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Review

Cajal's debt to Golgi

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Contents

We are accustomed to thinking of Camillo Golgi and Santiago Ramón y Cajal as contemporary scientists at war over the neuron doctrine. This is certainly how Cajal in his biography and later writings portrayed their relationship and Golgi did not help matters by his most unfortunate Nobel acceptance speech of 1906 (Golgi 1907) in which he emphasized in a contentious way his continuing belief in an outmoded view of the nervous system. Cajal's speech (Cajal, 1907), by contrast, was of a kind typical of that of any modern neuroscientist in which he outlined his past achievements in neurohistology and then proceeded to describe his ongoing experiments in nerve regeneration. The contrast between the two speeches is a reflection of the fact that Golgi's work on the organization of the central nervous system had essentially ended by 1883 when he turned mainly to investigations of malaria, while Cajal's which had commenced only in 1888 was still in full flight. As neuroscientists, therefore, they cannot be seen as contemporaries. In what follows, I shall attempt to present the case that Golgi's observations, made on tissue stained by his black reaction represented a fundamental breakthrough in the way in which the nervous system was viewed and that his observations provided a firm basis upon which Cajal was later able to build. While it would be wrong to say, as some have, that Cajal stood on the shoulders of Golgi, there can be little doubt that a number of Golgi's observations on the structure and organization of nerve cells not only transformed the way

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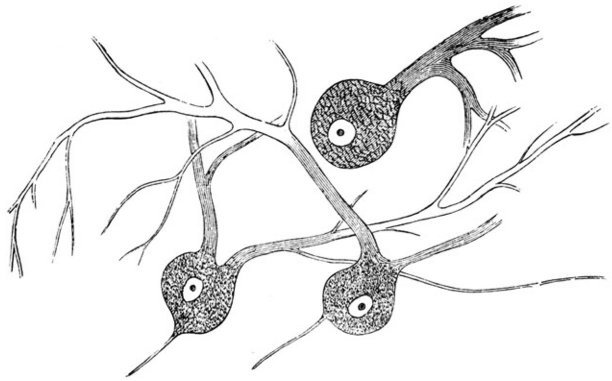

Fig. 1 – Drawings of Purkinje cells from the human cerebellum, showing the extent of detail that could be visualized in material fixed in chromium or osmium salts and stained with carmine. From Kölliker (1863).

in which contemporary scientists perceived nerve cells but also represented the starting points for Cajal's work in which in a few years he unraveled the intrinsic circuitry of just about every region of the nervous system.

Prior to Golgi's discovery of the black reaction (Golgi, 1873) and his publication of the first images of nerve cells obtained with it, knowledge of the form of nerve cells was remarkably primitive. Purkinje did not visualize the cells that now bear his name as the magnificent structures with their elaborate planar set of dendrites that we know today but as no more than globules that represented only the cell body. The later introduction of chromic acid as a fixative and carmine as a stain extended somewhat the knowledge of the nerve cell, notably in the hands of Deiters who identified branching dendrites (which he called protoplasmic processes) and the axon (called the axis cylinder). But the forms of the dendritic trees demonstrated by Deiters and others were incomplete and the collateral branches of axons had not been discovered. Kölliker's (1863) drawings of Purkinje cells (Fig. 1), while definitely an advance on what Purkinje himself had seen, are still primitive when compared with what we have subsequently learned about the structure of that neuron. Prior to his discovery of the black reaction, Golgi's drawings of nerve cells which he had observed in tissue fixed in potassium dichromate, chromic or osmic acid (Fig. 2) are little different from those of other contemporary histologists, sometime showing branching dendrites but often with no more than stumps of dendrites emerging from the soma and perhaps the axon hillock and initial segment.

