# History of Astrocytes

## Outline

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overview</td>
<td>2</td>
</tr>
<tr>
<td><strong>Neuron Doctrine</strong></td>
<td></td>
</tr>
<tr>
<td>Purkinje and Valentin’s <em>Kugeln</em></td>
<td>2</td>
</tr>
<tr>
<td>Scheiden, Schwann, and Cell Theory</td>
<td>3</td>
</tr>
<tr>
<td>Remak’s Remarkable Observation</td>
<td>4</td>
</tr>
<tr>
<td>The Neurohistology of</td>
<td></td>
</tr>
<tr>
<td>Robert Bentley Todd</td>
<td>5</td>
</tr>
<tr>
<td>Wilhelm His’ Seminal Contributions</td>
<td>7</td>
</tr>
<tr>
<td>Fridtjof Nansen: The Renaissance Man</td>
<td>8</td>
</tr>
<tr>
<td>Auguste Forel: Neurohistology, Myrmecology, and Sexology</td>
<td>8</td>
</tr>
<tr>
<td>Rudolph Albert Von Kölliker: Neurohistologist and Cajal Champion</td>
<td>10</td>
</tr>
<tr>
<td>Waldeyer and the Neuronlehre (Neuron Doctrine)</td>
<td>11</td>
</tr>
<tr>
<td>Conclusion</td>
<td>12</td>
</tr>
<tr>
<td><strong>Development of the Concept of Neuroglia</strong></td>
<td></td>
</tr>
<tr>
<td>Rudolf Virchow</td>
<td>12</td>
</tr>
<tr>
<td>Other Investigators Develop More Detailed Images of Neuroglial Cells</td>
<td>15</td>
</tr>
<tr>
<td>Camillo Golgi</td>
<td>16</td>
</tr>
<tr>
<td>Naming of the “Astrocyte”</td>
<td>18</td>
</tr>
<tr>
<td>Santiago Ramón y Cajal and Glial Functions</td>
<td>18</td>
</tr>
<tr>
<td>Glial Alterations in Neurological Disease: Early Concepts</td>
<td>21</td>
</tr>
<tr>
<td>Wilder Penfield, Pío Del Río-Hortega, and Delineation of the “Third Element”</td>
<td>22</td>
</tr>
<tr>
<td>Penfield’s Idea to Go to Spain</td>
<td>24</td>
</tr>
<tr>
<td>Types of Neuroglia</td>
<td>26</td>
</tr>
<tr>
<td>Penfield’s Description of Oligodendroglia</td>
<td>27</td>
</tr>
<tr>
<td>Coming Together: The Fruit of Penfield’s Spanish Expedition</td>
<td>30</td>
</tr>
<tr>
<td>Beginning of the Modern Era</td>
<td>33</td>
</tr>
<tr>
<td>References</td>
<td>34</td>
</tr>
</tbody>
</table>
OVERVIEW

In this introduction to the history of astrocytes, we wish to accomplish the following goals: (1) contextualize the evolution of the concept of neuroglia within the development of cell theory and the “neuron doctrine”; (2) explain how the concept of neuroglia arose and evolved; (3) provide an interesting overview of some of the investigators involved in defining the cell types in the central nervous system (CNS); (4) select the interaction of Wilder Penfield and Pío del Río-Hortega for a more in-depth historical vignette portraying a critical period during which glial cell types were being identified, described, and separated; and (5) briefly summarize further developments that presaged the modern era of neurogliosci-ence. In an endeavor of this kind, one must be sure to acknowledge those authors who have previously made strong attempts to synthesize information regarding the history of the concept of neuroglia. In particular, the efforts and publications of Helmut Kettenmann, Bruce Ransom, George Somjen, Alexei Verkhratsky, Arthur Butt, and Vladimir Parpura should be commended highly [1–7]. In contradistinction to the historical aspect of this chapter, see Chapter 2: Astrocytes in the Mammalian Brain will describe a more modern understanding of astrocytes in the mammalian brain.

NEURON DOCTRINE

Neuron theory or neuron doctrine asserts that nerve tissue is composed of individual cells which are anatomical and functional units called neurons. While generally attributed to Wilhelm Waldeyer who coined the term “neuron” in 1891 [8] and the neurohistological work of Santiago Ramón y Cajal [9], there were many scientific antecedents enabling the development of the neuron doctrine [10–13]. Microscopic imaging of the nerve cell in 1836 by Gabriel Valentin, visualization of the axon in 1837 by Robert Remak, development of “cell theory” by Schleiden and Schwann (1838–39), and discovery of the “Purkinje” cell by Jan Purkinje in 1839 were all seminal. The early proponents of the neuron doctrine, Wilhelm His, Fridtjof Nansen, and Auguste Forel, based partly on direct observations of Golgi-stained nerve cells and partly on credible generalizations, independently concluded that neurons are likely to be separate units [14–16]. In this section, we will explore the scientific antecedents to the neuron doctrine and the fascinating investigators involved.

Purkinje and Valentin’s Kugeln

Jan Evangelista Purkinje (1787–1869) (Fig. 1.1) served as the Chair of Physiology in Breslau (the modern-day Polish city, Wrocław) and made many valuable contributions to cell biology and neurobiology. Gabriel Valentin (1810–83) was one of his students assigned to study the nervous system. In 1836, Valentin described the nerve cell with remarkable accuracy using water-prepared tissue. He noticed small Kugeln (globules) which were clearly nerve cells. He described that the globule had a sharp outline (cell membrane) and an interior substance called parenchymasse (parenchyma) filled with viscous fluid containing numerous granules. Inside this was the nucleus and within it a small corpuscle (nucleolus).
The very first description of the nerve cell body was made. Valentin and his mentor Purkinje had generally implied the idea of a “cell” but it was Schwann who proposed the unified “cell theory” and extolled its significance.

**Scheiden, Schwann, and Cell Theory**

The development of the proposition, that there exists one general principle for the formation of all organic productions, and that this principle is the formation of cells, as well as the conclusions which may be drawn from this proposition, may be comprised under the term cell-theory, using it in its more extended signification, whilst in a more limited sense, by theory of the cells we understand whatever may be inferred from this proposition with respect to the powers from which these phenomena result [17,18].

Matthias Jakob Schleiden (1804–81) (Fig. 1.2) was a lawyer who went back to school to study medicine and eventually became a botanist. In 1838, Schleiden published a paper on the origins of the plant cell; despite some flawed conclusions, this paper contained the essential idea that plants are composed of cells [19]. Schleiden’s friend, the zoologist Theodor Schwann (1810–82) (Fig. 1.3), heard one of his ideas over dinner one October evening in 1838 [20]. Together, they went to the laboratory and examined sections of spinal cord that Schwann studied. Schleiden recognized the nuclei and their similarity to those of plant cells. That night, the first notion of a unified cellular theory was born. A year later, in 1839, Schwann published the cell theory for all living tissues (plants and animals) [17]. Interestingly, no mention of Schleiden was made! (Incidentally, it was Schleiden who influenced the young Carl Zeiss to form his subsequently very important optical firm, the fruits of which were much improved microscopes to the present day.)
1. HISTORY OF ASTROCYTES

Remak’s Remarkable Observation

In his 1838 thesis, Robert Remak (1815–65) (Fig. 1.4) described peripheral nerves as a “primitive band” (axon) within a thin walled “primitive tube” (myelin sheath). These bands were later referred to as the “band of Remak” and synonymous with axon or axis cylinder. He also described sympathetic nerves which lacked the tubular covering and called them primitive fibers. They were later referred to as the “fiber of Remak.” A third contribution was relationship between the nerve and the nerve fiber: he stated that “[t]he organic fibers
arise from the very substance of the nucleated globules.” Remak never conclusively backed his statement with evidence. It was a “remarkable observation” and in many ways years ahead of his time. The water preparation of these specimens and the associated swelling or shrinkage cast doubt over the accuracy of these findings. However, Remak was vociferous in his opinions and publicly acknowledged that his findings were contrary to Gabriel Valentin’s findings. In the pinnacle of his career, Remak was the first to suggest in 1855 that

1: Each multipolar anterior horn cell connects with one motor root fibre.
2: The other processes of the cells are (physically and chemically) different from that fibre.

