Golgi, Cajal and the Neuron Doctrine*

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ABSTRACT

Camillo Golgi and Santiago Ramón y Cajal shared the Nobel Prize in 1906 for their work on the histology of the nerve cell, but both held diametrically opposed views about the Neuron Doctrine which emphasizes the structural, functional and developmental singularity of the nerve cell. Golgi’s reticularist views remained entrenched and his work on the nervous system did not venture greatly into new territories after its original flowering, which had greater impact than is now commonly credited. Cajal, by contrast, by the time he was awarded the Nobel Prize, was already breaking new ground with a new staining technique in the field of peripheral nerve regeneration, seeing the reconstruction of a severed nerve by sprouting from the proximal stump as another manifestation of the Neuron Doctrine. Paradoxically, identical studies were going on simultaneously in Golgi’s laboratory in the hands of Aldo Perroncito, but the findings did not seem to influence Golgi’s thinking on the Neuron Doctrine.

Keywords: Cajal, Golgi, Perroncito, neurocytology, regeneration, neurotropism, catenary theory.

INTRODUCTION

The history of the Neuron Doctrine is one of paradoxes. The theory was born in an intellectual milieu that was dominated by the German intellectual tradition in biology, a tradition that had seen the development of the Cell Theory and which had produced some of the greatest names in the history of biological science – all of whom contributed to the foundations of the Neuron Doctrine. Yet, paradoxically, it was the contributions of two individuals, Golgi and Cajal, working outside that tradition and in countries out of the mainstream of European science, that provided the breakthroughs, the one in technique, the other in wealth of data and fundamental insight, that permitted the definitive formulation of the Neuron Doctrine by Waldeyer in 1891. Of other paradoxes, one that no student of the history of Neuroscience can fail to remark upon, although it has escaped the notice of others, is that the Neuron Doctrine was completely anatomical in its conception despite the fact that significant developments that could have substantially contributed to it and to its acceptance as the fundamental basis of nervous function were already occurring in physiology. By 1891 the finite delay in the spinal transmission of the monosynaptic knee jerk reflex had been measured, insights had been obtained into the nature of the nervous impulse and, in 1890, Langley and Dickinson had blocked transmission through the sympathetic ganglion by painting it with nicotine. Yet none of these observations that could have contributed to the Neuron Doctrine as an hypothesis of communication between nerve cells by contact rather than continuity seems to have entered into the consciousness of the chief actors in this drama. At least they did not enter into their writings; the first mention of Langley’s name, for example, in the writings of Cajal comes in the work, “¿Neuronismo o Reticularismo?” published in 1933, just before his death.

Perhaps the greatest paradox of all, and one that has been frequently commented upon is the
fact that the two most prominent figures in the establishment of the Neuron Doctrine, Golgi and Cajal, who shared the Nobel Prize for Physiology or Medicine in 1906, were at opposite ends of the spectrum of ideas associated with it, the one an ardent reticularist, the other an equally ardent connectionist from whose hands flowed most of the knowledge about the intrinsic circuitry of the brain centers that we continue to use to this day.

Neither of these two great scientists ever conceded much to the other in their writings, although they did accord one another the common courtesies natural to their times but so sadly missing from our own. The verdict of history must be, of course, that Golgi was in error but his reasons for disavowing the Neuron Doctrine throughout his career are by no means uninformed. I shall allude to some of them below. But this brings us to one of the most remarkable paradoxes of all, that I shall try to deal with in some detail. Exactly at the time of the Nobel Prize, and during the subsequent five or six years, when the Neuron Doctrine was entering its second phase and Cajal was working intensively to establish that severed peripheral nerves regenerated by sprouting of axons from the proximal stump and not by coalescence out of chains of Schwann cells, a scientist working in Golgi's laboratory, Aldo Perroncito, was coming to exactly the same conclusions and his findings were sometimes in advance of those of Cajal – much to the concern of the latter.

