CHAPTER

Cajal and the discovery of a new artistic world: The neuronal forest

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Abstract

The introduction of the staining method of Camillo Golgi in 1873 represented a giant step for neuroscience. Prior to this development, the visualization of neurons with the available histological techniques had been incomplete; it was only feasible to observe the cell body and the proximal portions of the dendrites and axon. However, with the Golgi method it was possible to observe neurons and glia with all their parts (cell body, dendrites, and axon in the case of neurons; cell body and processes in the case of glia). Due to the advantages of this method, all of a sudden it was possible to begin studying one of the great mysteries and critical issues of the organization of the nervous system—the tracing of the connections between neurons. Nevertheless, this method was not fully exploited until Santiago Ramón y Cajal arrived on the scene in 1888. It should be noted that, in Cajal's day, drawing was the most common method of describing microscopic images in the absence of the highly developed microphotography and other imaging techniques commonly available in today's laboratories. As a consequence, most scientific figures presented by the early neuroanatomists were their own drawings, providing an outlet for these scientists to express and develop their artistic talent. In the hands of Cajal, the Golgi method represented not only the principal tool that was to change the course of the history of neuroscience but also the discovery of a new artistic world, the neuronal forest.

Keywords

cerebral cortex, Golgi technique, drawing of neural elements, connections of neurons, history of neuroscience

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1 INTRODUCTION

Throughout the nineteenth century and at the beginning of the twentieth century, the study of the structure of the nervous system was marked by two milestones: the development of light microscopy and the discovery and improvement of anatomical methods. During this period, scientists sought to develop appropriate methods to analyze different aspects of the structure and function of the nervous system. Some of these methods were discovered by chance, whereas others were designed to resolve a given problem. Nevertheless, a good lesson from these early days is that the development of science depends not only on the methods available but also on the way that they are exploited. Often, methods were available to scientists but were not always used to their full potential until a researcher made an important discovery or an astute interpretation that generated new concepts. This was the case of the method discovered by Camillo Golgi (1843–1926), the *reazione nera* (Golgi method), which remained unexploited for many years until Santiago Ramón y Cajal (1852–1934) appeared, changing the course of the history of neuroscience. This period was important not only scientifically but also from an artistic point of view, since the brain was revealed to be a truly neuronal forest, where the beauty of the forms and groups of cells captivated both the scientific community and the general public alike.

This chapter first addresses the discovery of the Golgi method, thereafter the appearance of Cajal, and finally the artistic skills of the early neuroanatomists are emphasized, using their own words to describe the artistic emotions that they experienced when visualizing the neural elements. In particular, it focuses on the intriguing story and discoveries of Cajal himself, who, as a boy, wanted to become an artist through painting, but was prevented from doing so by his father until years later when he discovered a new artistic world, the neuronal forest.

2 THE GOLGI METHOD

On February 16, 1873, a revolution began in the world of neuroscience with the discovery of a new method of staining of the nervous system, the *reazione nera*, by the renowned Italian scientist Camillo Golgi (1843–1926). On this date, Golgi wrote the following letter to his friend Niccolò Manfredi (Mazzarello, 1999, p. 63):

> *I spend long hours at the microscope. I am delighted that I have found a new reaction to demonstrate even to the blind the structure of the interstitial stroma of the cerebral cortex. I let the silver nitrate react on pieces of brain hardened in potassium dichromate. I have obtained magnificent results and hope to do even better in the future.*

This technique allowed neurons and glia to be visualized, labeling them black (hence *reazione nera*) simply after "prolonged immersion of the tissue, previously hardened with potassium or ammonium dichromate, in a 0.50 or 1.0% solution of silver nitrate." The method was published in the *Gazzetta Medica Italiana* on August 2, 1873 (Golgi, 1873: *Sulla struttura della sostanza grigia del cervello* ("On the structure of the gray substance of the cerebrum")) and later this method was named "the Golgi method" in honor of its discoverer. For the first time, it was possible to observe neurons and glia in a histological preparation with all their parts (cell body, dendrites, and axon in the case of neurons; cell body and processes in the case of glia).

Although it was possible to completely visualize the morphology of a neuron before 1873 using the Deiters' method of mechanical dissociation (1865), this technique was very difficult to perform (Fig. 1). In the words of Cajal (1917, p. 71)[1]:

> *The procedure of mechanical dissociation . . ., applied to the analysis of the ganglia, of the retina, of the spinal cord or of the brain, the delicate operation of detaching the cells from their matrix of cement and of unravelling and extending their branched processes with needles, constituted a task for a Benedictine. What a delight it was when, by dint of much patience, we could completely isolate a neuroglial element, with its typical spider-like form, or a colossal motor neuron from the spinal cord, free and well separated with its robust axis cylinder and dendrites! What a triumph to capture the bifurcation of the single process [axon] from a dissociated spinal ganglia, or to clear a pyramidal cell from its neuroglial bramble thicket, that is, the noble and enigmatic cell of thought!*

