M. *Glial Physiology and Pathophysiology*; Wiley-Blackwell: Chichester, UK, 2013; 560p, ISBN 978-0-470-97852-8.
24. Retzius, G. *Biologische Untersuchungen. Neue Folge*; Volume vi. Mit 32 tafeln; Von Gustav Fischer: Stockholm, Sweden, 1894; 87p.
25. Lugaro, E. Sulle funzioni della nevroglia. *Riv. Patol. Nerv. Ment.* **1907**, 12, 225–233.
26. Schleich, C.L. *Schmerzlose Operationen: Örtliche Betäubung mit Indifferenten Flüssigkeiten*. *Psychophysik des Natürlichen und Künstlichen Schlafes*; Julius Springer: Berlin, Germany, 1894; 256p.
27. Butt, A.M.; Nedergaard, M.; Verkhratsky, A. Remembering Ben Barres. *Neuroglia* **2018**, 1, 2. [[CrossRef](#)]
28. Verkhratsky, A.; Nedergaard, M. Astroglial cradle in the life of the synapse. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **2014**, 369, 20130595. [[CrossRef](#)] [[PubMed](#)]
29. Pfrieger, F.W.; Barres, B.A. Synaptic efficacy enhanced by glial cells in vitro. *Science* **1997**, 277, 1684–1687. [[CrossRef](#)] [[PubMed](#)]
30. Eroglu, C.; Barres, B.A. Regulation of synaptic connectivity by glia. *Nature* **2010**, 468, 223–231. [[CrossRef](#)] [[PubMed](#)]
31. Frommann, C. *Untersuchungen über die Gewebsveränderungen bei der Multiplen Sklerose des Gehirns und Rückenmarks*; Verlag von Gustav Fischer: Jena, Germany, 1878.
32. Nissl, F. Ueber einige beziehungen zwischen nervenzellerkrankungen und gliissen erscheinungen bei verschiedenen psychosen. *Arch. Psychiat.* **1899**, 32, 1–21.
33. Merzbacher, L. *Untersuchungen über die Morphologie und Biologie der Abraumzellen im Zentralnervensystem*; Fischer-Verlag: Stuttgart, Germany, 1909.
34. Alzheimer, A. Beiträge zur kenntnis der pathologischen neuroglia und ihrer beziehungen zu den abbauvorgängen im nervengewebe. In *Histologische und Histopathologische Arbeiten über die Grosshirnrinde mit Besonderer Berücksichtigung der Pathologischen Anatomie der Geisteskrankheiten*; Verlag von Gustav Fischer: Jena, Germany, 1910; Volume 3, Band 3.
35. Robertson, W. A microscopic demonstration of the normal and pathological histology of mesoglia cells. *J. Mental Sci.* **1900**, 46, 724. [[CrossRef](#)]
36. Robertson, W.F. *A Textbook of Pathology in Relation to Mental Disease*; William F. Clay: Edinburgh, UK, 1900.
37. Del Rio-Hortega, P. Poder fagocitario y movilidad de la microglia. *Bol. de la Soc. Esp. de Biol.* **1919**, 9, 154.
38. Metz, A.u.S.H. Die hortegaschen zellen, das sogenannte “dritte element” und uber ihre funktionelle bedeutung. *Z. Neur.* **1924**, 100, 428–449.

39. Del Río-Hortega, P. Microglia. In *Cytology and Cellular Pathology of the Nervous System*; Penfield, W., Ed.; Hoeber: New York, NY, USA, 1932; Volume 2, pp. 482–534.
40. Del Río-Hortega, P. Estudios sobre la neuroglia. La glia de escasas radiaciones oligodendroglia. *Biol. Soc. Esp. Biol.* **1921**, *21*, 64–92.