GOLGI AND THE STRUCTURE OF NEURONS

Before turning to examine this second phase in the exposition of the Neuron Doctrine, let us examine some of the background prior to 1891, when Golgi made the majority of his contributions and Cajal first burst upon the scene. Prior to his discovery of the reazione nera in 1873, Golgi’s published representations of nerve cells were typical of those of the time when brain tissue was customarily teased apart after compression between cover glasses in water or alkali and when even with the added benefits of osmium tetroxide and carmine or gold chloride staining the most that could be revealed of a nerve cell was the soma, the proximal and with luck the secondary dendrites, and the initial segment of the axon. Some of Golgi’s early pictures of nerve cells can be seen, for example, in his 1871–1872 publications on the finer anatomy of the central nervous system in Rivista Clinica (Opera Omnia, pp. 5–70). After 1873, following the discovery of the black reaction, his drawings underwent a radical transformation, not only in their beauty but in the wealth of detail about individual nerve cells, hitherto undreamed of.

Among Golgi’s original observations that are often forgotten in the haste to debunk him for his failure to accept the Neuron Doctrine are: tracing of axons over longer distances than ever before; the first visualization of axon collaterals; the recognition of the dense axon plexuses of the central nervous system; the first demonstration of the full extent of the dendritic trees of neurons; the recognition of intrinsic (‘sensory’ or type II) and projection (‘motor’ or type I) neurons as separate classes. I believe that one can also identify dendritic spines even in his early drawings of Purkinje cells in the cerebellum, although he did not mention them.

Cajal was accustomed to accuse Golgi of adding the spines to his later drawings, e.g. in those which accompanied his Nobel lecture. I think that this was unfair. It is true that Golgi gave them little functional importance, mainly because he could see similar protrusions on neuroglial cells.

Out of Golgi’s studies prior to 1886 came a wealth of information not only about the structure of the nerve cell but also about the relationships among nerve cells that hold some of the keys to understanding their organization in an inter-communicating network. By demonstrating the free endings of the dendrites, he overthrew Gerlach’s belief in anastomotic union of these structures, but it also led him to a belief in their primary association with the brain surface or with blood vessels and to his considering them solely as nourishing channels for the cell soma. By demonstrating that some nerve cells were restricted to their brain region while others sent long axons into the white matter, he laid one
foundation essential for the construction of circuits for the flow of information through a region, something at which Cajal was later to excel. He has again been criticized, from Cajal on, for his use of the designators ‘sensory’ and ‘motor’ for the two classes of cell. This criticism is probably unjustified for, as he pointed out in his Nobel lecture, outside the spinal cord, he was largely using these terms by analogy with the cells of the cord.

Golgí’s attitude to the nervous network was especially conditioned by his observations of the remarkable density of the fiber meshworks of the granular layer of the cerebellum and plexiform layer of the dentate gyrus. In them, he saw the afferent axons and the axons of the intrinsic neurons lose themselves in progressively branching arborizations, while the collaterals of the efferent neurons furnished re-entrant inputs. In the meshwork he considered that the integrative properties of the nervous system had their seats. It is a concept but little removed from the ideas of Leydig and others on the nature and functions of the neuropil in the ganglia of invertebrates – ideas that played a substantial role in promoting the view of the nervous system as an anastomosing network.

In recognizing Golgi’s errors of interpretation, recent generations of neuroscientists have tended to neglect the importance of his observations on the basic structure of the nerve cell. That his technique met with a mixed response that sometimes tended to the disdainful is undoubtedly true. Cajal points out, for example, that Ranvier’s handbook of histological technique that he, Cajal, initially regarded as his technical bible, was dismissive of the merits of the Golgi technique. But it is incorrect to regard Golgi’s technique as having been ignored by other European scientists. As Ennio Pannese (1996) has pointed out, Boll had summarized in German Golgi’s original note soon after its publication in 1873; there were other brief reports of its use through the 1880s and during this period Golgi distributed some of his slides to other scientists such as Kölliker and Retzius (see G. Grant in this volume). The expedition of Fridtjof Nansen to the Naples marine station in 1887, with its side trip to visit Golgi, was probably the result of learning from Retzius of the merits of the new technique. Although not agreeing with many of Golgi’s interpretations, Kölliker in 1887 clearly stated the potential advantages of the technique and in that year, at the age of 70, visited Golgi in Pavia to learn the technique. It is reported that on returning to Würzburg and trying out the method for himself, he was to obtain only indifferent results. This did not prevent an enduring friendship from developing between Kölliker and Golgi, one cemented by frequent visits to Italy lasting into Kölliker’s 80s.