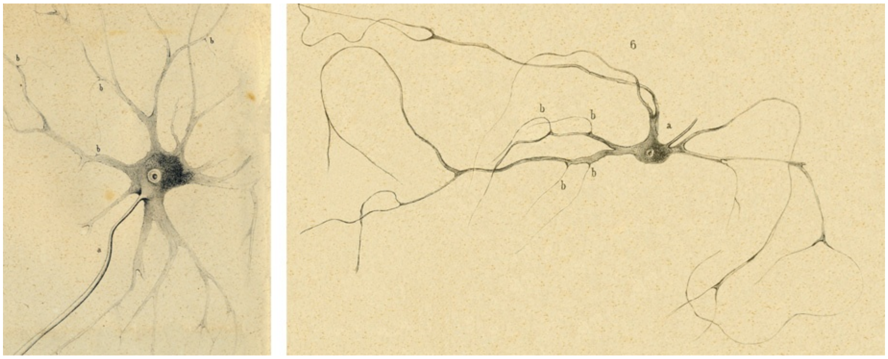

FIGURE 1

Drawings made by Otto Friedrich Karl Deiters (1834–1863) to illustrate nerve cells (spinal cord of the ox). Method of mechanical dissociation. He distinguished a principal axon (a) originated from the soma and several thin axons that arise from the dendrites (b, "second axonic system"). According to Cajal, the erroneous interpretation of this second axonic plexus was the germ of the reticular theory (see DeFelipe, 2010a). Taken from Deiters (1865).

[1]The author of the present chapter has translated any passages that were originally only available in Spanish.

FIGURE 2

The first illustration by Golgi of a Golgi-impregnated preparation of the nervous system. “Semi-schematic drawing of a fragment of a vertical section of the olfactory bulb of a dog”.

Taken from Golgi (1875).

Moreover, the advantage of the Golgi method was that it allowed the observation of many cells at once in a given section and *in situ*, without any possible artifacts that might be introduced by dissociation. Another important advantage of the Golgi method was that only a small portion of the neurons in a given preparation were stained, permitting individual neurons to be examined with the greatest morphological detail, allowing dendritic spines to be discovered. Thus, it was at last possible to characterize and classify neurons, and to potentially study their connections (Fig. 2). These characteristics of the Golgi method gave rise to another great advance, namely that of tracing the first accurate circuit diagrams of the nervous system (e.g., DeFelipe, 2002a,b).

An interesting aspect of these early days of the history of neuroscience is that for a long time after the discovery of the Golgi method, the vast majority of the scientific community failed to make the most of the opportunities it presented. Indeed, this method was not commonly referred to in most of the contemporary texts available at that time. Therefore, the slow progress in microanatomy was due not only to the lack of appropriate methods but also to the inability to exploit the methods available.

3 CAJAL ARRIVES ON THE SCENE

The Golgi method was not fully exploited until Santiago Ramón y Cajal (Fig. 3) arrived on the scene. He was born on May 1, 1852 in Petilla de Aragón, a small village located in Navarre (North of Spain) and died in Madrid on October 17, 1934. He studied medicine at the University of Zaragoza and was Professor of Anatomy and Histology at the Universities of Valencia, Barcelona, and Madrid. In addition to the many scientific articles and books he published, he also played a significant role in the development of science and culture in Spain, as shown by the publication of several nonscientific books (e.g., *Cuentos de vacaciones* ["Vacation stories"], Fortanet, Madrid, 1905) and two scientific magazines: *Revista trimestral de histología normal y patológica*, in 1888, and *Revista trimestral micrográfica*, in 1896 (later named *Trabajos del laboratorio de investigaciones biológicas de la Universidad de Madrid*). He was also a pioneer in the development of color photography and his book, *La fotografía de los colores* ("The photography of colors") (Moya, Madrid, 1912), is a masterpiece on the subject.

Cajal became involved in the study of the nervous system using the Golgi method after a meeting with Luis Simarro (1851–1921), a psychiatrist and neurologist who was also an enthusiast of histology. In 1887, Cajal visited the private laboratory of Simarro, who showed him a Golgi-impregnated preparation. Cajal was fascinated by this marvelous staining method and he immediately started using it to analyze practically the entire nervous system of several species. In his autobiography *Recuerdos de mi vida-Historia de mi labor científica* ("Recollections of my life—The story of my scientific work") (1917, p. 76), Cajal wondered why the

FIGURE 3

Cajal in his laboratory in Valencia (1885).

method of Golgi had not led to an explosion of excitement in the scientific community:

> *I expressed in former paragraphs the surprise which I experienced upon seeing with my own eyes the wonderful revelatory powers of the chrome-silver reaction and the absence of any excitement aroused in the scientific world by its discovery. How can one explain such strange indifference? Today, when I am better acquainted with the psychology of scientific men, I find it very natural.... Out of respect for the master, no pupil is wont to use methods of investigation which he has not learned from him. As for the great investigators, they would consider themselves dishonoured if they worked with the methods of others.*

The historical moment when Cajal discovered the properties of the Golgi method is beautifully described in several of his writings, especially in his classic book *Textura del sistema nervioso del hombre y de los vertebrados* ("Texture of the nervous system of man and the vertebrates") (Cajal 1899–1904) and in particular in the French version *Histologie du système nerveux de l'homme et des vertébrés* (Histology of the nervous system of man and vertebrates) (Cajal 1909–1911), which represents an excellent example of his typical vivid writing style and enthusiasm (DeFelipe, 2010a):