This is the first recorded, written statement alluding to the principle that a nerve cell gives rise to one process, different from the rest, which extends out to become an axon [13].

The Neurohistology of Robert Bentley Todd

Robert Bentley Todd (1809–60) (Fig. 1.5) was an anatomist, neurologist, and physiologist best known for the eponym “Todd’s paralysis” [21,22]. However, his neurohistological contributions are not well known [23–25].

Todd was aware of Schwann’s cell theory; however, he was not sure of the nature of the connection between “nerve vesicles” (cell bodies) and “nerve tubes”:

It is in vain, in the present state of our knowledge, to speculate upon the use of these caudate processes. Do they constitute a bond of union between the nerve-vesicles and certain nerve-tubes? or are they commissural fibers serving to connect the grey substance of different portions of the nervous centres? Until a more extended research has made us better acquainted with peculiarities of these vesicles in various localities, it would be premature to offer any conjecture concerning their precise relation to the other elements of the nervous centres. They exist, with different degrees development, in the locus niger of the crus cerebri, in the laminae of the cerebellum, in the gray matter of the spinal cord and medulla oblongata, and in the ganglions, and in the grey substance of the cerebral convolutions, in which latter situation they are generally of small size [24].
Todd appeared to maintain a neutral position and supported neither the reticular theory nor the neuron theory, holding that more study was required to draw a scientifically sound conclusion to this debate. Despite the subsequent excitement over the cell theory [11], it was not widely accepted at the time that cell theory could be applied to the nervous system [12]. Todd and Bowman also described the environment in which the nerve-vesicles exist in greater detail. While the nerve-vesicles are not physically touching, “they are either imbedded in a soft, granular matrix, as in the brain, or enveloped in a capsule of nucleated cells, as in the ganglia” [26]. Perhaps here they are describing supportive cells, such as astrocytes and oligodendroglia (not well distinguished until the work of Pío del Río-Hortega, one of Cajal’s disciples).

Todd and Bowman describe the cross-section of a myelinated axon or dendrite as a white substance of Schwann (not termed myelin until Virchow in 1858) forming a tube around the “axis cylinder” (not termed “axon” until Kölliker in 1896) and further suggested that a difference in function exists between the “white substance” and the “nerve tube”:

> It is a conjecture by no means devoid of probability, that the processes of the caudate vesicle may, after passing some way, become invested by the tubular membrane and by the white substance of Schwann [26].

While Todd and Bowman do describe the cytoarchitecture of the nerve-vesicle, they do not accurately describe its connections. Instead of suggesting that the axis cylinder is contiguous with the cell body, Todd and Bowman do not distinguish between the myelin sheath and the neurite processes, naming the two components the “fibrous nervous matter.” They do state that “the nerve-vesicles do not lie in immediate contact with each other” [26].
The exact nature of this “juxtaposition” was necessarily vague during Todd’s era, but this statement certainly seems closer to neuron doctrine than to reticularism (with no “admixture” of material). Todd was acutely aware of their inability to visualize actual nerve connections:

> How the fibres comport themselves with respect to the elements of the vesicular matter is not exactly known. It is certain, however, that the nerve-tubes frequently adhere to the sheaths of nerve-vesicles, and that many of them pass between the nerve-vesicles, probably to form a connection with more distant ones [26].

He recognized that they did not yet possess the proper histological techniques to study “the intimate connection of this granular sheath to the vesicle, and to its processes when they exist” [26]. It would not be until Ramón y Cajal fully utilized and perfected Camillo Golgi’s reazione nera (“black reaction” or silver stain, developed in 1873) that clear morphological evidence supporting the neuron doctrine could be adduced. Even so, as is well known, Golgi still defended reticularism in their joint (and contentious) Nobel ceremony of 1906.

While the overwhelmingly important work of Ramón y Cajal in solidifying the acceptance of the neuron doctrine has been well understood and described [10], the earlier events leading up to the acceptance of cell theory in the nervous system are not as often recognized [27]. In this, Robert Bentley Todd’s contributions have for the most part been overlooked. He proposed that the nerve-vesicle was the primary center for electrical activity in the brain and outlined its histology. At the time, Todd (and Bowman) did not have the appropriate techniques to elucidate further anatomic details of cell–cell interactions in the CNS; and furthermore the functional concept of the synapse was not introduced until Sherrington in 1897. Nevertheless, just as Todd’s electrical theory of epilepsy predated that of Hughlings-Jackson by 20 years [22], Todd identified the primacy of the “nerve-vesicle” more than 40 years before Waldeyer’s 1891 formulation of the neuron doctrine [8].

**Wilhelm His’ Seminal Contributions**

Wilhelm His Sr. (1831–1904) (Fig. 1.6) was a pupil of Robert Remak and a noted embryologist. He trained under several renowned developmental scientists such as Rudolf Virchow and Albert von Kölliker. As the professor of Anatomy and Physiology at University of Leipzig, His was the first to observe the development of the nerve cell. In 1886, he published that the nerve cell and its prolongations formed an independent unit [12]. Furthermore, he elucidated that the function of a nerve cell was based on proximity rather than continuity. In his studies on the peripheral nervous system, he observed that the nerve fiber was in close proximity to the muscle but not continuous [28]. He reasoned that the same underlying principle applied to the nerve cells in the CNS. In 1890, His coined the term “dendrites” to describe the branching, ramifying processes of the nerve cells [29]. Thus, he made a profound contribution to the neuron theory although his subsequent work focused exclusively on development and embryology. His son Wilhelm His Jr. (1863–1934) became a cardiologist and anatomist and discovered the eponymous “bundle of His.”
1. History of Astrocytes

Fridtjof Nansen: The Renaissance Man

Fridtjof Nansen (1861–1930) (Fig. 1.7) was a Professor of Zoology and Oceanography at the Royal Frederick University of Oslo. He made several remarkable contributions to neuroscience through his study of marine animals. Nansen embarked on an Arctic exploration to Greenland and after his return in 1882 he became the curator at the Bergen museum. Here, he interacted with experts studying the peripheral nervous system in marine animals. In 1886, he left to Pavia to train under the master neurohistologist Camille Golgi. He returned to Bergen museum and published his findings that nerve cells were bound by membranes and touched each other but were not fused to each other, an important step in the subsequent “neuron doctrine” [30]. He coupled this publication with unique illustrations with hand-etched figures [31]. Interestingly, he also postulated that neuroglia might be the “seat of intelligence, as it increases in size from the lower to the higher forms of animal” [30,32]. Eventually in 1887, he defended his dissertation and left for his second expedition to the Arctic where he was marooned for over a year [33]. After his rescue from the North Pole, his mind drifted to diplomacy and state affairs. He worked in the League of Nations, was its first president, developed the refugee passport, and helped establish monarchy in the newly independent Norway. He received the Nobel Peace prize in 1922 for his humanitarian work.

Auguste Forel: Neurohistology, Myrmecology, and Sexology

Auguste-Henri Forel (1848–1931) (Fig. 1.8) was a Swiss psychiatrist, trained under Von Gudden in Munich in neuroanatomy and psychiatry. He thus became a proponent of the
FIGURE 1.7  Fridtjof Nansen (1861–1930).

FIGURE 1.8  Auguste-Henri Forel (1848–1931).
Gudden method, which applied the technique of secondary degeneration to study the inter-
relationship between cortex and subcortical structures. After his medical training in Munich,
Forel took the position of Professor of Psychiatry at the Burghölzli asylum in Zurich. Here
in solitude, he pursued his neuroanatomical work and developed an interest in ant colonies
and their social structure. Using the Gudden method of controlled retrograde degeneration,
he published in 1887 that all nerve fibers were processes of cells [10].