CAJAL AND CONNECTIONS BY CONTACT

In 1887, Santiago Ramón y Cajal first learned of the Golgi technique from a Spanish amateur histologist, Luis Simarro, who had picked up the method in Paris (Cajal, 1917). From whom is not recorded but it is indicative of the widespread knowledge, if not universally successful implementation, of the new technique. Simarro, according to Cajal, after some early success, later gave it up. Not so Cajal himself, who in a flash of enlightenment (he says) recognized the importance of the method as a means of tracing connectivity in the nervous system. That he should have focused on connectivity from the outset is remarkable only if one thinks that histologists of that era had little concept of pathways underlying reflex action and supporting the flow of sensory information and motor commands. That this is patently untrue can be determined by reference to any textbook of the era from Claude Bernard on. Even the reticularists, of whom Golgi was one, were striving for a mechanism that would explain the fundamental capacity of one part of the nervous system to communicate with other parts.

Within the four years from his first application of the Golgi technique in 1887 to the formal enunciation of the Neuron Doctrine in 1891, Cajal had published some 45 papers (Pérez-de-Tudela, 1983) in which the foundations of the Neuron Doctrine were well and truly laid and on which Waldeyer was to draw heavily in making
the case for it. The reasons for Cajal’s success are several (De Felipe and Jones, 1992). Undoubtedly at the forefront was his improvement of the technique by introducing the procedure of multiple re-impregnations in the dichromate and silver solutions, controlling the level of impregnation by cutting sample sections at intervals throughout its course. Aiding and abetting this was his preference for the brains of immature animals and for those of birds and small mammals, in all of which the relative lack of myelin does not impede impregnation and the simplicity of the axonal plexuses permits individual axonal ramifications to be resolved. Once other scientists recognized the merits of these two approaches, the way was clear for widespread application and for rapid confirmation of Cajal’s observations. It is likely that their earlier difficulties in applying the stain had stemmed from concentrating most of their efforts, as Golgi had done, on the human brain.

Apart from his improvements in technique, it is likely that Cajal’s early success lay in his choice of highly laminated structures, such as the cerebellar cortex and retina, as the objects of his investigations. In these, the regularity of organization and the clear stratification of cell types and axonal plexuses facilitates the extraction of individual cells and their processes from the total pattern and permits their position in the circuitry running through the structure to be determined. In his first paper with the Golgi technique, on the cerebellum of birds, published in 1888, Cajal recognized that the axons of basket cells, seemingly conforming to Golgi’s type I cells, because they did not immediately breakup into plexuses typical of type II cells, actually ended by embracing the cell somata of Purkinje cells and thus could only exert their influence upon the white matter via the Purkinje cells. This was the key to his recognition of connections by contact rather than by protoplasmic continuity between neurons, and to his developing his analysis of the intrinsic structure of the nervous system in terms of circuits for the flow of information from neuron to neuron. Once the law of dynamic polarization, which stipulates that dendrites conduct towards the soma and the axon away from it, was formulated (Van Gehuchten 1892; Cajal 1892), Cajal could commence putting his typical arrows on his drawings, indicating the direction of afferent and efferent conduction through a center such as the cerebellum, retina or olfactory bulb. Our knowledge of the elementary circuitry of the nervous system at the cellular level stems from this era.

Cajal’s genius lay as much in his insights into the fundamental biology of the nervous system as in his technical innovations and the remarkable volume of work he produced in the early, formative years of the Neuron Doctrine. To claim that his success rested solely in his mastery of the Golgi stain would be inappropriate since, despite the failures alluded to above, there were contemporaries who were producing material as good as Cajal’s and often published in plates of greater elegance. Golgi himself was one and Cajal’s preparations of the retina published in 1888 and 1889 are surpassed in beauty by those of Tartuferi (1888). Tartuferi, like Golgi, however, was a reticularist.