The first images of neurons impregnated with the Golgi stain heralded the beginning of a revolution in how nerve cells were viewed. Those early drawings of Golgi (e.g. Golgi, 1875) present neurons for the first time in the form in which we are still accustomed to portraying them (Fig. 3). Golgi's disappointment at the slow recognition in print of the importance of his findings was real but it seems clear that this was less on account of disbelief than on the fact that few scientists could successfully employ his method to obtain similar results. It was only after Cajal much later, in 1888 and 1889, applied the stain in repeated impregnations, with longer immersion times and more concentrated reagents, and in infant animals in which myelination was less advanced than in adults, that others were able successfully to use it (DeFelipe and Jones, 1992). But at the time of Golgi's first publications no one with any eye for the nervous system could fail to appreciate the manner in which his stain had dramatically extended anatomical knowledge of the nerve cell, its dendrites and its axon. Outside the world of scientific publishing there were many who early on recognized that a revolution had occurred and attempted to implement the Golgi methods themselves. Fritjof Nansen in 1887, traveled to Pavia to learn the secrets of the method (Jones, 1994), and Luis Simarro who in 1887 was to give Cajal his first glimpse of a Golgi preparation (Cajal, 1917) applied while in Ranvier's laboratory in Paris. Neither of them published the results of their investigations, however, and others such as Kölliker, who also visited Pavia in 1888, after earlier correspondence with Golgi, were unable to get the stain to work. To the scientific establishment, the new revelations about nerve cells seemed to have come as the result of a freakish accident by a little known Italian. That other Italians, notably Tartuferi in his study of the retina (1887) could make preparations as compelling as those of Golgi made it clear that, given the correct application of the stain, nerve cells could be revealed in far greater detail than before. When Cajal reported his first observations in short communications in 1888 (Cajal, 1888a,

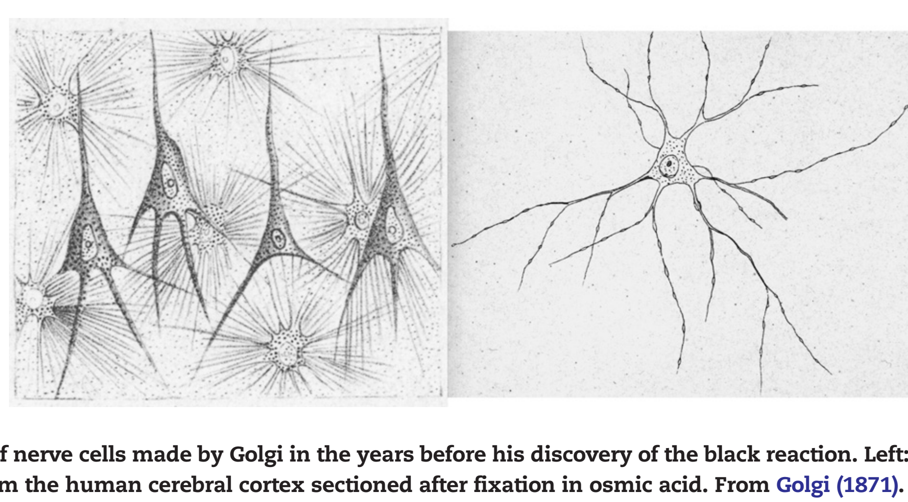

Fig. 2 – Drawings of nerve cells made by Golgi in the years before his discovery of the black reaction. Left: pyramidal cells and neuroglial cells from the human cerebral cortex sectioned after fixation in osmic acid. From Golgi (1871). Right: Ganglion cell from the retina of a horse, from a whole mount fixed in potassium dichromate and osmic acid. From Golgi (1872).