> *In summary, a method was necessary to selectively stain an element, or at most a small number of elements, that would appear to be isolated among the remaining invisible elements. Could the dream of such a technique truly become reality, in which the microscope becomes a scalpel and histology a fine [tool for] anatomical*

dissection? A piece of nervous tissue was left hardening for several days in Müller's pure liquid [potassium dichromate] or in a mixture of this [fixative] with osmic acid. Whether it was the distraction of the histologist or the curiosity of the scientist, the tissue was then immersed in a bath of silver nitrate. The appearance of gleaming needles with shimmering gold reflections soon attracted the attention. The tissue was cut, and the sections were dehydrated, cleared, and then examined [with the microscope]. What an unexpected spectacle! On the perfectly translucent yellow background sparse black filaments appeared that were smooth and thin or thorny and thick, as well as black triangular, stellate or fusiform bodies! One would have thought that they were designs in Chinese ink on transparent Japanese paper. The eye was disconcerted, accustomed as it was to the inextricable network [observed] in the sections stained with carmine and hematoxylin where the indecision of the mind has to be reinforced by its capacity to criticize and interpret. Here everything was simple, clear and unconfused. It was no longer necessary to interpret [microscopically] the findings to verify that the cell has multiple branches covered with 'frost,' embracing an amazingly large space with their undulations. A slender fibre that originated from the cell elongated over enormous distances and suddenly opened out in a spray of innumerable sprouting fibres. A corpuscle confined to the surface of a ventricle where it sends out a shaft, which is branched at the surface of the [brain], and other cells [appeared] like comatulids or phalangidas.[2] The amazed eye could not be torn away from this contemplation. The technique that had been dreamed of is a reality! The metallic impregnation has unexpectedly achieved this fine dissection. This is the Golgi method! … whose clear and decisive images enable us to cast off the famous net of Gerlach, the [dendritic] arms of Valentin and Wagner, as well as many another fanciful hypothesis.

During this period, Cajal brilliantly described the microorganization of almost every region of the central nervous system, and the results were summarized in the *Textura*. Furthermore, from the very onset of his studies with the Golgi method (Cajal, 1888; Fig. 4), Cajal made important discoveries and formulated fundamental theories regarding the development of the nervous system. For example, he discovered and named the axonal growth cone (*cono de crecimiento*) (Cajal, 1890) and also devised the hypothesis of chemotaxis or chemotactism (Cajal, 1893), later to be called neurotropism. At present, these early contributions represent two of the most exciting fields of research in neuronal development. Nevertheless, Cajal is better known for his vivid discussions in support of the Neuron Doctrine, which represented a radical change in the understanding of how the nervous system is organized, a subject that is discussed in Section 4.

[2]Comatulids are marine crinoid invertebrates like sea lilies and feather stars. Phalangidas (or opiliones), also known as water harvestmen, are arachnids that superficially resemble true spiders, but they have small, oval-shaped bodies and long legs. Cajal is probably referring to some neuroglial cells that, when stained with the Golgi method, have a morphology which resembles these invertebrates.

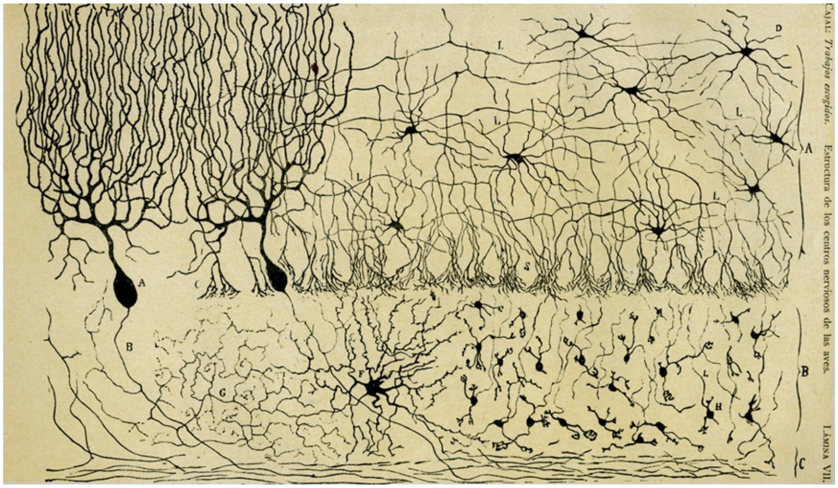

FIGURE 4

First illustration by Cajal of a Golgi-impregnated preparation of the nervous system (Cajal, 1888), whose legend states: "Vertical section of a cerebellar convolution of a hen. Impregnation by the Golgi method. A, represents the molecular zone, B, designates the granular layer, and C the white matter." In the text, Cajal said: "...the surface of [the dendrites of Purkinje cells] appears to be covered with thorns or short spines... (At the beginning, we thought that these eminences were the result of a tumultuous precipitation of the silver but the constancy of its existence and its presence, even in preparations in which the reaction appears to be very delicate in the remaining elements, incline us to believe this to be a normal condition)."