GOLGI’S VIEWPOINT

The reasons why Golgi and other individuals who made seminal contributions to the understanding of the structure of the nervous system, notably Nissl (1903), remained committed to a reticularist viewpoint in the face of overwhelming evidence against it, have never been fully explored. In Golgi’s case a focus on the densely intertwined mass of axons in the plexuses of the cerebellum and hippocampus may have influenced him. Possibly a conscious or unconscious commitment to the neuropil as the center for all integrative actions of the nervous system deriving from the earlier studies of Leydig and others on the ganglia of invertebrates in which the neuronal somata were seen as merely nourishing elements for the more important neuropil (see Shepherd, 1991), may have helped. We do not know what microscopes Golgi used in his early years, but it is unlikely that they were capable of resolving, separately, the arborizations of individual axons in the dense plexuses upon which Golgi placed so much importance. One point repeatedly made by Golgi and re-echoed in his
Nobel lecture of 1906 is his belief that recovery of function in the nervous system, such as occurs after a stroke, could only be possible if nerve cells were in continuity with one another. Another, is that structures such as the hippocampus and cerebellum function as single entities, their nerve cells operating in collective mass action and not as individual elements. It is this holistic view that led him to reject the concept of cortical localization of function.

In reading the 1906 Nobel lectures of Golgi and Cajal, one cannot help but remark on the contrast in styles, and it is difficult not to be saddened by that of Golgi. Golgi is clearly looking to the past, referring mainly to older work, defending an untenable position and presenting his case with an air of negativism. Cajal’s presentation is much more in line with that of a modern seminar in which the speaker rapidly summarizes his past work and quickly proceeds enthusiastically to describe his latest observations made with new techniques on fresh experimental paradigms. In Cajal’s case, it is the application of the reduced silver nitrate method, discovered independently by Cajal and Bielschowsky in 1902/1903, to the structure of the nerve cell and to the regeneration of peripheral nerves on which he had been working for two years. Throughout Cajal’s whole talk, however, runs the theme of the Neuron Doctrine and the individuality of the nerve cell. Cajal’s new studies on the peripheral nervous system were initiated to counter a growing threat to this fundamental tenet. It is a matter of great surprise that identical work was simultaneously going on in Golgi’s own laboratory at the hands of his nephew, adopted son, and successor in the Chair of Histology at Pavia, Aldo Perroncito.

CAJAL, PERRONCITO, AND REGENERATION OF PERIPHERAL NERVES

Cajal applied the reduced silver stain initially to examine the internal structure of the nerve cell and most of the material added to the French version of his textbook of the histology of the nervous system (1909, 1911) comes from application of the reduced silver technique, reflecting work done in the interim following publication of the original Spanish version (1899, 1904). In these studies, the neurofibrillar character of the nerve cell is clearly revealed. Cajal quickly realized, however, that the capacity to stain neurofibrils (now known to be bundles of intermediate filaments) offered the opportunity to study the growth of axons in the regeneration of severed peripheral nerves. He was conscious that recent developments in this field posed a threat to the universality of the Neuron Doctrine. With his usual insight, he also recognized that issues raised in the increasingly vehement debate about the nature of peripheral nerve regeneration had important implications for the normal development of the nervous system and for any hopes of promoting regeneration in the injured central nervous system.

Many years previously, the anatomist, Viktor Hensen (1864) had proposed that newly growing peripheral nerve fibers in the developing frog were formed by coalescence out of linear chains of Schwann cells. This ‘catenary’ theory was ruled out as a developmental process by the investigations of His (1887) and the later Golgi-based studies of Lenhossék (1890) and Cajal (1890) in which the growth cone was identified for the first time. However, it had also been proposed by Vulpian (1866) as the mechanism by which a new distal axon segment could be formed and reunited with its proximal stump in the process of peripheral nerve repair, the distal axon segment being formed within the chains of Schwann cells in the distal stump and later joined with the proximal segment. The idea was to be resurrected in the early 1900s by Albrecht Bethe (1901, 1903, 1907) and Hans Held (1907, 1909). The prevailing view dating to Waller (1850) and reinforced by Ranvier (1878) was that the proximal stump sprouted new axons that invaded the distal stump and restored continuity. To Cajal, the resurrection of the catenary theory was a serious threat to his theory of neurotropism, first formulated in his 1892 study of the retina, which depended on outgrowth of axons from single nerve cell somata under the influences of trophic signals and, by extension, demanded that repair of a cut peripheral nerve depended upon sprouting from the proximal stump.
and growth of the sprouts across the gap and down the distal segment of the nerve, under the same kinds of trophic influences.