41. Stallcup, W.B. The NG2 antigen, a putative lineage marker: Immunofluorescent localization in primary cultures of rat brain. *Dev. Biol.* **1981**, *83*, 154–165. [[CrossRef](#)]
42. Butt, A.M.; Hamilton, N.; Hubbard, P.; Pugh, M.; Ibrahim, M. Synantocytes: The fifth element. *J. Anat.* **2005**, *207*, 695–706. [[CrossRef](#)] [[PubMed](#)]
43. Andriezen, W.L. The neuroglia elements of the brain. *Br. Med. J* **1893**, *2*, 227–230. [[CrossRef](#)] [[PubMed](#)]
44. Wardell, W.M. Electrical and pharmacological properties of mammalian neuroglial cells in tissue-culture. *Proc. R. Soc. Lond. B Biol. Sci.* **1966**, *165*, 326–361. [[CrossRef](#)] [[PubMed](#)]
45. Hild, W.; Chang, J.J.; Tasaki, I. Electrical responses of astrocytic glia from the mammalian central nervous system cultivated in vitro. *Experientia* **1958**, *14*, 220–221. [[CrossRef](#)] [[PubMed](#)]
46. Tasaki, I.; Chang, J.J. Electric response of glia cells in cat brain. *Science* **1958**, *128*, 1209–1210. [[CrossRef](#)] [[PubMed](#)]
47. Kuffler, S.W.; Nicholls, J.G. The physiology of neuroglial cells. *Ergeb. Physiol.* **1966**, *57*, 1–90. [[CrossRef](#)] [[PubMed](#)]
48. Orkand, R.K.; Nicholls, J.G.; Kuffler, S.W. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **1966**, *29*, 788–806. [[CrossRef](#)] [[PubMed](#)]
49. Oberheim, N.A.; Goldman, S.A.; Nedergaard, M. Heterogeneity of astrocytic form and function. *Methods Mol. Biol.* **2012**, *814*, 23–45. [[PubMed](#)]
50. Oberheim, N.A.; Takano, T.; Han, X.; He, W.; Lin, J.H.; 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. [[PubMed](#)]
51. Oberheim, N.A.; Wang, X.; Goldman, S.; Nedergaard, M. Astrocytic complexity distinguishes the human brain. *Trends Neurosci.* **2006**, *29*, 547–553. [[CrossRef](#)] [[PubMed](#)]
52. Sosunov, A.A.; Wu, X.; Tsankova, N.M.; Guilfoyle, E.; McKhann, G.M.; Goldman, J.E. Phenotypic heterogeneity and plasticity of isocortical and hippocampal astrocytes in the human brain. *J. Neurosci.* **2014**, *34*, 2285–2298.
53. Zhang, Y.; Sloan, S.A.; Clarke, L.E.; Caneda, C.; Plaza, C.A.; Blumenthal, P.D.; Vogel, H.; Steinberg, G.K.; Edwards, M.S.; Li, G.; et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron* **2016**, *89*, 37–53. [[PubMed](#)]
54. Bushong, E.A.; Martone, M.E.; Jones, Y.Z.; Ellisman, M.H. Protoplasmic astrocytes in cal stratum radiatum occupy separate anatomical domains. *J. Neurosci.* **2002**, *22*, 183–192. [[PubMed](#)]
55. Colombo, J.A. Interlaminar astroglial processes in the cerebral cortex of adult primates: Further characterization. In *Proceedings of the 1st Int Conference on Glial Contributions to Behaviour*, Belfast, Ireland, 28 August–1 September 1995; pp. 117–118.
56. Colombo, J.A. Interlaminar glia and other glial themes revisited: Pending answers following three decades of glial research. *Neuroglia* **2018**, *1*, 3.
57. Colombo, J.A.; Reisin, H.D.; Jones, M.; Bentham, C. Development of interlaminar astroglial processes in the cerebral cortex of control and Down’s syndrome human cases. *Exp. Neurol.* **2005**, *193*, 207–217.
58. Galambos, R. A glia-neural theory of brain function. *Proc. Natl. Acad. Sci. USA* **1961**, *47*, 129–136. [[CrossRef](#)] [[PubMed](#)]
59. Bellini-Leite, S.; Pereira, A.J. Is global workspace a cartesian theater? How the neuro-astroglial interaction model solves conceptual issues. *J. Cogn. Sci.* **2013**, *14*, 335–360.
60. Caudle, R.M. Memory in astrocytes: A hypothesis. *Theor. Biol. Med. Model* **2006**, *3*, 2. [[CrossRef](#)] [[PubMed](#)]
61. Pereira, A., Jr.; Dos Santos, R.P.; Barros, R.F. The calcium wave model of the perception-action cycle: Evidence from semantic relevance in memory experiments. *Front. Psychol.* **2013**, *4*, 252. [[CrossRef](#)] [[PubMed](#)]

62. Pereira, A., Jr.; Furlan, F.A. Astrocytes and human cognition: Modeling information integration and modulation of neuronal activity. *Prog. Neurobiol.* **2010**, *92*, 405–420. [[CrossRef](#)] [[PubMed](#)]
63. Robertson, J.M. Astrocyte domains and the three-dimensional and seamless expression of consciousness and explicit memories. *Med. Hypotheses* **2013**, *81*, 1017–1024. [[CrossRef](#)] [[PubMed](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).