4 DRAWING OF NEURAL ELEMENTS: WHEN SCIENCE WAS ART

A remarkable aspect of the history of neuroscience is that, in Cajal's day, drawing was the most common method of describing microscopic images in the absence of the highly developed microphotography and other imaging techniques commonly available in today's laboratories. In general, the scientists used freehand drawings with various types of pencils, pens, watercolor dyes, Indian ink, and other common media, used separately or in a variety of combinations. These were drawn on different kinds of paper or cardboard, either directly or with the aid of a *camera lucida*. The type of camera is a plotting device attached to the microscope that allows the observer to outline the optical microscope image that is projected upon a drawing table. Thus, with this instrument the observer can visualize the paper, the pencil, and the histological preparation at the same time, allowing an accurate drawing of the objects to be produced. Readers interested in the various methods for reproducing microscopy images and the material used to generate these drawings can consult the work of

Cajal itself, in particular, his *Manual de histología normal y de técnica micrográfica* ("Handbook of Normal Histology and Micrographic Technique"), first published in 1889 (Cajal, 1889) and reedited over the years with additional and corrected content. An English version of this work was published with the help of his disciple, Jorge Francisco Tello (1880–1958) (Cajal and Tello, 1933).

Nevertheless, there was no section in most of Cajal's scientific articles describing the methods used in detail, and he reported the use of the camera lucida only occasionally. For example, in the paper of 1891 "*Sur la structure de l'écorce cérébrale de quelques mammifères*" ("On the structure of the cerebral cortex of certain mammals") (Cajal, 1891, p. 173), in the "Explanation of the plates," he wrote:

> *The majority of our figures have been made using the Zeiss camera lucida, with the objective C of that manufacturer, and employing sometimes the ocular 4, sometimes the ocular 2. Figs. 4 and 5 have been made with the very powerful E and Zeiss 1.30 apochromatic objectives.*

However, according to several testimonies of people who knew Cajal, it seems that he preferred direct drawing and only used the *camera lucida* as a last resort. Julian de la Villa, one of his former students, wrote the following paragraph on the occasion of the first centenary of the birth of Cajal (De La Villa, 1952, p. 24):

> *...the drawing was generated directly from the preparation; with the microscope on his left and the paper on his right, exact reproductions of [the preparations] began to appear. Although the camera lucida was known to him, because it was cumbersome to use, he preferred to avoid it.*

Also, it is important to point out that many of Cajal's illustrations were composite drawings, in particular the figures showing several cells. This is not only obvious by looking at the histological preparations that Cajal made himself and are now housed at the Cajal Institute (http://www.cajal.csic.es/ingles/legado.html) but also because Cajal himself stated this to be the case in some publications, such as "*La rétine des vertébrés*" ("The retina of vertebrates") published in 1893 (Cajal, 1893). In the "Explanation of the plates" section of this article (p. 247), he wrote:

> *The majority of our figures have been made using the camera lucida of Abbe with the Zeiss C objective. We have reproduced, in the figures of large size, cells found in different sections of the retina of the same animal. However, they are represented as if they were seen in a single plane.*

Thus, many of the illustrations by Cajal are composite drawings that synthetically show the complex texture of a given region of the nervous system. Although this method of illustrating the microscopic observations led to some skepticism (see Section 5), this really was one of the most important contributions of Cajal, as it required a combination of artistic talent and interpretation of the microscopic images in order to highlight the key features of the structure being studied through the exact copy of the most relevant elements of the microscopic images.

FIGURE 5

Watercolor by Cajal in 1865.

Taken from Cajal, A., 2007. María de los Ángeles Ramón y Cajal Junquera. In: Paisajes Neuronales: Homenaje a Santiago Ramón y Cajal (DeFelipe J., Markram H, and Wagensberg J). CSIC, Madrid.

As a consequence, drawing of neural elements became an art, providing an outlet for the early neuroanatomists to express and develop their artistic talent. This was the case of Cajal, whose boyhood dreams of becoming an artist (Fig. 5) were thwarted by his father's misgivings. He spoke about this in an interview in 1900 (M.ª Ángeles Ramón y Cajal; Speech presented in 1984 at the Ateneo de Madrid with the title *Coloquio sobre Ramón y Cajal en el 50º aniversario de su fallecimiento* ("Colloquium: Ramón y Cajal on the 50th anniversary of his death")):

> *Undoubtedly, only artists devote themselves to science .... I realized that if I wanted to make a name for myself as a painter, my hands needed to become precision instruments. I owe what I am today to my boyhood artistic hobbies, which my father opposed fiercely. To date, I must have done over 12,000 drawings. To the layman, they look like strange drawings, with details that measure thousandths of a millimetre, but they reveal the mysterious worlds of the architecture of the brain... Look [Cajal said to the journalist, showing one of his drawings] here I am pursuing a goal of great interest to painters: appreciating line and colour in the brain.*

Later, in his autographical *Recuerdos de mi vida-Mi infancia y juventud* ("Recollections of my life—My childhood and youth") (1901, pp. 84–86), Cajal shares with us the following amusing anecdote:

> *...my father, who was already averse to all kinds of aesthetic tendencies ... and wearied, no doubt, of depriving me of pencils and taking away my drawings, and*

> *seeing the ardent vocation towards painting which I exhibited, he decided to determine whether those scrawls had any merit promising their author the glories of a Velázquez .... As there was no one in the town sufficiently qualified in the art of drawing, the author of my days turned to a plasterer and decorator from afar, who had arrived in Ayerbe around that time ... to paint the walls of the church, damaged and scorched by a recent fire. ... I timidly displayed my picture ... the house painter looked at it and looked at it again, and after moving his head significantly and adopting a solemn and judicial attitude he exclaimed:*
>
> *"What a daub! Neither is this an Apostle, nor has the figure proportions, nor are the draperies right... this child will never be an artist." In fact, the opinion of this dauber of walls was received in my family like the pronouncement of an Academy of Fine Arts. It was decided, therefore, that I should renounce my madness for drawing and prepare myself to follow a medical career.*