As pointed out in our introduction to a new edition of Cajal’s Degeneration and Regeneration of the Nervous System (De Felipe & Jones 1991), there were two principal arguments raised by Bethe and others in support of the catenary (or ‘polygenic’) theory: (1) growing axons could never be histologically demonstrated crossing the gap or scar between the proximal and distal stumps of a cut peripheral nerve; (2) reinnervation of a distal stump and restoration of motor function could occur even when obstacles were introduced that prevented re-union of the proximal and distal stumps. The first of these arguments was clearly based upon the inability of available methods to stain individual axons, contemporary workers having to rely upon the staining of the Schwann sheath or the myelin sheath, both of which appear only after axons have crossed the gap. The first applications of the reduced silver stain at the hands of Cajal (1905) and Perroncito (1905a) were to resolve this issue by clearly demonstrating the sprouting of proximal cut axons and the early presence of naked, regenerating axons with growth cones, crossing the gap and invading the distal stump.

The second argument was for a time perhaps more cogent, although from a modern perspective it can be seen as indicative of the enormous capacity of growing or regenerating peripheral axons to circumvent obstacles in reaching their appropriate targets. A number of ingenious experiments by Cajal resolved the issue by demonstrating the capacity of regenerating axons to correct inappropriate orientations, negotiate obstacles, and reach their targets by highly devious routes. This turned out to be the supreme vindication of the neurotropic theory.

Volume I of Cajal’s two volume work, Estudios Sobre la Regeneración del Sistema Nervioso, first published in 1913, contains one of the most extensive studies of the histological phenomena associated with degeneration and regeneration of peripheral nerves ever published. It can easily be viewed as the definitive word on the subject but this is to ignore the contributions of a number of other contemporary investigators. Among these were Aldo Perroncito in Golgi’s laboratory and an active Romanian group led by the neurologist, Georges Marinesco. Apart from the competition between the proponents of the catenary and sprouting schools, which was at times highly polemical, there was considerable competition between those who were most active in the application of the reduced silver technique to the issue of peripheral nerve regeneration. Perroncito was definitely ahead of Cajal in describing the earliest phenomena occurring in the central stump of a divided nerve. Between 1905 and 1909, Perroncito published a series of relatively short papers on his observations in the Bulletin of the Medico-Surgical Society of Pavia (Perroncito, 1905a,b, 1906, 1908, 1909), a more extensive summary and review of the whole field appearing in German in 1907 (Perroncito, 1907). He points out in a footnote to his December 1905 paper that the work formed part of his thesis and that, just as he was about to publish it, Cajal (1905) and Marinesco and Minea (1905) had independently published short preliminary communications on the same subject. Cajal, ever sensitive to issues of priority, wrote on the first reprints sent to him by Perroncito that they were received on 28th September and 4th of December 1905 and on the latter that it followed his own presentation to the Society of Biology and his two published notes of 1905 (Cajal, 1905). In writing about this in his 1913 book he was forced to concede, however, that although published in September, it was the May number of the Journal that carried the first paper.

Perroncito, working on dogs in which he had cut the sciatic nerve, independently made the same observations as Cajal on the retraction of the proximal ends of cut axons, the sprouting of new branches, the naked axons making their way across the gap, their entry into the distal stump, and the degenerative changes occurring in the distal stump. He was ahead of Cajal in observing sprouting occurring as early as twenty four hours after nerve section and sprouts invading the gap between the stumps on the second day. He is also responsible for first describing the unusual ‘apparatuses of Perroncito’ (Cajal’s name), a peculiar mixture of bundles, rings, spi-
ral and bulbs formed on the ends of early re-
generating nerve fibers. Although he perhaps
saw the earliest outgrowth of new sprouts, he
did not agree with Cajal about the growth cones,
seeing them as responses to obstructions or indi-
cative of aborted regeneration.

