

available at www.sciencedirect.comwww.elsevier.com/locate/brainresrev
**BRAIN
RESEARCH
REVIEWS**

Review

A historical reflection of the contributions of Cajal and Golgi to the foundations of neuroscience

Juan A. De Carlos, José Borrell*

Instituto Cajal (CSIC), Avenida del Dr. Arce nº 37, E-28002-Madrid, Spain

ARTICLE INFO

Article history:

Accepted 14 March 2007

Available online 5 April 2007

Keywords:

Cajal

Golgi

Nobel Prize

ABSTRACT

In 1906, the Spaniard Santiago Ramón y Cajal and the Italian Camillo Golgi shared the Nobel Prize in Physiology or Medicine, in recognition of their work on the structure of the nervous system. Although both were well-known scientists who had made a large number of important discoveries regarding the anatomy of the nervous system, each defended a different and conflicting position in relation to the intimate organization of the grey matter that makes up the brain. In this communication we will review the importance of Cajal's studies using the method of impregnation discovered by Golgi, as well as the relevant studies carried out by Golgi, the concession of the Nobel Prize and the events that occurred during the Nobel conferences. In summary, we will précis the important contribution of both scientists to the founding of modern Neuroscience.

© 2007 Elsevier B.V. All rights reserved.

Contents

1. Introduction	8
2. Neuroscience before Cajal and Golgi	9
3. The Golgi method	9
4. The main discoveries of Cajal	10
5. The Nobel prize	14
6. The main discoveries of Golgi	15
7. Summary	15
Acknowledgments	15
References	15

1. Introduction

Santiago Ramón y Cajal was awarded the Nobel Prize in Physiology or Medicine in 1906. For the first time in its history,

this prize was awarded to two individuals and Cajal shared this honour with Camillo Golgi, an Italian scientist from the University of Pavia. According to the Karolinska Institute, both

* Corresponding author.

E-mail address: borrell@cajal.csic.es (J. Borrell).

scientists were favoured with this prize in recognition of their work on the structure of the nervous system. However, each of them had their own conception of the nervous system. While Golgi believed that the nervous system was comprised of a diffuse network formed by the anastomosis of the axonal processes, Cajal defended the individuality of the nerve cell. As a result, the corresponding Nobel lectures represented a defence of these two conflicting theories, the reticular doctrine and the neuron doctrine. However, this award was possible thanks to the Golgi discovery of a new histological method to impregnate tissue that was capable of staining the entire nerve cell (Golgi, 1873). Unfortunately, this method (“La reazione nera”) did not have a big impact on the scientific world at the time, since French and German scientists followed a severe discipline whereby specialists were dishonoured when working with other people’s methods (Cajal, 1917). Cajal learnt how to use this method in 1887 when Professor Luis Simarro, a famous Spanish psychiatrist, showed him some preparations impregnated with this histological stain. Cajal was highly impressed by these preparations and he began to use the Golgi method in his own laboratory. However, although this method has uncountable advantages, it was too unpredictable. Thus, Cajal came to the conclusion that if he wished to better understand the nervous system he would have to define the conditions of the chrome-silver reaction more strictly, and adapt it to each particular circumstance. He modified the method, varying the length of immersion of the tissue in the osmium-bichromic solution according to the nervous structure that he wished to study, the animal that he was using, and the age of the animal. He devised the method of double impregnation that produced results that he could not possibly have hoped for, and when he realized that the majority of myelinated fibres were not impregnated, he decided to use young animals and even embryos, where myelination had still not occurred. This was his ontogenetic or embryological method, which proved to be tremendously fruitful and helped him to make innumerable discoveries. Regarding the qualities and excellence of this method, he used to say:

“If the stage of development is well chosen, the relatively small nerve cells stand out completely in each section. The terminal ramifications of the axis cylinder are most clearly depicted and they can be seen to be perfectly free. The pericellular nests, that are the interneuronal articulations, appear simple, gradually acquiring intricacy and extension. In summary, the fundamental plan of the histological composition of the grey matter rises before our eyes with admirable clarity and precision” (Cajal, 1917).

Applying these modifications he was able to produce splendid images and it was precisely these images which convinced him that the laws regulating the morphology and the connections of the nerve cells in the grey matter were not as Golgi had claimed. Moreover, the use of the ontogenetic method took him to study of different aspects of the development of the nervous system. In the present article we shall review some of the main contributions of Cajal and Golgi that can be considered as the foundations of modern neuroscience.