I think all the systems of fibres and the so-called networks of fibres of the nervous system are no more
than nervous processes of cells that ramify to a greater or lesser distance in the form of ramifications within
other ramifications, but not anastomosized [34].

Interestingly, Forel and Wilhelm His both began writing their independent assertions,
unaware of each other’s work, that later formed the tenets of the neuron doctrine in 1886.
But, due to a publishing delay, Forel’s work was printed and disseminated later than
Wilhelm His’ work.

During his service at Burghölzli asylum, Forel was fascinated by his studies on myrmec-
ology and even named his home as La Fourmilière (The Ant Colony) and gradually shifted
his focus away from neurohistology. In his later years, he suffered a stroke on his right side,
learned to use his left hand and authored several controversial publications in sexology,
eugenics, and penal law related to mental illnesses [35].

Rudolph Albert Von Kölliker: Neurohistologist and Cajal Champion

Rudolph Albert von Kölliker (1817–1905) (Fig. 1.9) was a prolific neurohistologist and
embryologist and among other things named the “axon.” He is well known for his role in
the dissemination of Cajal’s observations, but Kölliker was initially a firm proponent of the
reticular theory. In 1889, the German Anatomical Society meeting was held at the University of Berlin and both Cajal and Kölliker were present. At that time, Kölliker was already a respected scientist and a giant in the field of histology. In this meeting, Cajal was assigned a table for demonstration of his neurohistology slides. Cajal’s phenomenal staining of the nerve cells made a lasting impression on Kölliker’s mind, despite Cajal’s broken German and halting French [36]. Kölliker invited the young Spaniard to a private dinner and introduced him to several renowned German histologists. The extraordinary contribution to the neuron doctrine by Kölliker was his vocal support and enthusiastic promulgation. Despite being in his seventies, Kölliker wrote to several histologists extolling Cajal’s observations, publicly abandoned the erroneous reticular theory and adopted the neuron theory [9]. Even more extraordinary is the fact that Kölliker himself translated some of Cajal’s work from Spanish to German, accelerating the acceptance of Cajal’s work.

**Waldeyer and the Neuronlehre (Neuron Doctrine)**

Heinrich Wilhelm Gottfried von Waldeyer-Hartz (1836–1921) (Fig. 1.10) published over 269 papers spanning a range of topics from gross anatomy, histology, physiology, pathology, anthropology, education, history to the arts [37]. In a series of review articles in 1891 titled “Über einige Neuere Forschungen im Gebiete der Anatomie des Centralnervensystems” (On some recent research in areas of the anatomy of the CNS) published in Deutsche Medizinische Wochenschrift (German Medical Weekly), Waldeyer founded the word “neuron” from the Greek word “sinew.” The doctrine of separate, distinct entities as the building blocks of the CNS had been formulated by others before him based on histological works of Ramón y Cajal and Nansen [38]. However, Waldeyer’s new term “neuron” bestowed this doctrine and its functional entity awareness and consequent acceptance of the concept.
Waldeyer was probably directly influenced by having read Kölliker’s recent German translation of Cajal’s works. He had a specific talent for coining new scientific terms. He had also named “chromosome,” “plasma cells,” and “gastric canal” before coining the term “neuron” [37,39,40]. Waldeyer was the Chair of Anatomy and Professor at the prestigious research institution of Berlin University, a military surgeon and a consulting physician for Emperor of Prussia Friedrich III, which indubitably helped the new term “neuron” to gain prominence. Waldeyer unified diverse and controversial data into a single coherent theory and published in a popular, widely read journal (notably without contributing a single original observation) and yet obtained worldwide and long-standing recognition for the discovery of the “neuron” [41].

Conclusion

The neuron doctrine was based on a series of observations that culminated in the concept of the neuron as the single independent unit in the nervous system. It is clear from the work described above that several important investigators laid important groundwork for the neuron doctrine. Jan Purkinje, Gabriel Valentin, Robert Remak, and Robert Bentley Todd made important contributions from 1836–45. However, none of these scientists directly formulated the neuron doctrine. Nearly half a century later, Wilhelm His, Fridtjof Nansen, and Auguste Forel independently published documents anticipating the neuron doctrine within a span of 1 year (1886–87). Interestingly, these three investigators inferred similar conclusions despite different methodologies such as retrograde degeneration, development of nerve cells, and marine biology. Thus, while Kölliker gets credit for championing Cajal’s work after 1889 and Waldeyer published the neuron doctrine in 1891, it is clear that in the development of neurohistology in the 19th century, these previous scientists played a crucial part.

DEVELOPMENT OF THE CONCEPT OF NEUROGLIA

Rudolf Virchow

Ich habe bis jetzt, meine Herren, bei der Betrachtung des Nervenapparatus immer nur der eigentlich nervösen Theile gedacht. Wenn man aber das Nervensystem in seinem wirklichen Verhalten im Körper studieren will, so ist es ausserordentlich wichtig, auch diejenige Masse zu kennen, welche zwischen den eigentlichen Nerventheilen vorhanden ist, welche sie zusammenhändig dem Ganzen mehr oder weinger seine Form gibt.

[Hitherto, gentlemen, in considering the nervous system, I have only spoken of the really nervous parts of it. But if we would study the nervous system in its real relations in the body, it is extremely important to have a knowledge of that substance also which lies between the proper nervous parts, holds them together and gives the whole its form in a greater or lesser degree] [42].

Rudolph Virchow (1821–1902) (Fig. 1.11) was primarily a pathologist who studied diseased tissue. The above quote comes from a lecture that Rudolf Virchow delivered to medical students at the Charité Hospital in Berlin on April 3, 1858. He had previously introduced the actual term Nervenkitt (neuroglia) 2 years earlier as a “connective substance, which
FIGURE 1.11 Rudolf Virchow (1821–1902) and the concept of neuroglia. (A) Portrait of Rudolf Virchow in the 1850s. Rudolf Virchow was born on October 13, 1821, in Schivelbein, which was then under the rule of the Prussian kingdom and now is a city of Swidwin in Poland. He studied medicine in Berlin and worked as a pathologist at the Charité. After the failure of the 1848 revolution, in which he had actively participated, he was forced to leave Berlin and to move to Würzburg, where he became Professor of Pathology. He returned to Berlin in 1856 and occupied the Chair in Pathology for the rest of his life. He had a broad interest in science, ranging from cancer research and neuroscience to anthropology and, as the editor-in-chief of Virchow’s Archiv, he could oversee it all. Rudolf Virchow was not only a highly influential scientist, but was actively engaged in different aspects of political and cultural life. He initiated laws for meat inspection at slaughterhouses and, as a member of the German parliament, he was instrumental in installing a modern sewage system in the city of Berlin, borne out of the recognition that there is a relationship between infections and hygienic conditions. His interest in anthropology let him to participate in excavations carried out by his friend Heinrich Schliemann in Troy, and it was Virchow who convinced Schliemann to donate the treasures of Priam to the city of Berlin. Virchow assembled a tremendous collection of pathologic specimens and, at the end of his life, he opened a Pathologic Museum, not only for students and medical practitioners, but also for the public. He died in Berlin on September 5, 1902. (B) The frontispiece of Cellular Pathology, published in 1858. (C) Lecture 13 (“Spinal cord and the brain”) from Cellular Pathology, where the name neuroglia was first coined. Source: Reproduced with permission from Verkhratsky A, Butt AM. Glial physiology and pathophysiology. Oxford, UK: Wiley-Blackwell, 2013 (Figure 1.13).
forms in the brain, in the spinal cord, and in the higher sensory nerves a sort of Nervenkitt (neuroglia), in which the nervous system elements are embedded” [43]. Thus, in his invention of the term neuroglia in 1856, Virchow thought of the neuroglia (nerve glue) as a true connective tissue (Zwischenmasse or “in between tissue”) rather than containing individual cell types. Note however, from above that cell theory had already been introduced by Schleiden and Schwann in 1838–39.