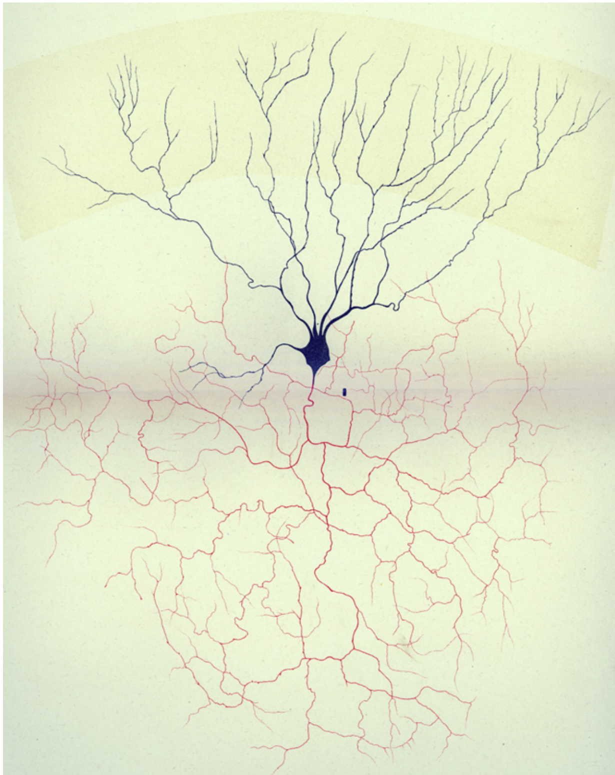

Fig. 3 – Golgi cell from the cerebellar cortex of a neonatal cat, showing the stereotypy of cell form along with the free ending dendrites and axon collaterals demonstrable by the Golgi technique. The shadow across the middle of the figure is formed by the fold in the original plate. From Golgi (1882, 1903).

b, c), he did not claim to be seeing the full extent of nerve cells for the first time but rather that he had been able to replicate the findings of Golgi on the cerebellum and of Tartuferi on the retina. His interpretations of what he saw, however, were to be quite different from those of Golgi and Tartuferi.

The facts that Golgi gave to the scientific world and that Cajal was able so brilliantly to build on, were three. Cajal himself makes a point of these in his early publications of 1888–1891 (Cajal, 1888a,b,c, 1889a,b), and amplified them in his *El nuevo concepto de la histolgía de los centros neviosos* of 1892. He was to repeat the debt that he owed to Golgi in this regard throughout all of his publications up to and beyond his great *Histologie du Système Nerveux de l'Homme et des Vertébrés* (Cajal, 1909, 1911). Although pointing out even in the earliest papers that he disagreed with Golgi's portrayal of the nervous system as an anastomotic network, and in later works disparaging Golgi for this, he never failed to remark on how Golgi's observations had led him to his conclusions about neuronal connectivity.

To Cajal, there were two fundamental observations of Golgi that led Cajal to make his new interpretations of the organization of the nervous system: (i) every nerve cell possesses an axon that gives rise to numerous very fine collateral branches (Fig. 4); and (ii) the dendrites, although branching extensively, do not form a network and end freely (Fig. 5). The collateral branches of the axon had never previously been seen and although Golgi felt that the free ending dendrites might contact blood vessels in order to gain nourishment, that was of less consequence than the lack of a dendritic network which had been emphasized by earlier workers such as Gerlach (1872). Both of these points were to form the foundations of all Cajal's work on the intrinsic circuitry of the nervous system. To them it is necessary to add a third, implicit in Cajal's writings but never stated in this form, namely that nerve cells can have a stereotyped structure that is defined by size and by the nature of the dendritic ramifications and repeated in the same region of different animals and even of different species. Without the revelations of the Golgi technique, this had hitherto been impossible fully to appreciate. Nerve cells could now be given standardized names; some such as the Purkinje cell or the pyramidal cell of the cerebral cortex were already in existence but others were novel and Cajal was to be more inventive than Golgi in this regard. Golgi's classification of nerve cells into large type I or motor and smaller type II or sensory cells was based on his determination of whether in the case of the first, the axon became continuous with a myelinated fiber of the white matter or, in the case of the second, remained within the vicinity of the cell. But it was Golgi's commitment to the network theory that led him into errors here. To take as an example the cerebellar cortical cells that Cajal was later to call basket cells, Golgi considered that their axons branched within an anastomotic plexus of collaterals within the granule cell layer from which axons emerged to enter the white matter. To him, therefore the basket cells were Type II cells. But as soon as Cajal in his first study of the cerebellar cortex in 1888 recognized that the axons of the basket cells did not immediately lose their individuality in breaking up into a network of branches but ended instead around the cell bodies of Purkinje cells, he was able to say that the only way these cells could communicate with the white matter was indirectly via the axons of the Purkinje cells. This was one of the revelations that led him towards the concept of interneuronal connections by contact and to the flow of activity from cell to cell. From this beginning he was able to describe the intrinsic circuitry of just about every region of the nervous system.