2. Neuroscience before Cajal and Golgi

It can be said that before Cajal and Golgi, the discipline that is now called neuroscience did not exist. The cell theory was enunciated early in 1839 by the botanist M.J. Schleiden and the zoologist T. Schwann, and it stated that all tissues in the body are composed of individual cells. However, it was R. Virchow who introduced the concept of biological individuality, considering the cell as the elementary component that specifies living matter. Further, Virchow and Remak were the scientists that definitively demonstrated that every cell always comes from another cell (*Omnis cellula e cellula*). However, brain tissue was not considered to conform to the rules of the cell theory in part because there was no good histological method to stain nervous structures. Furthermore, the composition of the nervous system was more complicated than the tissues in other areas of the body and the difficulties in establishing the relationship between nerve cells, nerve fibres and terminal branches delayed the application of the cell theory to this system for half a century (Shepherd, 1991). In 1871, the German Josef von Gerlach put forward the “the reticular theory” that was the most prevalently accepted theory until Cajal’s discoveries. This theory stated that the grey matter of the nerve centres was made up of a dense mesh of thin filaments that join up to form the nerve fibres that lead to the white matter and reach the spinal cord. Gerlach also postulated that the central nerve endings would not end freely, but would rather continue with protoplasmic processes. This theory was rapidly accepted by the scientific community, including by the patriarch of German histology A. von Kölliker, later a fervent supporter of the neuronal theory. Similarly, the scientist from Pavia, Camillo Golgi, also believed in this theory with one modification, he thought that the network was formed by the anastomosis of the axonal fibres. Later, Golgi continued to defend this theory even when the neuron doctrine had become widely accepted (López-Muñoz et al., 2006). However, thanks to the discovery of a new histological method to impregnate nerve tissue, Golgi made many interesting contributions to our understanding of the structure of the nervous system.

3. The Golgi method

The method discovered by Camillo Golgi was published in 1873 (Golgi, 1873) and as pointed out above, it was popularized by Cajal who modified it and introduced a series of refinements relating to the species and the ages of the animals to study, as well as the brain structures to examine.

Basically, the Golgi method involves preparing pieces of nervous tissue 4–5 mm in thickness, either from fresh material or from blocks of fixative-perfused brains. These tissue blocks are immersed for several days (1 to 7 days) in a solution of 2.4% potassium dichromate, to which 1 g of osmium tetroxide has been added. After this treatment, the pieces are briefly rinsed twice with 0.75% aqueous silver nitrate until they no longer produce a brownish precipitate. Subsequently, the tissue is placed in fresh 0.75% silver nitrate solution for 1–2 days at room temperature before

finally superficially embedding the material in paraffin and obtaining 100–250 μm thick sections using a sliding microtome (Valverde, 1993).

The nerve cells and processes stained by the Golgi method become filled with a fine, opaque precipitate of silver chromate that renders the neuron and neuroglia cells clearly visible against the transparent yellow/orange background. In good preparations, the impregnated cells are seen in their entirety and their axonal and dendritic processes can be followed for long distances in thick sections or even in serial, adjacent sections (Figs. 1A, 2A, 3C).

In Cajal words: “What an unexpected sight! Sparse, smooth and thin black filaments or thorny, thick, triangular, stellate, or fusiform black cells could be seen against a perfectly translucent yellow background! One might almost liken the images to Chinese ink drawings on transparent Japanese paper [...] this is the Golgi method” (Cajal, 1899).

4. The main discoveries of Cajal

In 1887, when working as a full professor at the University of Valencia, Cajal was appointed to the selection committee of the public examinations for professors in descriptive anatomy in Madrid. He went to the Spanish capital and took advantage of his trip to visit Dr. Luis Simarro, the well-known psychiatrist who was well versed in histology. Simarro, like Cajal in Valencia, used to give classes in a laboratory that he had set up in his own house and he showed Cajal some preparations of nervous tissue impregnated with the Golgi method. This innovation impressed Cajal, who put this new method into practice upon his return to Valencia. A few months later, in 1888, Cajal was appointed as Chair of Histology and Histochemistry in Normal and Pathological Anatomy at the Faculty of Medicine in Barcelona, to where he moved with his family.