It is also interesting to draw attention to what Cajal wrote in *Recuerdos de mi vida-Historia de mi labor científica* ("Recollections of my life—The story of my scientific work") (1917, pp. 155–156), referring to the intellectual pleasure he felt when observing and drawing from his histological preparations providing a fascinating bridge between science and art:

> *My work began at nine o'clock in the morning and usually lasted until around midnight. Most curiously, my work caused me pleasure, a delightful intoxication, an irresistible enchantment. Indeed, leaving aside the egocentric flattery, the garden of neurology offers the investigator captivating spectacles and incomparable artistic emotions. In it, my aesthetic instincts were at last fully satisfied.*

Who could have imagined that the forest that Cajal painted when he was only 13 years old (shown in Fig. 5) would later lead on to drawings illustrating the neuronal forest that constitutes the brain? Cajal had found a new world of infinite forms with an extraordinary beauty in the study of the brain. These artistic skills and emotions were also shared by Pío del Río-Hortega (1882–1945) and Fernando de Castro (1896–1967), as well as by other famous disciples of Cajal and many other important pioneers in neuroscience, including Otto Friedrich Karl Deiters (1834–1863), Rudolf Albert von Kölliker (1817–1905), Theodor Meynert (1833–1892), Louis Antoine Ranvier (1835–1922), Camillo Golgi (1843–1926), Gustav Magnus Retzius (1842–1919), Aleksander Dogiel (1852–1922), and Alois Alzheimer (1864–1915). Of course, in addition to the Golgi method, other staining techniques were available in Cajal's time, and many others were developed over the years, using different fixation and staining protocols to analyze specific architectonic aspects of the nervous system, and the morphology and cytology of neurons and glia. A variety of chemicals (pyridine, methylene blue, mercuric chloride, osmic acid, silver solutions, etc.) have been used in different procedures to visualize particular elements selectively. Examples include methods to visualize different types of glial cells and not neurons, and other procedures to label mainly neurons with their dendritic and axonal processes, as well as the techniques that stain the neurofibrils but not other cytoplasmic organelles. Other selective staining methods were developed that allowed the examination of the different types of organelles in the perikaryon (e.g., Nissl bodies, mitochondria, neurofibrils, Golgi apparatus, inclusions such as pigments, fat, and lipids). The collective work of many scientists made the improvement of these methods possible and facilitated the analysis of all regions and cells of the nervous system from many different perspectives. Over the years, this work has unveiled all that we now know about the structure of the nervous system. As Peters et al. (1991, p. 14) summarized in the classic book *The Fine Structure of the Nervous System*: Our image of the nerve cell at the light microscope level is like a collage of many overlapping views, patiently accrued during a century of study.

5 SCIENTIFIC "ART" AND SKEPTICISM

An interesting point regarding the representation of the microorganization through drawings is that clearly the observers did not reproduce the entire field of the histological preparations that they viewed through the microscope, but rather they drew only those elements that they thought were important for what they wanted to describe. As such, these illustrations were not necessarily free of technical errors, and they may have been subject to the scientists' own interpretations. Indeed, this subjectivity made fellow scientists reluctant to accept their findings at times.

This skepticism is illustrated well by the response speech that Professor Arthur van Gehuchten (1861–1914) delivered at an event marking his 25 years of teaching service at the University of Louvain. In this speech, van Gehuchten describes the historic moment when Rudolf Albert von Kölliker (1817–1905)—one of the most influential neuroscientists of the time—discovered Cajal in the famous Congress of the German Society of Anatomy, held at the University of Berlin in October 1889. The section of the speech where van Gehuchten (1913, pp. 32–33) describes this event highlights the difficult situation that Cajal (and other scientists) had to face:

> *The facts described [by Cajal] in his first publications were so strange that the histologists of the time . . . received them with the greatest skepticism. The distrust was such that, at the anatomical congress held in Berlin in 1889, Cajal, who afterwards become the great histologist of Madrid, found himself alone, provoking around him only smiles of incredulity. . . I can still see him taking aside Kölliker, who was then the unquestioned master of German histology, and dragging him into a corner of the demonstration hall to show him under the microscope his admirable preparations, and to convince him at the same time of the reality of the facts which he claimed to have discovered. This demonstration was so decisive that a few months later the Würzbourg histologist [Kölliker] confirmed all the facts stated by Cajal.*

Kölliker was so impressed with the findings of Cajal that he stated (Cajal, 1917, p. 147):

> *The results that you have obtained are so beautiful that I am planning to immediately undertake a series of confirmatory studies by adopting your methodology. I have discovered you, and I wish to make my discovery known in Germany.*

Cajal, encouraged by the success of the Congress of Anatomy and by the beauty of the histological preparations, was trapped forever by the fascinating world of the nervous system.

6 INTERPRETATION OF THE MICROSCOPIC WORLD

Although all scientists of Cajal's era had the same microscopes and produced similar histological preparations, the crucial difference was in the pursuit and interpretation of details that went unnoticed by others. A remarkable example was the different interpretation of the connections between neurons and the debate about the existence of dendritic spines. At that time, the most common view regarding the organization of the nervous system was that it consisted of a diffuse network of nerves formed by the anastomosing branches of nerve cell processes, with the cell somata principally playing a role in nourishment (Reticular Theory).