The secret of Cajal's success in the early phase of his career lay as much in his choice of regions in which to work as upon his repeated impregnation method and his choice of the brains of small and immature animals for staining (DeFelipe and Jones, 1992). It is not without significance that the first regions that he chose to study were the retina and the cerebellar cortex (Cajal, 1888a, b, c; Cajal, 1891) for here the stereotypy of cell types, their arrangement in distinct layers and the evident flow of information from input to output in each gave him the opportunity to view them from a circuit based perspective. He was led to these regions by the work on the retina of Dogiel (1888) and Tartuferi (1887) who, in using methylene blue or Golgi staining, had identified the layer specific cell types and by that of Golgi (1882) who had done the same for the cerebellar cortex. In recognizing first the free endings of the basket cells on Purkinje cells in the cerebellum (Cajal 1888c) and of the bipolar cells of the retina on the dendrites of ganglion cells (Cajal 1888a,b), Cajal was led immediately to his view of connections by contact and to the cellular basis of connectivity. His viewpoint was further developed after his discovery that the granule cells of the cerebellum gave rise to the parallel fibers which Golgi had not seen (Cajal 1888c) and his identification of the mossy and climbing fibers (Cajal 1889a), for their organization allowed him

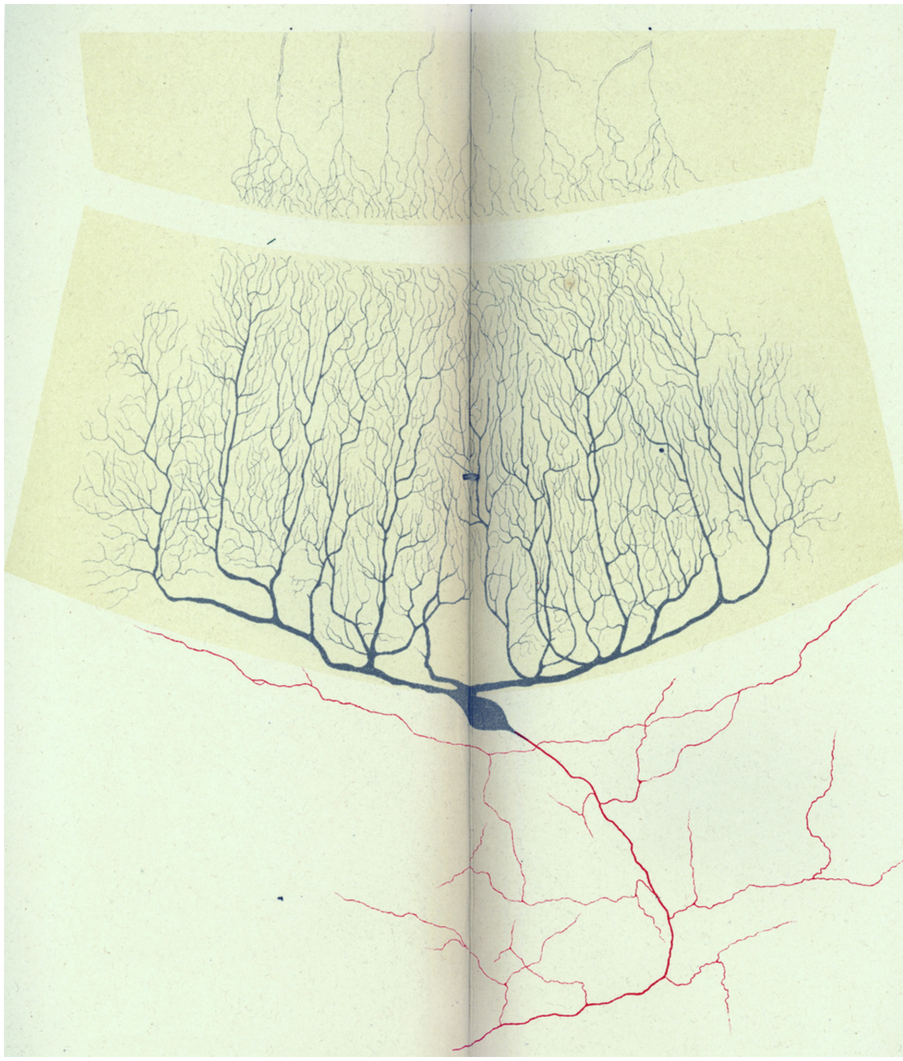