Twenty of Virchow’s lectures delivered between February and April 1858 were published as Cellular pathology [42]. This contained Virchow’s first illustrations of neuroglia (Fig. 1.12). In this volume he also explains that “the real cement, which binds the nervous elements together, and that in all its properties it constitutes a tissue different from the other forms of connective tissue, has induced me to give it a new name, that of neuroglia (nerve cement)” [42]. Neuroglia is variously translated as “nerve cement,” “nerve glue,” and “nerve putty” [4–6]. This would seem to indicate merely an acellular matrix, however, he goes on to add elsewhere that “where neuroglia is met with, it also contains a certain number of cellular elements” [42]. Ultimately, as Kettenmann and Ransom indicate [1], the term neuroglia came to refer to neuroglial cells as its cellular constituents became more and more apparent.

At the same time (1858), Virchow was the one to introduce the term myelin to refer to the fatty sheath surrounding “axis cylinders” (not termed “axon” until Kölliker): “It is this substance, for which I have proposed the name of medullary matter (Markstoff), or myeline, that in extremely large quantity fills up the interval between the axis-cylinder and the sheath in primitive nerve fibers” [42].

**FIGURE 1.12** Virchow’s illustrations of neuroglia. (A) Ependyma and neuroglia in the floor of the fourth ventricle. Between the ependyma and the nerve fibers is “the free portion of the neuroglia with numerous connective tissue corpuscles and nuclei.” Numerous corpora amylacea are also visible, shown enlarged below the main illustration (ca). E, ependymal epithelium; N, nerve fibers; v, blood vessels. (B) Elements of neuroglia from white matter of the human cerebral hemispheres. a, free nuclei with nucleoli; b, nuclei with partially destroyed cell bodies; c, complete cells. Source: Reproduced with permission from Verkhratsky A, Butt AM. Glial physiology and pathophysiology. Oxford, UK: Wiley-Blackwell; 2013 (Figure 1.14).
Other Investigators Develop More Detailed Images of Neuroglial Cells

Heinrich Müller (1820–64) was a pathologist who performed a thorough analysis of retinal histology in Kölliker’s department. He noted that the retina contains radial fibers [44], which Kölliker called Müller fibers in 1852 [45]. He provided detailed histological images of retinal glial cells (thereafter called Müller cells) [44,46].

Several years later, Max Schulze (1825–74) provided even more detailed and precise images of retinal glial cells (Fig. 1.13) [47].

Otto Deiters (1834–63) was the first to provide histological images of stellate cells which resemble astrocytes [48]. Deiter’s cell was synonymous with astrocyte for many years. Upon Deiters’ early death at the age of 29 from typhoid fever, his friend Max Schulze published Deiters’ findings 2 years later [48]. In it he clearly identifies stellate cells from both white and gray matter (see Kettenmann and Ransom for details) [1,48].

Karl Bergmann (1814–65) discovered fibers in the molecular layer of the cerebellum and described them as processes of glial cells, subsequently termed “Bergmann” glia [49].

In 1869, the anatomist Jakob Henle (1809–85) (yes he of the loop of Henle in the kidney) and his son-in-law Friedrich Merkel (1845–1919) described networks of stellate cells (presumably astrocytes) in the gray and white matter of the spinal cord [50].

**FIGURE 1.13** Retinal “Müller” glial cells. Müller fiber of the sheep retina, inspected by Max Schulze with a microscope from Amici. yyy, brush-like fibrils extending from the outer Müller fiber in the outer granular layer; xx, internal limiting membrane; a, opening in the limiting membrane; b, very delicate network of fenestrated membranes similar in the ganglion cell layer; cc, network in the so-called molecular layer; ddd, nuclei as part of the Müller fibers; ee, cavity in which the nuclei or the cells of the internal granular layer is located. Source: From Schulze, 1859. Image kindly provided by Prof. Helmut Kettenmann, Max Delbruck Center for Molecular Medicine, Berlin. Reproduced with permission from Verkhratsky A, Butt AM. Glial physiology and pathophysiology. Oxford, UK: Wiley-Blackwell; 2013 (Figure 1.15).
CAMILO GOLGI

Camillo Golgi (1843–1926) (Fig. 1.14), born in Brescia, went to Pavia for medical school and then assumed the title of Extraordinary Professor of Histology and then in 1881 Chair of General Pathology at the same institution. He is associated with supporting the reticular theory of brain organization (contra the neuron doctrine). Golgi performed the first in-depth histological observations of neuroglia in the 1870s [51,52]. Golgi described cells with the features of astrocytes and oligodendrocytes, and was the first to describe glial–vascular contacts or “end feet”:

Studying thin slices of treated cortical substance (osmium treated for 4 to 6 h) with addition of glycerin with the microscope, revealed numerous, long, fine and never arborized prolongations originate…Many (prolongations) extend to the vessel walls, including capillaries, vessels of medium size (particularly to those) and directly attach to the walls of the capillaries or to the lymphatic border of vessels with larger diameter…The surround of the vessels is demarcated by a dense, regular network of fibers [53].

Based on these findings, which clearly suggest astrocyte endfeet, Golgi theorized that glial cells provide the bridge between parenchyma and vasculature (Fig. 1.15). This led to his concept of metabolic support and substance exchange, which remains quite valid today.

Of course, Golgi is best known for his development of the reazione nera (“black reaction” or silver nitrate stain, developed in 1873) that allowed visualization of entire neurons and glial cells. Others such as Cajal and Río-Hortega (see below) would subsequently use and modify Golgi’s techniques to develop specific stains for glial cells [54,55].

FIGURE 1.14 Camillo Golgi (1843–1926).
FIGURE 1.15 Neuroglial cells stained by the silver-chromate technique and drawn by Camillo Golgi (Golgi, 1903). Top panels show individual star-shaped astrocytes and astroglial networks. At the bottom right, astrocytes forming numerous contacts (the end feet) with brain capillaries are demonstrated. The bottom left panel shows the drawing of white matter with numerous cellular processes oriented parallel to axons, which most likely represent oligodendrocytes. Source: Reproduced with permission from Verkhratsky A, Butt AM. Glial physiology and pathophysiology. Oxford, UK: Wiley-Blackwell; 2013 (Figure 1.16).
1. HISTORY OF ASTROCYTES

Naming of the “Astrocyte”

Stellate CNS glial cells were named “astrocytes” (from the Greek *astron*—star and *kutos*—container or vessel, later cell, thus literally “star cells”) by Michael von Lenhossék from Würzburg (1863–1937) in 1893:

> I would suggest that all supportive cells be named spongiocytes, and the most common form in vertebrates be named spider cells or **astrocytes**, and use the term neuroglia only cum grano salis [with a grain of salt], at least until we have a clearer view...Astrocytes are the small elements, which form the supportive system of the spinal cord. They are star shaped and indeed no other comparison describes their form so clearly [56].

Andriezen further classified them into fibrous glia (*Langstrahler*, “long projectors,” found in white matter) and protoplasmic glia (*Kurzstrahler*, “short projectors,” found in gray matter) [57].

It is noteworthy that around this time, in the last decade of the 19th century, just 3 years after the term “neuron” was coined by Waldeyer in 1891 and “astrocyte” by Lenhossék in 1893, that in 1894 Carl Ludwig Schleich (1859–1922) hypothesized for the first time that neuronal–glial interactions are important for brain function. In his book *Schmerzlose Operationen* (literally “Pain-free Operations”), which was mainly a treatise on the principles of local anesthesia, he also hypothesized that interactions among glial cells and neurons determine the status of excitation in the CNS (Fig. 1.16) [58]. In particular, according to Schleich, excitation from neuron to neuron via intercellular gaps is checked by generally inhibitory glial cells filling these interneuronal gaps. In particular, glial swelling was postulated to inhibit and glial shrinkage to promote neuronal excitation, a prescient idea but exactly converse to what is now known about the excitability-promoting effects of astrocyte swelling such as release of glutamate! Nevertheless, Schleich was the first to (correctly) promote the role of glial cells in controlling neuronal excitation, and recent findings in this field clearly indicate profound effects of glia on excitability. A related prescient hypothesis was that of Ernesto Lugaro, who was the first to propose (in 1907) that astrocytes function to take up and metabolize chemical transmitters at “neuronal articulations” (synapses) [59]; this was experimentally confirmed by Mennerick and Zorumski in 1994 [60], and we now know that astrocyte glutamate transporters are responsible for approximately 90% of glutamate reuptake from the extracellular space [61].