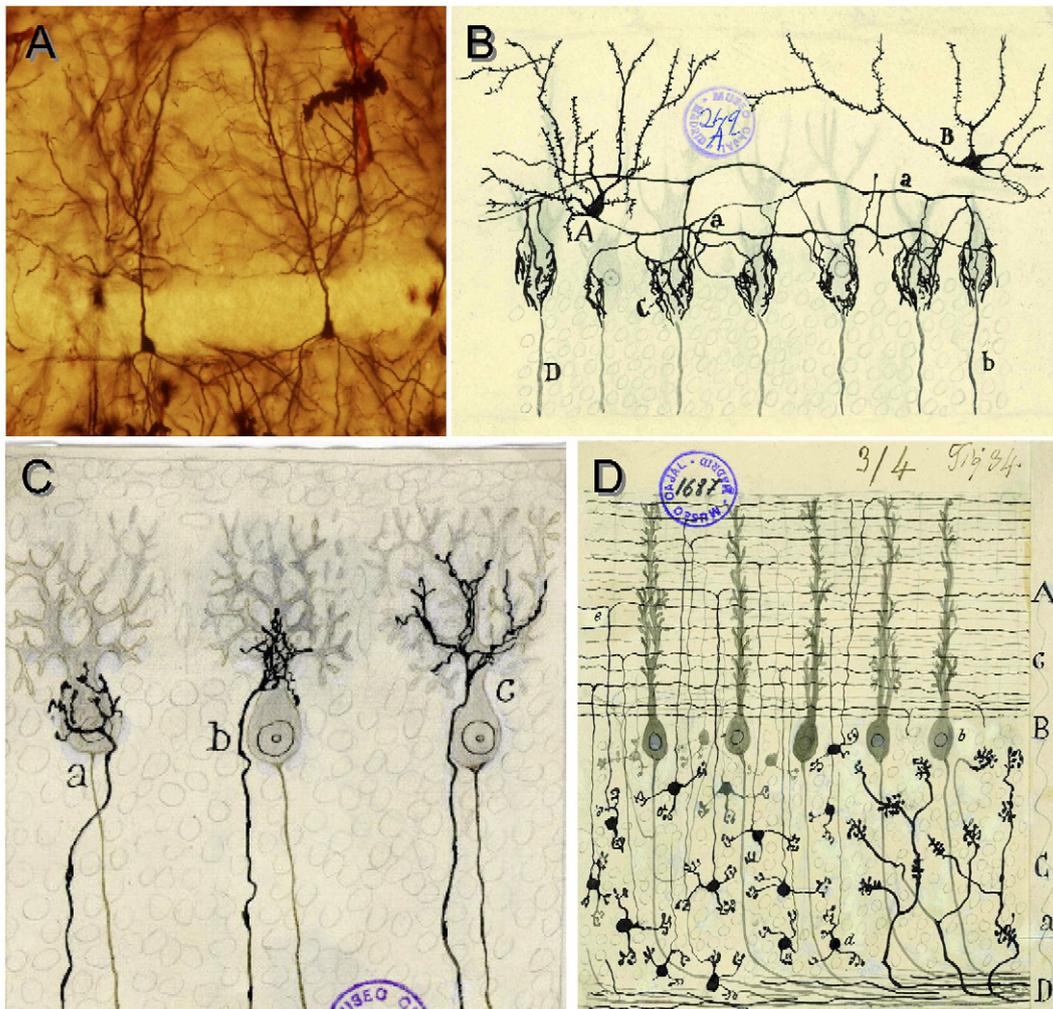


Fig. 1 – (A) Example of the Golgi method: mouse hippocampal preparation made by the authors (10 \times). (B) Transverse section of a cerebellar lamella, where A and B are stellate (basket) cells of the molecular layer, whose axons (a) produce terminal nets around the Purkinje cells (D). (C) The climbing fibres come from the pontine region reach the bodies of Purkinje cells and climb along their dendritic trunks. (D) Longitudinal section of a cerebellar convolution showing the different layers of this structure: the molecular layer (A), the Purkinje cell layer (B), the granular layer (C) and the white matter (D). From the deep pontine nucleus, the mossy fibres enter (a) and they arborise in the granular layer. Granule cells (d) send ascending axons that bifurcate (e) in the molecular layer. B–D are reproductions of Cajal’s original drawings from the “Cajal Legacy” (Instituto Cajal, Madrid).