Cajal, although highly complimentary in re-
ferring to Perroncito in his later (1913) book on
degeneration of peripheral nerves, in 1905 and
1906 was watchful of this work emerging from
Golgi’s laboratory. The pages of many of the
reprints sent to Cajal by Perroncito and currently
in the library of the Instituto Cajal, are covered
with comments in Cajal’s handwriting, a number
of which reflect a desire to assert his own claims
to priority. The truth is that many of Cajal’s ob-
servations emerged in very short formats and his
definitive work appeared well after the publica-
tions of Perroncito and even after those of
Marinesco, whose work with Minea in 1905
contained many of the same observations as that
of Perroncito. (It is to Minea (1909) that we owe
the introduction of that now popular term
‘neuroplasticity’). Although we may express
distaste for Cajal’s at times unseemly competi-
tiveness, and possibly deny him full priority for
these findings, there can be little doubt about the
monumental character of his overall, definitive
work. Nor can one deny him credit for those
fundamental biological insights that were part of
his genius. Apart from recognizing that nerve
regeneration had to be a recapitulation of nerve
growth in development, he was able to see, far
more clear sightedly than others at the time, that
regeneration, the reconstitution of a nerve and
the reestablishment of connections with appro-
priate nerve endings, such as motor end plates
and muscle spindles, must involve a variety of
chemical signals, some trophic, some tropic,
some local, some long distance, some non-spe-
cific, others specific, some chemical, others me-
chanical. This was all part of his theory of neu-
rotropism, a term he obtained from Forssman
(1900). At one point in his 1913-1914 book, he
even hints that he had some comprehension of
the necessity for interactions between tropic
molecules and receptors on the growth cones.
Although not completely alone in conceiving the
neurotropic theory, Marinesco, for example, also
alluding to attractant properties of Schwann
cells for growing axons, Cajal’s was by far the
major contribution.

By 1908, Perroncito was as embroiled as
Cajal in the debate with Albrecht Bethe, Profes-
sor of Anatomy at Strassburg, who was the chief
polemicist on the side of the catenary or poly-
genic theory of nerve regeneration. Papers by
Bethe published in 1907 personally attacked the
work of both investigators, at one point accusing
Perroncito of ‘supine ignorance’ of the facts of
physiology. Despite the new evidence emerging
from the Italian, Spanish and Romanian labora-
tories, Bethe continued to belabor the idea of
nerve regeneration as the reunion of chains of
supporting cells. These papers met with as vig-
orous a response from Perroncito (1908) as they
did from Cajal (1907). Perroncito terming the
attacks ‘indecent’. Gradually, Bethe was to give
ground, admitting first the phenomenon of
sprouting and some invasion of the distal stump
and finally, in 1922, that all new fibers came
from the proximal stump. In return, Cajal re-
moved certain personal comments about Bethe
from the second edition of his autobiography
(De Felipe and Jones, 1991). It was only Cajal,
however, who continually linked the contro-
versy to the Neuron Doctrine and the singularity
of the nerve cell as the unit of organization of
the nervous system. One can detect nothing in
the writings of Perroncito or Golgi from that
time to suggest that the drama of peripheral
nerve regeneration being played out to a large
extent in his own laboratory over influenced
Golgi’s thinking on the Neuron Doctrine. The
Neuron Doctrine does not warrant mention in
Perroncito’s lengthy German review in 1907,
whereas it forms the entrée for Cajal’s whole
work on regeneration.

CONCLUSION

As recognized by the Nobel Committee, Camillo
Golgi and Santiago Ramón y Cajal extended the
investigation of the nerve cell to a level of reso-
lution far beyond that achieved by any of their
predecessors, the one by his introduction of a
vastly improved analytic technique, the other by
his mastery of that technique, the breadth and depth of his work and his fundamental biological insights. Golgi was not alone among contemporary scientists in remaining wedded to the reticular theory but this may have been as much due to his laying aside study of the nervous system in favor of investigations in other fields, as to his stated reasons. Cajal continued to adopt new techniques as they became available and new experimental preparations that would enable him to continue to push forward the frontiers of neurohistological analysis. He was not alone in revealing the histological basis of peripheral nerve repair. But it was he who clearly derived from it the underlying biological mechanisms that governed this process, and who foresaw their relevance to the development of the nervous system and to any hope of promoting regeneration of the injured central nervous system. While Aldo Perroncito, working contemporaneously in Golgi’s laboratory might have also had some insight into these mechanisms and their relevance, the most important aspect of the findings in these studies, clearly apparent to Cajal from the outset, namely their significance in relation to the Neuron Doctrine, seems to have eluded both he and his master.

REFERENCES