Santiago Ramón y Cajal and Glial Functions

The great Spanish neurohistologist Santiago Ramón y Cajal (1852–1934) (Fig. 1.17) was born in a small village in the north of Spain and studied medicine in the Faculty of Medicine in Zaragoza. In 1883, Cajal was appointed Professor of Descriptive and General Anatomy at the University of Valencia. In 1887, he moved to the University of Barcelona, where he was appointed to the Chair of Histology and Pathological Anatomy. In 1892, he was appointment to the same Chair of Histology and Pathological Anatomy at the University of Madrid, where he remained until retirement.
FIGURE 1.16  Carl Ludwig Schleich (1859–1922) and the neuronal–glial interactions hypothesis. Schleich was a pupil of Virchow and surgeon who introduced local anesthesia into clinical practice. In 1894, he published a book *Schmerzlose Operationen*, the frontispiece of which is shown in the right upper panel. Apart from describing the principles of local anesthesia, this book also contained the first detailed essay on interactions in neuronal–glial networks as a substrate for brain function. Mid panels show original drawings from this book, depicting intimate contacts between glial cells and neurons, and the lower panel shows, in a schematic manner, Schleich’s theory of neuroglia controlling information flow in neuronal networks. Source: Reproduced with permission from Verkhratsky A, Butt AM. Glial physiology and pathophysiology. Oxford, UK: Wiley-Blackwell; 2013 (Figure 1.24).
Between 1887 and 1903, Cajal was able to apply the Golgi method to describe in detail almost every part of the CNS (Fig. 1.18). In his pioneering summary treatise Histology of the Nervous System, published in Spanish in 1899–1904 and translated into French in 1909 and 1911 and into English only in 1995 [62], Cajal queried: “What is the function of glial cells in neural centers? The answer is still not known, and the problem is even more serious because it may remain unsolved for many years to come until physiologists find direct methods to attack it” [62]. At the time, microglia and oligodendrocytes, the mysterious “third element” that his disciple Pío del Río-Hortega (1882–1945) (Fig. 1.19) would be instrumental in deciphering, were not yet described. Cajal dismissed Golgi’s hypothesis that glial cells carry important nutritive functions by virtue of the proximity to capillaries on the one hand and neuronal cell bodies on the other. Similarly he did not subscribe to Weigert’s (1895) “filling theory” in which glia are entirely passive, just filling spaces between neurons [63]. Held (1904) suggested that glial fibers form a syncytial network (prefiguring the modern knowledge that astrocytes are interconnected by gap junction networks) [64]. Cajal rejected the notion of a syncytium (as he did with neurons in support of the neuron doctrine against reticularism), and instead he subscribed to the “isolation theory” (developed by his brother Pedro) in which astrocytes act as physical insulation [62]. Ironically of course, Cajal’s disciple Pío del Río-Hortega was ultimately to describe oligodendrocytes (see below) and proposed that they were the source of CNS myelin. Of course, Cajal’s studies provided overwhelming evidence in favor of the neuron doctrine, which contrasted with the reticular theory [13]. Nageotte (1910) described secretory granules in glial cells and suggested an endocrine function for glia [65]; Cajal (1913) agreed that protoplasmic astrocytes may be secretory [66]; and Cajal’s disciple Achúcarro (1915) hypothesized that secretions from glial cells might enter the bloodstream to influence distant organs [67].
Glial Alterations in Neurological Disease: Early Concepts

Following developments in the concepts of neuroglia, neuropathologists started to recognize glial alterations in tissues from patients with neurological diseases. Carl Frommann (1831–92) found changes in glial cell morphology in the vicinity of demyelinating plaques [68]. Andriezen indicated that astrocytes “exhibit a morbid hypertrophy in pathological conditions” [57]. Franz Nissl (1860–1919) described two pathological cell types: Stäbchenzellen (rod cells), which appeared in demented patients’ brains; and Körnerzellen (granule cells) or Gitterzellen (lattice cells) which were associated with disruption of the blood–brain barrier [69,70]. Alois Alzheimer (1864–1915) described ameboid change in glial cells in response to acute and chronic neurologic diseases, such as epilepsy, syphilis, and the disease that...
bears his name [71]. Nicolás Achúcarro, a Basque-born psychiatrist and neurohistologist who was a colleague and alumnus of Cajal, advanced the idea that dysfunction of neuroglia may itself produce brain diseases [72]. Critically for epilepsy and brain injury, the role of astrocytes in glial scar formation was recognized by Cajal, Río-Hortega, and Penfield in the 1920s (see below) [73]. Cajal’s later career (from 1903 through his death in 1934) was devoted largely to the investigation of pathological processes within the nervous system. This work was summarized in another classic of neuroscience, Degeneration and Regeneration of the Nervous System [74].


WILDER PENFIELD, PÍO DEL RÍO-HORTEGA, AND DELINEATION OF THE “THIRD ELEMENT”

To the modern neuroscientist, Wilder Penfield (1891–1976) (Fig. 1.20) needs little introduction. Many are familiar with the basic outline of his legendary career (Table 1.1). Penfield is justifiably famous for two main achievements: his tremendous contributions to the functional mapping of the human brain in the surgical treatment of epilepsy and the founding of the Montreal Neurological Institute. However, lesser known but seminal as well was his initial neuropathological work examining and characterizing glial cells. As the function of glial cells increasingly is seen as complimentary rather than subsidiary to neurons [75–77], it seems appropriate to look back at Penfield’s important contributions to

TABLE 1.1  Some Significant Events in the Life of Wilder Penfield

<table>
<thead>
<tr>
<th>Year(s)</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1904</td>
<td>At 13 years, Penfield discovers existence of Rhodes Scholarship</td>
</tr>
<tr>
<td>1913</td>
<td>Graduation from Princeton</td>
</tr>
<tr>
<td>1914</td>
<td>Awarded Rhodes Scholarship</td>
</tr>
<tr>
<td>1915–16</td>
<td>Medical student at Oxford, exposed to William Osler and Charles Sherrington</td>
</tr>
<tr>
<td>1916–18</td>
<td>Medical student at Johns Hopkins Medical School</td>
</tr>
<tr>
<td>1918–19</td>
<td>Surgical internship at Peter Bent Brigham Hospital, Boston</td>
</tr>
<tr>
<td>1919–20</td>
<td>Returned to Oxford for postgraduate study, works with Sherrington assistants, Cuthbert Bazett on decerebrate rigidity and Harry M. Carleton on neurocytology</td>
</tr>
<tr>
<td>1921</td>
<td>Joins staff as neurosurgeon at Presbyterian Hospital, New York (surgical chief: Allen Whipple)</td>
</tr>
<tr>
<td>1924</td>
<td>Leaves Presbyterian Hospital to go to Madrid</td>
</tr>
<tr>
<td>September 1924</td>
<td>Returns to New York and Presbyterian Hospital, meets William Vernon Cone and inaugurates Laboratory of Neurocytology</td>
</tr>
<tr>
<td>1928</td>
<td>Works with Otfrid Foerster in Breslau, Germany for 6 months</td>
</tr>
<tr>
<td>1928</td>
<td>Arrives in Montreal (Royal Victoria Hospital and McGill University)</td>
</tr>
<tr>
<td>December 11, 1928</td>
<td>Operates on sister Ruth for oligodendroglioma</td>
</tr>
<tr>
<td>1932</td>
<td>Publishes Cytology and Cellular Pathology of the Nervous System, a 3-volume text with contributions from world-leading authorities, many of whom Penfield knew personally</td>
</tr>
<tr>
<td>September 27, 1934</td>
<td>Opening of the Montreal Neurological Institute</td>
</tr>
</tbody>
</table>
the early characterization of glial cells. The modern reader may be surprised that Penfield was, together with his Spanish mentor Pío Del Río-Hortega, the first to properly describe oligodendroglial cells. In this section, we review Penfield’s trip to Spain and the contributions arising from his fruitful albeit short time in the Residencia des Estudiantes in Madrid, 1924 [78].