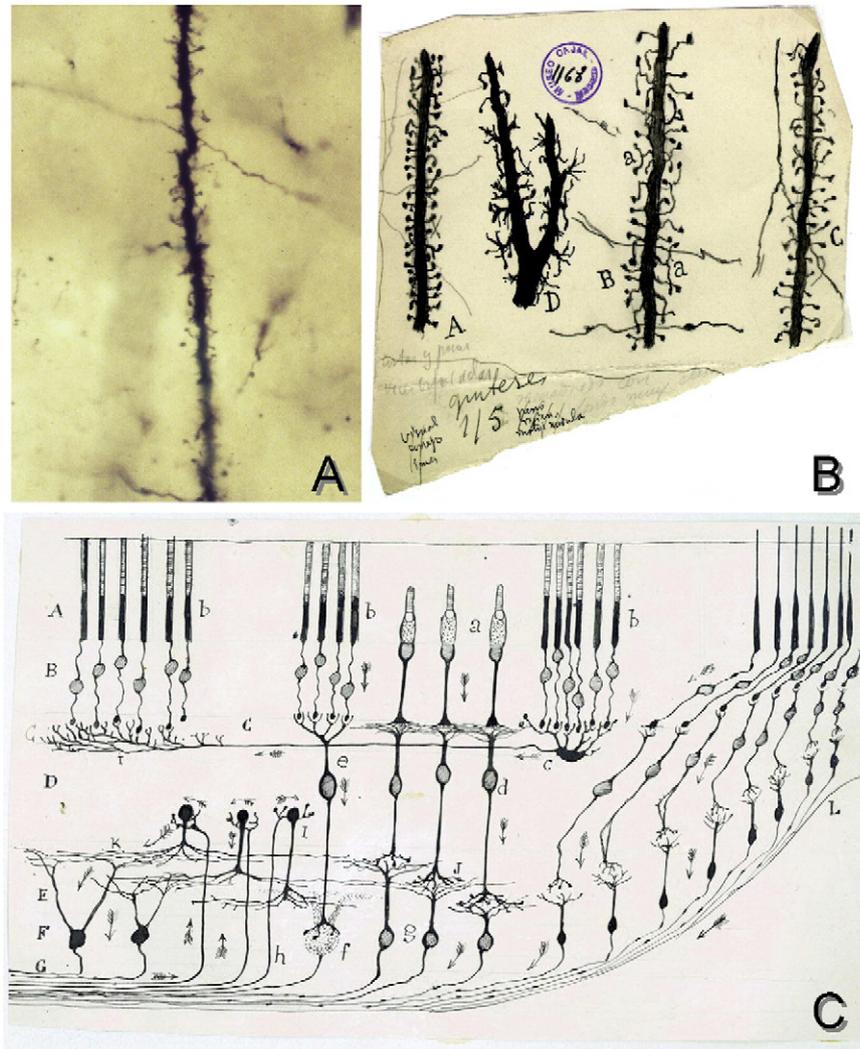


Fig. 2 – (A) Photomicrograph taken by the authors from one of Cajal’s original preparations with the Golgi method, showing dendritic spines. (B) Cajal’s original drawing showing his pictorial representation of the dendritic spines. (C) Cajal’s diagram showing the connections between the different avian retinal neurons, and the course of the nerve impulse (arrows). Cajal Legacy (Instituto Cajal, Madrid).

This was to be the most fruitful period of Cajal’s life...as he used to say “[...] and then 1888, my greatest year arrived [...] my year of fortune [...]”.

Using the Golgi method, Cajal began a systematic study of the nervous system, thereby confirming the usefulness of the method developed by the Italian professor. Cajal realized that the terminal and collateral fibres in the grey matter did not form a diffuse network but rather, they remained free, establishing simple contacts with the neuronal cell body and the dendrites of other adjacent nerve cells. What he observed in his preparations he drew methodically and accurately, without missing a single detail. In reference to his histological drawings, Cajal explained what a combined image is in a letter that he sent to his pupil Fernando de Castro on July 19 1927 and which is still conserved:

“In a combined image, all the cells are copied with precision; the only trick (already used by Golgi, van Gehuchten, Retzius) consists of uniting in a single drawing the elements collected in

*several sections of the same region. Without this trick, my book on neural centres would have required more than 3000 figures, and that at a time of economic penury in which a dozen engravings knocked off balance my domestic budget”.*¹

The first nervous structure studied by Cajal was the developing cerebellum of birds, where he discovered how the axon of some small stellate cells located in the molecular layer ends freely over the soma of the Purkinje cells, making pericellular contacts (Fig. 1B). Later, Kölliker was to name these contacts “terminal nests”. He also described for the first time a type of afferent fibre, the mossy fibres, which establish a relationship with the small dendrites of the granule cells (Fig. 1D). Furthermore, he identified a collection of very short processes that arise at right angles from any part of the dendritic surface that he named dendritic spines (Figs. 2A, B).

¹ This letter is conserved by Fernando Guillermo de Castro, only son of Professor de Castro.