One year after his meeting with Simarro, Cajal published his first important article based on the results obtained with this method in the avian cerebellum (Fig. 4). In this study titled *Estructura de los Centros Nerviosos de las Aves* ("Structure of the avian nerve centres") (Cajal, 1888), Cajal made two great contributions. First, he described the existence of dendritic spines (which he also named). These elements were considered by Cajal and other scientists to be fundamental structures present in certain neurons (spiny cells, such as pyramidal neurons and Purkinje cells), while others, like Golgi, considered them to be mere histological artifacts (DeFelipe and Jones, 1988). At present, dendritic spines generate particular interest as they are highly plastic and are the main postsynaptic site for excitatory synapses in the cerebral cortex. Second, Cajal confirmed Golgi's conclusion that dendrites end freely. But, in contrast to Golgi, Cajal came to the decisive conclusion that this also applies to axons and their branches. Cajal's early studies with the Golgi method were so decisive for the Neuron Doctrine that they represented the main core of the review published by von Waldeyer-Hartz (1836–1921) in the journal *Deutsche Medizinische Wochenschrift* in 1891. In this article, the term "neuron" was introduced to denominate the nerve cell and, at last, the so-called Neuron Doctrine became popular.

Certainly, the two opposite views regarding the connections of neurons implied rather different functional consequences. According to the Reticular Theory, nerve currents would flow through a continuous network of neuronal processes, whereas for the Neuron Theory these currents passed from one cell to the next by contact "in much the same way that electric current crosses a splice between two wires" (Cajal, 1909–1911) (Fig. 6). Thus, the new ideas about the connections between neurons led to novel theories about the relationship between neuronal circuits and brain function (DeFelipe, 2010b).

Cajal was very proud to be recognized as one of the scientists who had contributed most to the victory of the Neuron Doctrine in its battle against the Reticular Theory. Except for some prestigious researchers, such as Golgi or the well-known

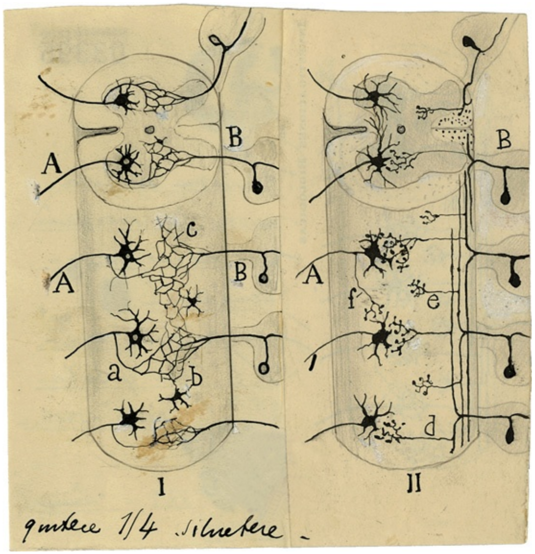

FIGURE 6

Cajal's drawing to explain the differences between the neuron and the reticular theories. The figure legend states "Scheme to compare the concept of Golgi regarding the sensory-motor connections of the spinal cord (I) with the results of my investigations (II). A, anterior roots; B, posterior roots; a, collateral of a motor root; b, cells with a short axon which, according to Golgi, would intervene in the formation of the network; c, diffuse interstitial network; d, our long collaterals in contact with the motor cells; e, short collaterals." This figure was reproduced as Fig. 9 in *Recuerdos de mi vida-Historia de mi labor científica* ("Recollections of my life—The story of my scientific work") (Cajal, 1917).

Franz Nissl (1860–1919; Nissl, 1903), the Neuron Doctrine was by the end of the nineteenth century the most accepted theory to explain the organization of the nervous system, in which the neuron was considered as the anatomical, physiological, genetic, and metabolic unit of the nervous system (Jones, 1994, 2006; Shepherd, 1991). The many, fundamental contributions of Cajal (1933) to the neuron doctrine were summarized by himself in several articles and books, and especially in "*Neuronismo o Reticularismo*" ("Neuronism or Reticularism?" published in 1933).

The misinterpretation of Golgi regarding the connections of nerve cells represents the basis for the popular belief in the scientific community that the contributions of Golgi were mainly only methodological. However, he made many significant discoveries. Perhaps one of the most important was the discovery in 1898 of an "internal reticular apparatus" in the nerve cells (Golgi, 1898), which was later named the Golgi apparatus or Golgi complex in his honor (Bentivoglio, 1999). As occurred with other discoveries at that time, the existence of this organelle was the subject of intense debate since some authors thought that this was an artifact of the method of staining, whereas other scientists believed that it was a real organelle which was found not only in neurons but in most eukaryotic cells as well. Many years later, after the introduction and development of electron microscope techniques in the 1950s, this discovery was confirmed, proving that Golgi was right (Bentivoglio, 1999).

Thus, during this period, the drawings used to illustrate their publications were often considered to be essentially artistic or erroneous interpretations rather than accurate copies of the histological preparations. Indeed, this issue also makes it difficult for us to interpret some of these figures today. This early period represents a captivating page in the history of neuroscience marked by scientific "art" and skepticism, when the drawings were subject to different interpretations and considered to be good or bad depending on the artistic talent of the scientists and their artistic assistants and on their ability to extract the relevant information. Once again, Cajal explained the importance and implications of this in the *Textura* (Cajal 1899–1904, vol. 1, p. X): A good drawing, like a good microscope preparation, is a fragment of reality, scientific documents that indefinitely maintain their value and whose study will always be useful, whatever interpretation they might inspire.