Penfield’s Idea to Go to Spain

In 1923, a young Wilder Penfield was attempting to stain brain scars in the laboratories of the New York Presbyterian Hospital in the hope of identifying the mysterious cause of posttraumatic epilepsy in both animal models and humans. Penfield had begun this project 2 years earlier, when he began teaching medical students at the Columbia College of Physician and Surgeons under the authority of William C. Clarke, professor of surgical pathology. Clarke challenged Penfield immediately, asking “Wouldn’t you like to see how the nerve cells, and all the other cells that surround them and nourish them, behave when… you make an incision in the brain? What is the cause of epilepsy?” [79].

Penfield was fascinated by epilepsy from his very first exposure to the CNS, mentioning that as early as his undergraduate years, he “had filled [his] index cards with notes from the writings of Hughlings Jackson” [79]. Clarke gave Penfield his first opportunity to begin studying the disease. He recognized immediately, however, “that the methods we use show only half the picture” [79], and indeed 2 years later, Penfield met a “dead end” in his work due to the inability to stain the nonneuronal cells of the CNS. Penfield believed these cells were crucial to demonstrating the healing process of the brain and in helping elucidate why a “healing scar so often leads to epilepsy” [79].

It was then that Penfield recalled the trouble he had staining neurons in the laboratory of another famous mentor he had worked with while on a Rhodes Scholarship, Sir Charles S. Sherrington of Oxford University (1857–1952) (Fig. 1.21), who had admonished him “Don’t give up until you have tried the methods of Ramón y Cajal” [79]. (Sherrington had invited Cajal to stay at his home during Cajal’s Croonian Lectureship in 1894 and the awarding of an honorary degree from Oxford; Cajal was rumored to have turned the guest bedroom into a histology laboratory! [80]) Remembering the brilliant success he had met with using Cajal’s staining techniques, Penfield immediately went to the New York Academy of Medicine to read Cajal’s articles in the hopes of adopting his staining techniques once again, only this time for glia rather than neurons. Though the techniques proved fruitful, Penfield was not able at first to emulate the beautiful stains of Cajal’s greatest disciple, Pío Del Río-Hortega, nor was he able to completely interpret the results of the stains he used.

In January of 1924, Penfield decided to approach the man who had recruited him to the Presbyterian Hospital, surgical chief and professor of surgery Allen O. Whipple (Fig. 1.22), in the hopes of securing funds to travel to Spain and study under Pío Del Río-Hortega. Río-Hortega had published detailed drawings of the nonneuronal cells Penfield stated were “no more than ghosts” [79] in his preparations. Though Penfield was unsure how Whipple would respond, Whipple was quite supportive and decided to call upon Mrs. Percy Rockefeller. He had operated on her daughter free of charge and was able to secure a generous grant from Mrs. Rockefeller. With the help of a few other benefactors, Whipple

FIGURE 1.22  Allen O. Whipple (center) and his surgical team, including Wilder Penfield (far left), c.1924 at the Presbyterian Hospital. William V. Cone is standing behind Penfield. Whipple was known for developing the pancreaticoduodenectomy (“Whipple procedure”) and describing Whipple’s triad (diagnostic triad for insulinoma). Source: Reproduced with permission from Gill AS, Binder DK. Wilder Penfield, Pío del Río-Hortega, and the discovery of oligodendroglia. Neurosurgery 2007;60(5):940–8.
secured enough funds to allow for Penfield, his wife, and two children to spend 6 months in Madrid. Here, Penfield hoped to work closely with Río-Hortega to “study the brain of man, and then move on to the effects of disease on the brain” [79]. Interestingly, Penfield and family set sail for Spain before receiving any word or invitation from Río-Hortega. Penfield wryly describes finally receiving Río-Hortega’s one-word imperative, “venga” (“come”), while halfway across the Atlantic [79].

Types of Neuroglia

It was not until the publication of Golgi’s method in 1886 [81,82] that a means for the more exact study of nerve cells and neuroglia was made possible. Cajal’s pioneering modifications of Golgi’s technique made possible the detailed anatomical study of the CNS. In fact, Cajal’s modifications and studies were so fundamental that many, including Sherrington and Penfield, regarded Cajal (Fig. 1.23) as the true father of neuroanatomy [79]. His studies on glial cells came after his celebrated studies of neurons. Using his new gold chloride-sublimate method, Cajal demonstrated the morphology of the protoplasmic neuroglia as well as the fibrous neuroglia of the white matter in 1913 [66,83]. Cajal also recognized that the “satellites” and “interfascicular cells” were of a different class from neuroglia, which he termed “the third element” [66].

However, the remainder of the “adventitial” or nonnervous cells remained unstained until Cajal’s disciple Pío Del Río-Hortega (1882–1945) (Fig. 1.19) described a method of using silver carbonate to stain neuroglia and connective tissue in 1918 [84,85]. For the first time, this clearly distinguished two cell types with distinct cytoplasmic expansions, which Río-Hortega termed microglia and oligodendroglia. Thus the nonneural interstitial cells

FIGURE 1.23  Santiago Ramón y Cajal (1852–1934) in his laboratory.
could be divided into four classes: (1) fibrous neuroglia; (2) protoplasmic neuroglia; (3) microglia; and (4) oligodendroglia. Oligodendroglia were so named because they exhibited fewer (Greek oligo—few) and smaller branches (Greek dendro—branch) than astrocytes, and were later called oligodendrocytes (as astroglia are astrocytes). Río-Hortega would go on to focus his research efforts on microglia, elucidating their genesis, function, and pathologies in remarkably precise fashion [86]. He indicated that microglia are histiocytes of mesodermal origin as opposed to the epithelial origin of “classical” glia. Regarding microglia he stated: “Since it is of different ancestry and its characteristics differ from those of the nerve cells (first element) and the neuroglial astrocytes (second element) (containing astrocytes and oligodendrocytes), the microglia constitutes the true third element of the central nervous system” [87].

However, there was still much debate on the existence of oligodendroglia as a distinct CNS cell type, due in part to the difficulty involved in staining these cells. In fact, Cajal himself was unable to produce an effective and reproducible stain, leading him to dismiss these cells altogether as a true class of glial cells and to declare that the “third element” was made up exclusively of microglia. Given Cajal’s enormous influence, his dismissal was perhaps just as important as any staining difficulty with respect to the further characterization of oligodendroglia. This put a strain on the relationship between Cajal and Río-Hortega. On Penfield’s arrival in Spain, there had been no resolution to this debate (Fig. 1.24).

**Penfield’s Description of Oligodendroglia**

Penfield noted that prior to the method of Río-Hortega, oligodendroglia were very difficult to stain completely. The first to stain oligodendroglial cells was the Scottish investigator William Ford Robertson (1867–1923), who employed a platinum method to describe cells that he termed mesoglia [88,89]. However, in his term mesoglia, he had just described a group
of cells he believed to be of mesodermal origin. Penfield went back to examine an original preparation of Robertson and compared it with sections stained by Río-Hortega’s method. He was able to verify that the “mesoglia” of Robertson were indeed identical to the “oligodendroglia” of Río-Hortega. Based on this, Penfield suggested that the term “mesoglia” be abandoned.