Fig. 4 – Purkinje cell from the human cerebellum, stained by the Golgi technique and showing for the first time the planar dendritic tree and the axon collaterals. From Golgi (1882, 1903).

to plot the input–output connections of the cerebellar cortex. It is not surprising that in the 45 papers published by Cajal between 1888 and 1891 when the Neuron Doctrine was enunciated by Wilhelm Waldeyer, the word "connections" appears either in the title or as part of a lengthy subsection of every paper (Jones, 1994).

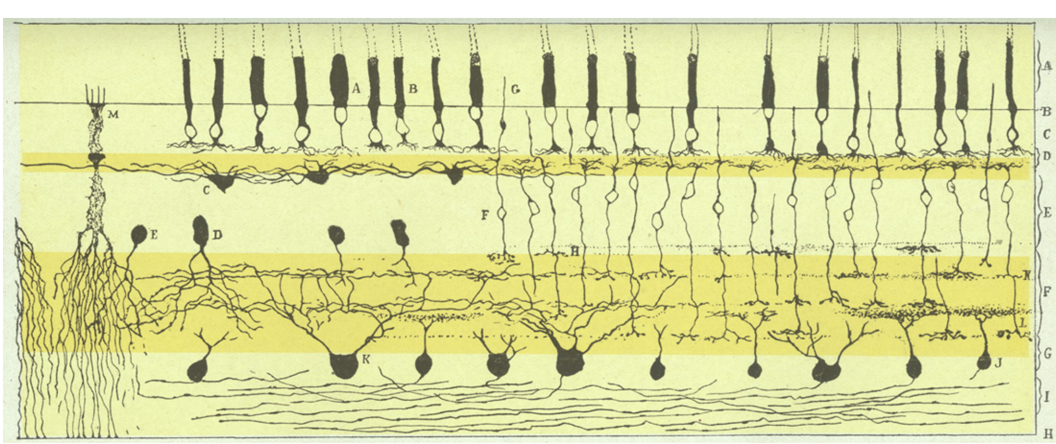

Fig. 5 – One of Cajal's first drawings of a Golgi preparation, from the retina of a chicken, demonstrating the links made by the bipolar cells between the photoreceptors and ganglion cells. From Cajal (1888).

If it was the stereotyped forms and locations of nerve cells in the retina and cerebellar cortex that gave Cajal the base upon which to build his connectionist view of cerebral circuitry, when he turned in 1890 to the cerebral cortex, it was the axon collaterals that gave him a basis for building circuits therein (Cajal, 1891). He was impressed by two types of collaterals: those that branched off fibers in the white matter and ascended into the cortex while the parent axon continued on in the white matter, and those given off by cells in the cortex itself. The first included callosal, corticocortical and thalamocortical fibers while the second formed elements of intracortical circuitry (Cajal, 1891, 1892, 1894, 1899a,b). In his early papers on the cortex, Cajal can be quite tiresome in his enthusiastic descriptions of collaterals.