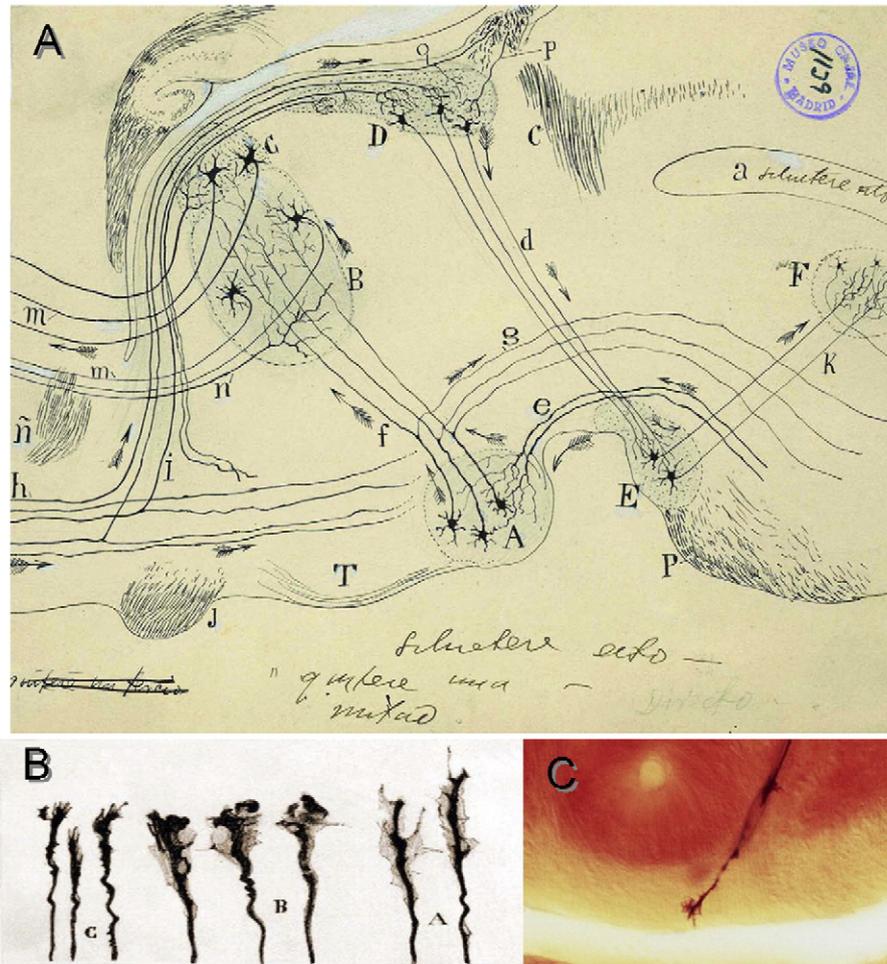


Fig. 3 – (A) One of Cajal’s drawings to illustrate the afferent and efferent pathways associated with the mammillary body, habenula and anterior thalamic nuclei. Arrows point to the course of the nerve impulse. **(B)** Cajal’s drawing of growth cones impregnated with the Golgi method from the spinal cord of a 4-day-old chick embryo. **(C)** Photomicrograph of one of Cajal’s original preparations showing a Golgi impregnated growth cone in the early chick spinal cord. Cajal Legacy (Instituto Cajal, Madrid).

These three important anatomical features were presented in his first paper on the nervous system (Cajal, 1888a). Cajal made his first discoveries using the Golgi method and some structural details, such as the dendritic spines, were also confirmed with the Ehrlich methylene blue method, ruling out any possibility that these appendices were artefacts of the silver precipitate. In Figs. 2A and B, we can see a photograph of such dendritic spines taken from an original preparation of Cajal and one of his drawings demonstrating these structures. Accordingly, we can see both what Cajal saw and how he interpreted it. What remains is for us to understand the significance that Cajal attributed to the anatomical data that he discovered. Indeed, this was Cajal’s greatest asset. Cajal was a tireless worker, a histologist and in this respect a perfectly normal man. However, what really made him special was his inquisitive nature or more precisely, the manner in which he interpreted his observations. In this sense, he was exceptionally talented. Cajal saw that dendritic spines were normally found on the largest cells, generally on pyramidal cells in the cerebral cortex or Purkinje cells in the cerebellum. The large number of spines increased the surface area of the dendrites and he thought that these appendices might be

where the contacts with the axons of neighbouring cells could be made. As he knew that nerve cells are incapable of dividing, he sustained that if Mother Nature had provided us with only a limited number of brain cells, she had given us the priceless gift of being able to restructure, ramify and intensify the expansion of these elements. In this way, we could almost infinitely combine reflex associations and create ideal systems. That is, Cajal insisted that we could create new contacts and ramifications, as well as generating more complex connections between nerve cells. In other words, Cajal was visualising dynamic features where they could not actually be seen, in the histological preparations of fixed nervous tissues. The merit in this was all the greater if we consider that Cajal could not visualise the nervous tissue as we can see it today when applying our modern technology.

Concomitant with his first paper on the cerebellum, he also published in the same journal and in the same volume, another study on the avian retina. Here, he described the different cell morphologies and the free endings of the axons of the photoreceptors, cones and rods in the external plexiform layer (Fig. 2C) (Cajal, 1888b). These observations in the retina supported his first discoveries made in the cerebellum. A few

months later, Cajal published another important work presenting more data from the cerebellum of birds, and that included an interesting description of the parallel fibres and the identification of their parental cells, the granule cells (Fig. 1D). Another fortunate finding described in this work was that the climbing fibres come from the pontine region of the brain. These afferent fibres reach the Purkinje cell bodies and run over their dendrites to which they adapt closely (Fig. 1C) (Cajal, 1888c).