Penfield then learned the method of Río-Hortega for staining oligodendroglial cells and added his own modifications. Using the “ammoniacal silver carbonate” method of Río-Hortega, originally developed by Achúcarro [90], the results were variable. In 1924, Penfield reported on his modifications to the method, and used it to stain oligodendroglia in the CNS of rabbits.

The remainder of the staining procedure consisted of washing, toning, fixing, dehydrating, and clearing the specimen. Toning, originally described by Cajal, consisted of substituting gold for silver. With his modifications, Penfield had finally succeeded in developing a reliable stain for oligodendroglia. Don Pío, having been shown Penfield’s exceptional slides, remarked, “Casi mejor que yo” (almost better than I could do). Penfield later reminisced that he “might have laughed at his use of the word ‘almost,’” but that “he could expect no higher praise” [79].

Río-Hortega then asked Penfield to publish his results confirming oligodendroglia as the remaining cell type of the “third element.” Penfield studied and drew many of the oligodendroglia that he stained (Fig. 1.25). While noting that neuroglia and oligodendroglia both possessed “the asteroid body with expansions, centrosome and Golgi apparatus of similar appearance” [91], he nevertheless was able to distinguish many characteristics of oligodendroglia. He noted that oligodendroglial nuclei are larger than those of microglia but smaller.
than those of neuroglia. He also commented on the ability to distinguish neuroglia from oligodendroglia by the presence of “sucker feet” (modern-day “vascular endfeet”) on the former group of cells [91]. Penfield also stated that with his improved methods he could get a better view of oligodendroglial cell cytoplasm, showing that the “expansions” of cytoplasm were directed along the length of the “neuron cable system” (ie, along white matter tracts) (Fig. 1.26). In addition to studying white matter oligodendroglia (termed “interfascicular glia” by Río-Hortega), Penfield carefully described oligodendroglia in gray matter as well. Penfield clearly showed that “perineuronal satellites” included both oligodendroglia and microglia. Similarly, he showed that “perivascular satellites” could also be either oligodendroglia or microglia. Critically, he noted that while the cell bodies of these two types of perivascular satellites were “applied closely to the blood-vessel,” neither one were like neuroglia in this respect: with neuroglia it was the “neuroglia expansions that are applied to the neuron and vessel.” Interestingly, he may have simultaneously underestimated neuroglia and overestimated neurons in claiming that “oligodendroglia forms by far the most numerous group of cells in the central nervous system, after nerve cells” [91].

In 1924, Wilder Penfield published his work from La Residencia in a seminal article in the journal Brain, “Oligodendroglia and its relation to classical neuroglia” [91]. In this article, he paid homage to his mentors Cajal and Río-Hortega:

> In spite of untiring study of the central nervous system which has demonstrated the intricate morphology of neurones and neuroglia, a very numerous body of small cells (the third element of Cajal) continued to be refractory to staining methods...The brilliant studies of Del Río-Hortega show that these cells possess complicated expansions. By demonstrating their detailed structure, he was able to show that they fall into two groups, differing in form and function. One group, which he chose to denominate microglia, is of mesodermal origin, and the other, oligodendroglia, composing the more numerous portion of the cells, he believes to be of ectodermal origin [91].

1. HISTORY OF ASTROCYTES

In this historic paper, Penfield summarized his work along with the work of Cajal and Río-Hortega in formulating an overall classification of the interstitial cells of the nervous system (Table 1.2). This gross classification has changed remarkably little since.

### Table 1.2  Penfield’s Formulation of the Interstitial Cells of the Central Nervous System (1924)

<table>
<thead>
<tr>
<th>Interstitial Cells</th>
<th>In White Matter</th>
<th>In Gray Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroglia, classical (ectodermal)</td>
<td>Fibrous</td>
<td>Protoplasmic</td>
</tr>
<tr>
<td>Oligodendroglia (probably ectodermal)</td>
<td>Interfascicular</td>
<td>Perineuronal (satellite)</td>
</tr>
<tr>
<td>Microglia (probably mesodermal)</td>
<td>Present (no subdivision)</td>
<td>Present and numerous (no subdivision)</td>
</tr>
</tbody>
</table>

Coming Together: The Fruit of Penfield’s Spanish Expedition

When he initially used Río-Hortega’s stains, before leaving for Madrid, Penfield explained the results were, “very exciting, but also very confusing. What I saw was difficult to interpret” [79]. In a short 6 months in Madrid, not only did Penfield perfect a specific stain for oligodendroglia and describe this “third element” in the seminal 1924 Brain paper, but with Río-Hortega also moved from “pure” neuroscience to neuropathology in studying the reaction of glial cells to injury. In an article entitled “Cerebral cicatrix: the reaction of neuroglia and microglia to brain wounds” [73], they provide several observations regarding cellular changes following simple stab wounds:

The formation of a simple cicatrix in the brain presents the following stages: The first cellular change is observed in microglia cells which begin their phagocytic activity early and continue it for a long period. Later, the neuroglia astrocytes about the wound become swollen and those closest to the area of destruction or to obliterated vessels undergo clasmatodendrosis. There follows rapid amitotic division of the other astrocytes and the cells then become fibrous and arrange themselves typically in a radial fashion about the wound. Most of their expansions, and particularly the robust ones, are arranged like the spokes of a wheel with the site of the former stab as the hub…. A connective tissue core forms at the center, connective-tissue collagen fibrils are laid down and the wound contracts [73].

It is noteworthy that their basic idea of these injury stages and formation of the “cicatrix” (contracting scar), is essentially correct. Compare, for example, a modern neuropathology textbook:

In regions of tissue damage hematogenous monocytes infiltrate the CNS and phagocyte dead cells and necrotic debris. Swelling of astrocytes is a relatively rapid response. With time, reactive astrocytes proliferate and insinuate long cytoplasmic processes into the adjacent brain parenchyma, which appear as fibrils in appropriately stained preparations [92].

Others have recently verified “clasmatodendrosis,” their term for the loss of distal astrocytic processes, in degenerative disorders such as Alzheimer’s disease [93–95]. Though the details of gliosis and microglial response were not complete, the numerous observations and conjectures put forth by Río-Hortega and Penfield in this single publication are astonishing. “Cerebral cicatrix” [73] was actually intended by Penfield to be a combined publication with his 1924 Brain paper. The latter paper was to be a combined publication with
the former. However, “Pío procrastinated. This was his old-time enemy. I could not get our results into print for 3 years, not until 1927” [79].

In 1927, back at the New York Presbyterian Hospital, along with his research associate William V. Cone (Fig. 1.27), Penfield decided to write a “textbook on the general principles of neuropathology without describing specific diseases” [79]. (Cone was actually a remarkably astute innovator and scholar whom, if not for his reluctance to write papers, would undoubtedly be widely known to every modern neurosurgeon [96].) Penfield thought that moving beyond a simple description of various diseases to a mechanistic description of disease, pathophysiology was an essential step in the eventual treatment of various neurological diseases. However, Penfield “realized, far too often, that someone else, somewhere in the world, could write a better chapter. I wrote to several to see if they would do a chapter for us. I was surprised when the invitations were readily accepted, since my name carried no prestige as editor” [79]. Numerous sources [97–100] belie this self-effacing claim: Penfield was widely known and respected, even in 1927, by many of the most eminent scientists of the day. In any event, all of his requests to contribute to Cytology and Cellular Pathology of the Nervous System were accepted, save for Cajal who “alone refused, saying he had advancing arteriosclerosis, the histologist’s way of describing old age” [79]. Río-Hortega also gave Penfield pause, for he had a habit of not responding to letters and telegrams. Penfield describes bombarding Río-Hortega “with letters and finally, received a telegram
from him followed by a letter. ‘Of course I will write for your book. How could you think otherwise’” [79].