Golgi, in his studies on the cerebral cortex, had identified the pyramidal cell as the source of fibers leading from cortex to white matter and had demonstrated the presence of numerous intracortical collaterals on these fibers (Fig. 6). These collaterals were to form an important element in Cajal's thinking about cortical circuitry. In his first studies on the cortex Cajal emphasized the pyramidal cell as the source of output from the cortex and, on identifying the terminations of the fibers coming from the thalamus, proceeded to investigate how these inputs might be linked to the output cells by the intracortical connections formed by the collaterals of pyramidal cell axons and by the short axons of intrinsic neurons. His first proposals on this subject appeared in the *Nuevo Concepto* of 1892 and in his Croonian lecture of 1894 and were elaborated further in his *Textura del Sistema Nervioso del Hombre y de los Vertebrados* (Cajal, 1899, 1904) and its French translation, the *Histologie* (Fig. 7) (DeFelipe and Jones, 1988). In summarizing his views in the latter two works, Cajal identified a series of intracortical and corticocortical "arcs" intercalated between "the pathways of reception and

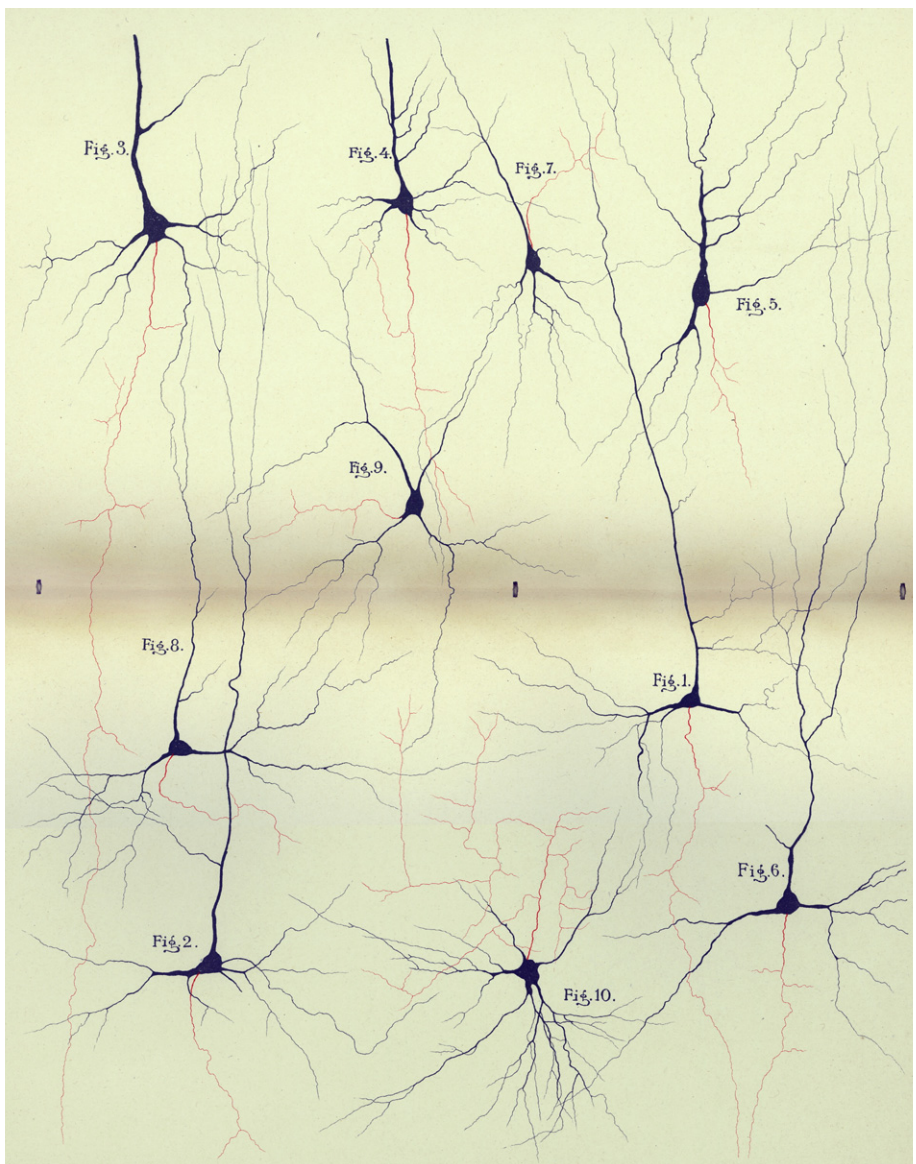

Fig. 6 – Pyramidal cells and non-pyramidal cells stained by the Golgi technique in the human cerebral cortex, showing the axon collaterals on the pyramidal cells. From Golgi (1882, 1903).

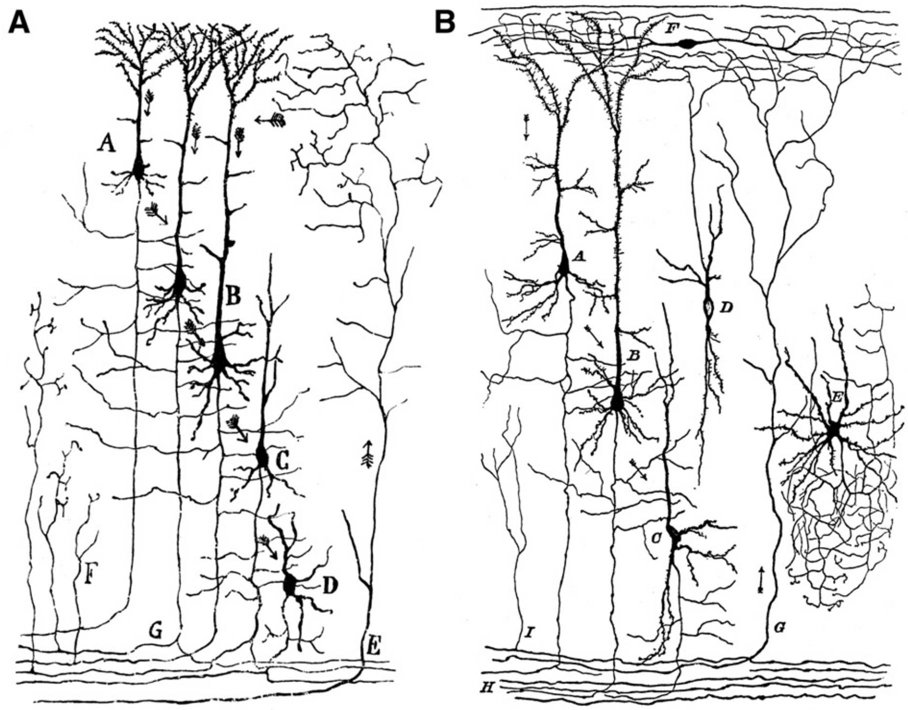

Fig. 7 – Early drawings made by Cajal in order to illustrate his views of the circuitry of the cerebral cortex, with his typical arrows indicating the direction of impulse propagation from cell to cell by means of the collaterals of pyramidal cell axons. A is from his *Nuevo concepto* (Cajal 1892) and B is from his Croonian lecture (Cajal 1894). In these days before his identification of the thalamic afferents he draws the afferent fibers (E in A, G in B) as reaching layer I of the cortex. In this case he was probably visualizing corticocortical or callosal fibers. Collaterals of the white matter are represented by F in A and I in B.