All those early discoveries, together with those that he made in the following year (1889) working mainly on the medulla, led him to formulate the neuronal doctrine, a theory that contrasted with the reticularist belief that was strongly and vehemently defended by several authors such as Gerlach and Golgi. In his autobiography, Cajal summarized the four laws that can be considered as the starting point for the formulation of the neuron doctrine:

“The laws governing the morphology and connections of the nerve cells in the gray matter, which first became patent in my studies on the cerebellum, and that were confirmed in all the organs that I successively explored, I may formulate as follows:

- (1) *The collateral and terminal ramifications of every axon cylinder end in the gray matter, not in a diffuse network as maintained by Gerlach, Golgi and most other neurologists, but with free arborisations arranged in a variety of ways (pericellular, baskets or nets, climbing branches, etc.).*
- (2) *These ramifications are very closely associated with the bodies and dendrites of the nerve cells, a contact or articulation being established between the receptive protoplasm and the ultimate axonal branchlets.*
From the anatomical laws formulated, two physiological corollaries arise:
- (3) *Since the final rootlets of the axis cylinders are closely associated to the bodies and dendrites of the neurons, it must follow that the cell bodies and their protoplasmic processes enter into the chain of conduction, that is to say that they receive and propagate the nervous impulse. This contrasts with the opinion of Golgi, according to whom these parts of the cell perform a merely nutritive role.*
- (4) *The continuity of substance between cell and cell being excluded, the view that the nerve impulse is transmitted by contact as in the junctions of electric conductors, or by induction as in induction coils, becomes inescapable” (Cajal, 1917).*

In those years, Cajal had already made many discoveries that supported this new concept of the individuality of the neuron, a concept suggested by His, Nansen and Forel although they had never demonstrated it. This brought them into conflict with the majority of the scientific community that held to the old theory of the diffuse network. However, the problem that Cajal faced was that, apart from some Spanish professors, nobody was aware of his discoveries, since the few international articles that he had managed to publish had been largely ignored. Who was to pay attention to the daring theories of an unknown professor from a Spanish University?

For this reason, he decided to attend the German Anatomical Society Congress celebrated that year (1889) in Berlin. He requested financial support from the University but this was denied. However, having decided to go, he paid the costs of the

trip out of his own pocket. In order to defend his theories against the antineuronists, he took with him on this journey his “Zeiss” microscope and a box containing his best preparations. There he succeeded in capturing the attention of Professor Kölliker, the patriarch of German histology, who was impressed with the discoveries of the Spanish professor and who said: “I have discovered you and I wish to make my discovery known in Germany”. From then on things begin to change for Cajal, who began to receive due recognition for his research. But before he received the Nobel Prize in 1906, Cajal was to discover an important number of anatomical details by working with different species of small mammals at early stages of development (De Castro et al., 2007). Through his studies aimed at obtaining further support for his neuronal theory, Cajal studied the early development of distinct types of nerve cells. Thus, he saw how the nerve cell first sends out an axon or primordial process, and that only later does it produce dendrites and nerve collaterals. The entire process appears to be continuous with the cell body and it gradually increases in length, maintaining its individual nature until it reaches the adult size and it finally connects with other elements (muscular, epithelial or nervous) with which it maintains a physiological relationship.

But, how does an axon grow? From coronal sections of medulla of a 3 day old chick embryo, Cajal realized in 1890 that the axonal termination looked like a conical concentration of protoplasm, endowed with amoeboid properties (Figs. 3B, C). Once more, Cajal saw a dynamic picture in the static images of fixed tissue. He reasoned that the growth cone could move and that it could be compared to a living battering-ram, albeit soft and flexible, which advances mechanically, pushing aside the obstacles that it comes across until it reaches its peripheral destination. Furthermore, Cajal postulated that the growth cone probed the pathway to determine where the nerve fibre had to grow, and that it was attracted to or repelled by certain chemical substances secreted by the cells it encounters along its route (Cajal, 1890a,b). This is now known to be true and it gave rise, in its day, to his theory of chemotropism formulated in 1892.

Another of Cajal’s major discoveries, the so-called Law of Dynamic Polarization, was deduced from the study of many preparations. This law was presented at the medical congress celebrated in Valencia in 1891 and it states that the nerve cell is polarized (Cajal, 1891). This polarization is such that the nerve impulse is received by the dendrites of the cell, which is then transported to the cell body, and through the axon the signal can then be transmitted on beyond the cell. In this way, Cajal predicted the direction of the nerve impulse in all the systems he studied and he represented the direction of the impulse in his histological drawings by arrows. In some systems the direction is easily predicted, such as in the olfactory or visual system where the signal must travel from outside to in. Thus, in the retina, the nerve impulse forms in the visual receptor cells (the rods and cones), that must transmit it to a second cell (the bipolar neurons) and from these to a third cell, the ganglion neurons (Fig. 2C). These latter neurons are responsible for the transport of the impulse along the optic nerve towards the brain, where it reaches the lateral geniculate body in the thalamus, and from there it is passed on to the visual cortex located in the occipital lobe. However,



Fig. 4 – (A) Santiago Ramón y Cajal in the library of the “Laboratorio de Investigaciones Biológicas” (ca. 1930). In the left-upper part there is a picture of the Helmholtz gold medal, one of the more renowned prizes and one of which he was especially proud. Cajal was very popular in his country as can be seen by the use of his portrait on a 50 pesetas bill (paper currency, bottom-left). (B) Picture of a microscope, some colorants and some histological tools used by Camillo Golgi, conserved in the Museum of Pavia University, Italy (upper-left). Golgi also was a very popular scientist in his own country and we show a commemorative stamp produced by the University of Pavia to celebrate the centenary of the discovery of his impregnation method, the “reazione nera” (bottom-left).

in other systems such pathways are not so obvious. Thus, one example of Cajal’s understanding of these systems is the correct interpretation of the direction of the nerve impulse in more complex structures, such as in the hippocampus or at the mesencephalic level, in the distinct long tracts, such as the mamilo-thalamic, mamilo-tegmental, habenulo-interpeduncular tract (Fig. 3A).