The Golgi method, together with the extraordinary variety of other techniques used at the time to unravel the complex organization of the nervous system, opened up a beautiful microscopic world, with an almost infinite combination of forms and multiple colors. The coming together of art and science was also vividly described by Del Río-Hortega (1933, p. 200):

> *After using a technical process of those that required the careful combination of several complementary colours: red and green, yellow and blue, the histologist finally got a true picture from which three sources of pure emotion could be derived: that which stems from the beauty of the landscape itself, with its polychromatic nature, its tones and [depth]; that which emanates from the observer himself, who feels the hidden satisfaction of achieving his purpose; and that which emerges from the novelty of the details resolved, [that is] the discovery of ignored truths.*

Indeed, as shown in Fig. 7, many of the illustrations of these great scientists and artists can be considered to belong to different artistic movements, such as modernism, surrealism, cubism, abstraction, or impressionism.

7 THE BRAIN AS A NEURONAL FOREST

It does not require a great deal of imagination to see some neurons as trees, such as the pyramidal cells of the cerebral cortex and the Purkinje cells of the cerebellar cortex. Given their high density and arrangement, they seem to constitute a thick forest (Figs. 8 and 9). This is why Cajal, as well as other scientists, often referred to trees and forests in their descriptions of the brain and, in particular, of the cerebral cortex. Another beautiful example is the following comment from Cajal regarding cortical

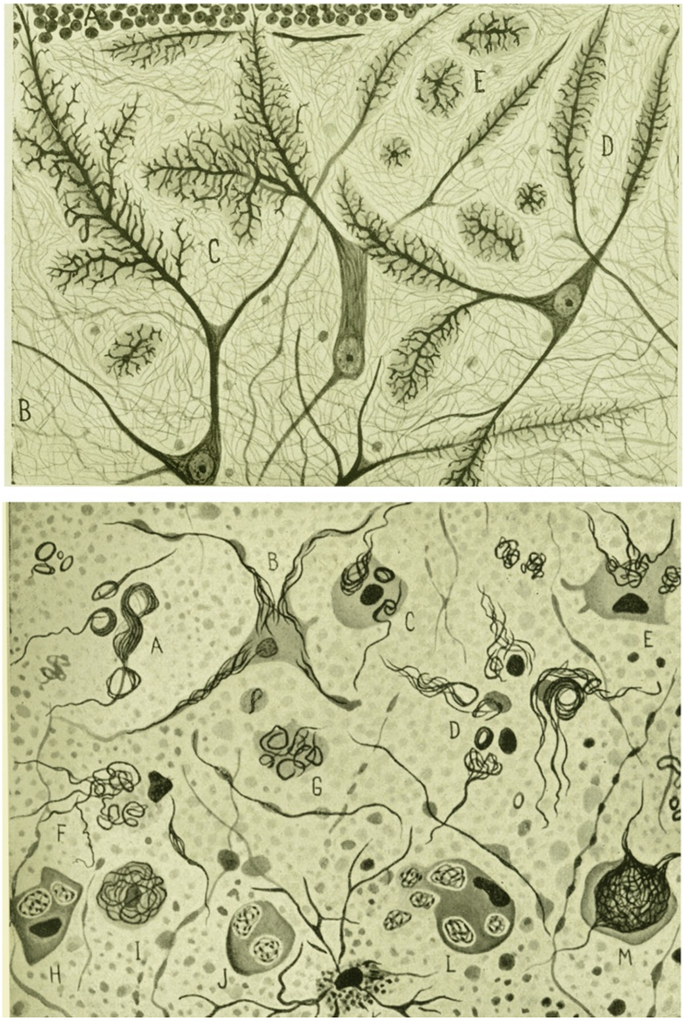

FIGURE 7

Drawings taken from Del Río-Hortega to illustrate different cellular elements of the hippocampal formation and neocortex. Upper panel, cells in the dog fascia dentate (Del Río-Hortega, 1918); lower panel, neuroglial cells of the aging human cerebral cortex (Del Río-Hortega, 1918–1919).

plasticity (Cajal, 1894, pp. 159–160): The cerebral cortex is similar to a garden filled with innumerable trees, the pyramidal cells, which can multiply their branches thanks to intelligent cultivation, send their roots deeper and producing more exquisite flowers and fruits every day.