Cytology, dedicated to Cajal, was finally published in 1932 with 26 eminent contributors. It proved to be an instant and influential success. It was the first tome written on neuropathology from a basic science perspective, a common staple of many pathology texts written today both for graduate and medical study. Penfield describes the reaction he received after the first edition went out of print:

> When, eventually, the first edition of this reference book went out of print, I received letters of inquiry from all over the world. But I was too busy making clinical use of what I had learned to undertake a second edition. At long last, in 1965, Hafner, New York, reprinted it without change [79].

> For such a text to be reprinted in original form more than 30 years after its first publication is a testament to its lasting influence.

Penfield credits the time he spent in Europe, specifically in Madrid, providing him the “keys to understanding” [79]. At a time when glial cells were just being described and differentiated by Río-Hortega and himself, Penfield immediately studied their reaction to injury, and their potential role in epilepsy. In his autobiography No Man Alone, he stated, “if one desired to throw new light on the effect of disease, or injury, and on the process of healing in the brain, the best hope lay in the study of the nonnervous cells, using Hortega’s little-tried methods” [79]. More than 80 years after this statement was made, recent evidence is accumulating for a critical glial contribution to epilepsy [75,101].

Penfield was humble in acknowledging his mentors. He credited Sherrington with influencing his scientific thinking “more than anyone else” [102], saying “I looked through his eyes and came to realize that here in the nervous system was the great unexplored field—the undiscovered country in which the mystery of the mind of man might someday be explained” [79]. In the obituary he wrote for Río-Hortega in 1945, Penfield makes no mention of his own role in the authentication of oligodendroglia or the elucidation of microglia, giving full credit to Río-Hortega [103].

In deep irony, in the late 1920s Penfield’s older sister Ruth Inglis developed an oligodendroglioma, a tumor of the same cell type that Penfield had substantiated in Madrid. Interestingly, on describing a tumor resection in a letter to his mother in 1921, Penfield had stated, “Brain surgery is a terrible profession. If I did not feel it would become very different in my lifetime, I should hate it” [79]. Despite craniotomies for resection of her tumor by Penfield on December 11, 1928 and for recurrence by Harvey Cushing on November 6, 1930, Ruth ultimately died in September 1931.

This vignette outlining Penfield’s contributions to the elucidation of Cajal’s “third element” provides a wonderful example of critical neuroscience research performed by a neurosurgeon. Penfield later made seminal contributions to epilepsy surgery, the mapping of the intact human brain, and the founding of the Montreal Neurological Institute [97–100,104,105]. As an Oslerian “medico-chirurgical neurologist” [98], Penfield indeed embodied the ultimate combination of neuroscientist and neurosurgeon. When comparing physician investigators to pure scientists, Penfield offers advice, that is, as true today as it was then: “We have our practical purposes. We must select our weapons and plan our researches with the patient and his unique problems in mind” [79].
Could the “something else” needed to pull together disparate facts, harmonize apparent contradictions, and put an end to our journeys down blind alleys just be the physiological properties of that other cell population of the brain, the glia? [106]

Despite Río-Hortega’s worldwide recognition for describing oligodendrocytes and microglia, his dispute with Cajal undermined his reputation in Spain. Ultimately a reconciliation between Río-Hortega and Cajal took place in 1928, when Cajal was 77 years [107]. Cajal passed away in 1934, and Río-Hortega continued his research until his death in 1945 [85].

In 1952, Alan Hodgkin and Andrew Huxley published a series of classic papers in which they described, analyzed, and modeled the ionic conductances occurring during the nerve action potential, a seminal event for modern neurophysiology [108–114]. The modern era in glial physiology began with several key discoveries in the late 1950s and 1960s. Walter Hild and colleagues obtained microelectrode recordings from cultured astrocytes for the first time in 1958 [115], and demonstrated electrophysiological properties distinct from neurons in 1962 [116]. Already in 1961, Robert Galambos advanced a “glial-neural theory of brain function” [106]. In 1965, Leif Hertz was the first to emphasize the importance of glia in uptake of extracellular potassium released by active neurons [117]. The Swedish neurophysiologist Holger Hydén described “a two-cell collaboration responsible for brain activity” in 1960 [118], and together with his colleagues demonstrated alterations in gene expression in the neuron–glia unit during learning [119], degenerative brain disease [120], and exposure to psychoactive drugs [121,122]. The great Stephen Kuffler (Fig. 1.28), working with David Potter, showed that glial cells have lower resting membrane potential than neurons and did not generate action potentials in 1964 [123]. Later, working with Richard Orkand

FIGURE 1.28  Stephen W. Kuffler (1913–80).
and John Nicholls, Kuffler demonstrated electrical coupling between glial cells in 1966 [124]. These experiments also led to formulation of the potassium spatial buffering hypothesis [125]. Milton Brightman and Tom Reese identified structures connecting astrocytes (now known to be gap junctions) in 1969 [126].

Nevertheless, glial cells were still thought to be passive participants in CNS function, playing a supportive role. However, in 1984 the research teams of Helmut Kettenmann and Harold Kimelberg identified glutamate and GABA receptors in astrocytes and oligodendrocytes [127–129]. In the 1980s, Glenn Hatton and colleagues showed that pituicytes (pituitary astrocytes) actively modulate hormone secretion, a direct glial–neuronal interaction in the neurohypophysis [130]. In 1990, Ann Cornell-Bell and colleagues found that networks of astrocytes can communicate via propagating calcium waves [131]. In 1994, Maiken Nedergaard, Philip Haydon, and Vladimir Parpura discovered that astrocytes can stimulate calcium elevations in adjacent neurons when grown in co-culture [132,133]. Thus, bidirectional communication between astrocytes and neurons was already clearly documented in the mid-1990s. Further work, some summarized later in this book, has indicated that astrocytes express a multitude of neurotransmitter receptors and ion and water channels; that there is marked astrocyte heterogeneity within and between distinct brain areas; and that there are major new roles for astrocytes in both physiology and disease.

References
[47] Schultz M. Observationes de retinae structura penitiori. Bonn, Germany: Published lecture at the University of Bonn; 1859.
1. HISTORY OF ASTROCYTES

[66] Deleted in Review.
REFERENCES

[86] Rezaie P, Male D. Mesoglia & microglia—a historical review of the concept of mononuclear phagocytes within


[88] Robertson WF. The normal histology and pathology of the neuroglia (in relation specially to mental diseases).
J Ment Sci 1897;43:733–52.

[89] Robertson WF. A microscopic demonstration of the normal and pathological history of mesoglia cells. J Ment
Sci 1900;46:724.

[90] Achúcarro N. Nuevo método para el estudio de la neuroglia y del tejido conjuntivo. Bol Soc Esp Biol
1911;1:139–41.


[93] Sahlas DJ, Bilbao JM, Swartz RH, Black SE. Clasmatodendrosis correlating with periventricular hyperintensity


brain barrier and glial cells in white-matter lesions in cerebrovascular and Alzheimer’s disease patients.


[99] Preul MC, Feindel W. “The art is long and the life short”: the letters of Wilder Penfield and Harvey Cushing.

[100] Preul MC, Feindel W. Origins of Wilder Penfield’s surgical technique: the role of the “Cushing ritual” and

2005;11(9):973–81.


[104] Almeida AN, Martínez V, Feindel W. The first case of invasive EEG monitoring for the surgical treatment of


Superior de Investigaciones Científicas; 1985.


[110] Hodgkin AL, Huxley AF. The dual effect of membrane potential on sodium conductance in the giant axon


[112] Hodgkin AL, Huxley AF. Currents carried by sodium and potassium ions through the membrane of the giant

[113] Hodgkin AL, Huxley AF. Movement of sodium and potassium ions during nervous activity. Cold Spring

[114] Hodgkin AL, Huxley AF, Katz B. Measurement of current-voltage relations in the membrane of the giant

[115] Hild W, Chang JJ, Tasaki I. Electrical responses of astrocytic glia from the mammalian central nervous system

ASTROCYTES AND EPILEPSY
1. HISTORY OF ASTROCYTES