Cajal’s popularity increased day by day and he received many prizes and much recognition for his studies. However, his most important discoveries, those that led him to postulate the neuron doctrine, were made between 1888 and 1891. In 1900, the International Congress of Medicine met in Paris and decided to award him the Moscow Prize. As a consequence of receiving this prize, the government approved the creation of a state subsidised laboratory where Cajal could work. This laboratory was situated in Madrid, in an annex of the Dr. Velasco’s Anthropological museum and it was named the “Laboratorio de Investigaciones Biológicas” (Biological Research Laboratory). Some years later, in 1905, Cajal was honoured with the Helmholtz gold medal by the Royal Academy of Sciences of Berlin, an award that had been instituted in 1892. This medal was awarded every 2 years to the author who had made the most important discoveries in any field of human knowledge. As such, Cajal was indeed tremendously proud of receiving this prize (Fig. 4A).

5. The Nobel prize

One day in October, 1906, Cajal received a telegram in his house, sent from Stockholm and written in German. It said merely: “*Carolinische Institut verliehen Sie Nobelpreis*”.

Cajal was awarded the Nobel Prize in Physiology or Medicine and he shared this prize with his “scientific enemy” Camillo Golgi (Fig. 4B), in recognition of their work on the structure of the nervous system. In Cajal’s own words: “*What a cruel irony of fate to pair, like Siamese twins united at the shoulders, scientific adversaries of such contrasting character*” (Cajal, 1917). Golgi was a defender of the reticular theory and a problem arose when both scientists gave their corresponding lectures since each of them defended their own particular point of view. While by this time the neuronal theory had become more universally accepted, there were still some scientists that defended the reticular theory. In essence, it can be said that the reticular theory or that of the diffuse network, was mainly defended by Gerlach, who sustained that the nerve cells anastomose their dendritic trees forming a network, and by Golgi who claimed that the network was formed of the anastomised cell axons. In contrast, the neuronal theory defended the anatomical, physiological and biochemical independence of the neuron, such that these cells

were interrelated with one another by contact and not by continuity. Cajal illustrated his lecture with many drawings of his histological preparations, describing the facts objectively and presenting the logical consequences that had led him to postulate the neuronal doctrine. However, Golgi while recognising the possible validity of the neuronal theory, preferred to defend the reticular theory to which he had always adhered. The posture of Golgi caused certain ill-feeling among those present and profoundly upset Cajal, who some years later wrote:

“The misfortune was that in defending his extravagant lucubration he made such an immoderate display of pride and self-worship that it produced a deplorable effect upon those assembled. Not even incidentally did he allude to the almost innumerable neurological works that had appeared outside Italy, and even in Italy itself. Neither the anatomist from Pavia, nor even his compatriot Lugaro had added anything of interest to his discoveries from former times. Likewise, he considered it unnecessary to correct any of his old theoretical errors, or the lapses in his observations. The noble and most discrete Retzius was in consternation; Holmgren, Henschen, and all the Swedish neurologists and histologists looked at the speaker with stupefaction. I was trembling with impatience as I saw that the most elementary respect for the conventions prevented me from offering a suitable and clear correction of so many odious errors and so many deliberate omissions” (Cajal, 1917).

6. The main discoveries of Golgi

Having raised the issue of the discrepancy between the Italian and Spanish scientists, we should add that the feelings of Cajal towards his colleague from Pavia were not eternally soured, since he was later to write: “[...] I admire the work of Golgi and his scientific character and I have the utmost respect and consideration for him. It is precisely his fruitful initiative to which we owe the precious method that enables us to see with such clarity the intimate scheme that constitutes the nervous centres” (Cajal, 1917).

Camillo Golgi is widely known for his description of the existence of a diffuse nervous network formed by the anastomosis of axonal collaterals, where nerve transmission spreads through the whole nervous system by continuity (Golgi, 1891). Furthermore, we should not forget that Camillo Golgi is not renowned amongst the scientific community only for the discovery of the chrome-silver impregnation method, but that he also made other such important contributions, such as:

- The description of two fundamental types of nerve cells, still named after him as Golgi type I (motor) neurons and Golgi type II (sensory) neurons.
- Golgi noticed that an intracellular structure existed in neurons that he designated as the “internal reticular apparatus”, and that is known today as the “Golgi apparatus”.
- In 1878 he described the “tendinous sensory corpuscles” that bear his name: “Golgi tendon organ”.
- Description of Muller–Golgi tubules, which are the canaliculi of the parietal cells of the gastric glands.