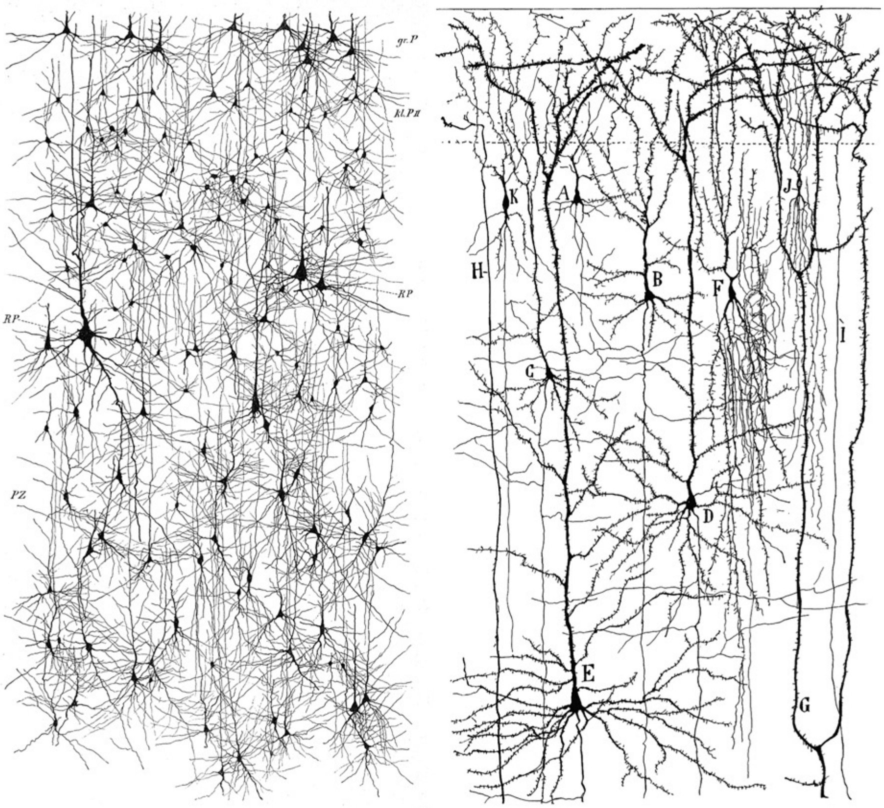

FIGURE 8

Drawings of the human cerebral cortex (Golgi method), illustrating a forest-like appearance.

Taken from von Kölliker (1893) (left) and Cajal (1899) (right).

These neuronal forests truly represent an unlimited source of artistic and poetic inspiration to scientists and others since they reveal a fantastic and virtually unknown world of forms, a brain microuniverse with an aura of mystery (DeFelipe, 2010a). Indeed, Del Río-Hortega (1933, p. 193) described the relationships between neurons, glia, and blood vessels perfectly: In the landscape of the brain, there are endless irrigation canals—blood vessels—and on their banks, the bush-like cells—glia—collaborate in nerve function.

Finally, it is worth pointing out that trees have also served as artistic symbols in a variety of contexts to describe texts or concepts. For example, multicolored drawings of trees have been used to describe cognitive alterations in biblical texts, as can be seen in one of the trees shown in a miniature belonging to the Mozarabic Beato of San Miguel de Escalada (León, Spain), in work by the monk Maius in the tenth century. In this miniature, King Nebuchadnezzar II of Babylon (sixth century BC) is shown

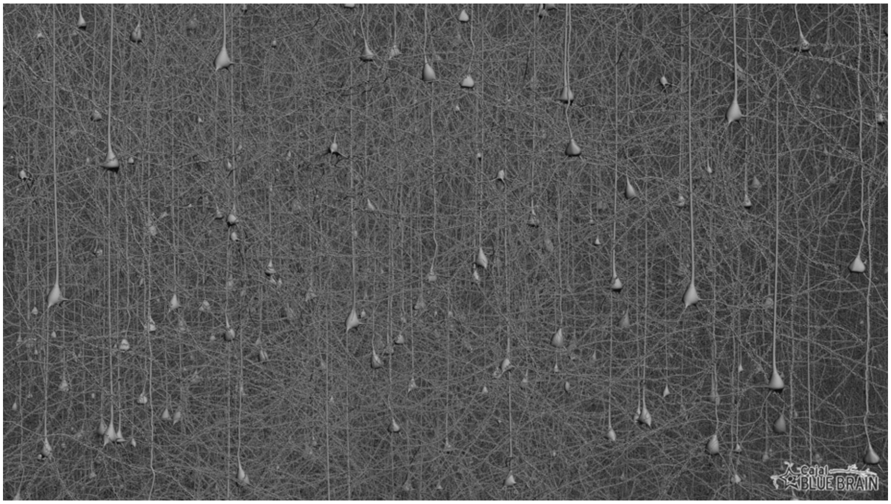

FIGURE 9

Computer-generated image to illustrate the complexity of the cerebral cortex, which resembles a neuronal forest.

This image was taken from the video "Bosque Neuronal" (Cajal Blue Brain Project; http://cajalbbp.cesvima.upm.es/) created (in alphabetical order) by Sofía Bayona, Ruth Benavides-Piccione, Juan Pedro Brito, Eva Cortés, Javier DeFelipe, José Miguel Espadero, Susana Mata, Luis Pastor, Ángel Rodríguez, and Luis Miguel Serrano.

with what might be dementia, eating grass, "like the beasts in the field" and seemingly walking "on all fours," because of the extreme bending of his trunk (camptocormia). Today, it is thought that his condition that might be at least in part due to parkinsonism associated with Lewy body disease (Martín-Araguz, 2006). As a main theme in the illustration, a tree is shown whose trunk represents the kingdom with its inhabitants (the birds in its branches), and a cut at the base of the trunk, possibly showing the risk posed to the kingdom by the king's disease (DeFelipe 2010a; Martín-Araguz, 2006). At present there is considerable interest in examining the possible alterations to the tree-like pyramidal cells and their possible alterations associated with brain disease, as well as their role in memory, learning, and cognition. Here, we can also imagine an appealing bridge between literature, artistic drawings, and neuroscience.

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