- He also defined the Golgi–Mazzoni corpuscle used to describe an encapsulated structure, similar to the Paccini corpuscle, but found only in the fingertips.
- He described the Golgi–Rezzonico filaments in the nerve fibres.
- Golgi also provided many fundamental contributions to the study of malaria, elucidating the cycle of the malarial agent, Plasmodium, in red blood cells.

It is evident that Camillo Golgi was a very good scientist and clinician. The large number of discoveries attributed to him made him worthy of the Nobel Prize.

7. Summary

Cajal dedicated the last years of his life to bring together all the anatomical evidence that unequivocally demonstrated the Neuronal Doctrine. Thus, in 1933, a year before he died, he published an extensive monograph under the title “Neuron Theory or Reticular Theory?” (Cajal, 1933), defending the point of view for which he had fought for his entire life as stated in the opening lines of this work: “I propose to describe briefly what I have seen during fifty years of work and what any investigator can verify for himself”. We can consider this work as his scientific testament. Finally, Cajal’s neuronal theory became accepted and he is currently considered as the father of the modern Neuroscience. Golgi fail to correctly interpret his preparations stained with the “reazione nera” but gave his name to numerous anatomical discoveries. For this reason, Golgi remains one of the most cited names in Biology. Furthermore, he discovered the method to impregnate the cellular components of the nervous system, which for the first time, facilitated the opportunity to systematically study this tissue.

In summary, 100 years after the award of the Nobel Prize to these scientific revolutionaries, we can consider that if the studies of Camillo Golgi on the structure of the elements of the nervous system marked an era, either due to the method he developed or the results he obtained, the works of Santiago Ramón y Cajal were of such intensity and were so fruitful in terms of the new discoveries they produced, that we could consider him the greatest anatomist of the nervous system in his own right, and essentially the founder of modern neurobiology.

Acknowledgments

This work has been supported by grants BF12003-00139 and BFU2006-01898 from the Spanish Ministerio de Educación y Ciencia (MEC).

REFERENCES

- Cajal, S. Ramón y, 1888a. Estructura de los centros nerviosos de las aves. Rev. Trimest. Histol. Norm. Patol. 1, 1–10.
- Cajal, S. Ramón y, 1888b. Morfología y conexiones de los elementos de la retina de las aves. Rev. Trimest. Histol. Norm. Patol. 1, 11–16.

- Cajal, S. Ramón y, 1888c. Sobre las fibras nerviosas de la capa molecular del cerebelo. *Rev. Trimest. Histol. Norm. Patol.* 2, 33–49.
- Cajal, S. Ramón y, 1890a. Notas anatómicas. I. Sobre la aparición de las expansiones celulares en la médula embrionaria. *Gac. Sanit. (Barcelona)* 12, 413–419.
- Cajal, S. Ramón y, 1890b. A quelle époque apparaissent les expansions des cellules nerveuses de la moelle épinière du poulet. *Anat. Anz.* 5, 609–613.
- Cajal, S. Ramón y, 1891. Significación fisiológica de las expansiones protoplásmicas y nerviosas de las células de la sustancia gris. *Rev. Cienc. Méd. Barc.* 27, 1–15.
- Cajal, S. Ramón y, 1899. *Textura del Sistema Nervioso del hombre y de los vertebrados*, Tomo I, Moya, Madrid.
- Cajal, S. Ramón y, 1917. *Recuerdos de mi vida*, Moya, Madrid.
- Cajal, S. Ramón y, 1933. ¿Neuronismo o reticularismo? Las pruebas objetivas de la unidad anatómica de las células nerviosas. *Arch. Neurobiol.* 13, 1–144.
- De Castro, F., López-Mascaraque, L., De Carlos, J.A., 2007. Cajal: lessons on brain development. *Brain Res. Rev.* doi:10.1016/j.brainresrev.2007.01.011.
- Golgi, C., 1873. Sulla struttura della sostanza grigia del cervello. *Gazzetta Medica Italiana. Lombardia* 33, 244–246.
- Golgi, C., 1891. La rete nervosa diffusa degli organi centrali del sistema nervoso. Suo significato fisiologico, *Rendiconti del R. Istituto Lombardo di Scienze e Lettere* vol. 24, 594–603 and 656–673.
- López-Muñoz, F., Boya, J., Alamo, C., 2006. Neuron theory, the cornerstone of neuroscience, on the centenary of the Nobel Prize award to Santiago Ramón y Cajal. *Brain Res. Bull.* 70, 391–404.
- Shepherd, G.M., 1991. *Foundations of the Neuron Doctrine*. Oxford University Press, New York.
- Valverde, F., 1993. The rapid Golgi technique for staining CNS neurons: Light microscopy. *Neurosci. Protocol* (93-050-01).