emission", that is between the thalamocortical fibers and the pyramidal cells. As he saw it, "the single channel represented by an afferent fiber is broken down into an infinity of secondary channels that traverse almost the whole gray matter of the hemispheres along variable radii". The first "short or principal arc" (Fig. 8) is made up of the afferent axon ending directly on the dendrites of pyramidal cells mainly in the third and fifth layers, and by this direct route sensory impulses are rapidly transformed into reflex outputs such as movements. But the communication is not between single elements but between a group of afferent fibers and a more numerous constellation of pyramidal neurons. This leads to an "avalanche of conduction" that is further amplified and extended to other output neurons by the collateral branches of the projection axons that spread excitation to other cells of the same layers (Figs 7, 8). The second, "intragriseal arc with intercalation of cells with ascending axons" (Fig. 9), begins with terminations of the afferent fibers upon small pyramidal and stellate cells located in layer IV (D in Fig. 7B) whose ascending axons contact the dendritic tufts of layer III and V pyramidal cells. The collaterals of the pyramidal cells will carry the activity to other groups of pyramidal cells located in the same gyrus but at a considerable distance from the territory of arborization of the afferent fiber. This arc of activity would be further extended by the intercalation of the axons of horizontal cells located in layer I (F in Fig. 7B) carrying the activity to even more distant pyramidal cells, including those located in other gyri. The third "intraareal intragriseal arc" (Fig. 10) is formed by pyramidal cells whose axons terminate in the association areas of the same hemisphere, extending the influence of the original afferent input to areas concerned with higher level cortical function. An extension of this arc is the "interhemispheric arc" whereby activity is carried across the corpus callosum by the axons of pyramidal cells.

Cajal emphasized, however that the role of the non-pyramidal cells whose short axons remained within the cortex was more than to form a link between input fibers and output cells. If this were so, he says, then the arrangement of the axons of these cells would direct the afferent impulses into many "useless detours". Rather, he saw the short axon cells as serving as "condensers or accumulators of nervous energy" which would serve to amplify impulses passing through the various arcs described above and perhaps convert it into some kind of lingering trace that persists after the initial activity has passed. Not a bad idea when made in the absence of knowledge about inhibition or long term synaptic plasticity.

Golgi's gift to Cajal, who called him without condescension "the savant of Pavia", cannot be underestimated and should be viewed from a perspective that is unencumbered by their contentious squabbling over the neuron doctrine, a controversy that Cajal was only too eager to promote. When viewed from a more objective perspective, Golgi must be seen as having anteceded Cajal and to have given to the scientific world a vision of neurons and their organization in the central nervous

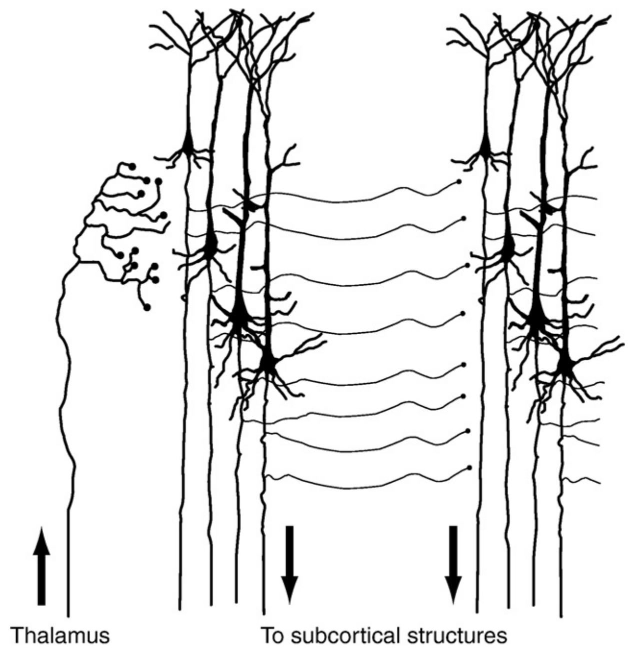

Fig. 8 – Schematic view of the first of Cajal's intracortical and corticocortical "arcs" intercalated between "the pathways of reception and emission". This first "short or principal arc" is made up of a thalamic axon ending on the dendrites of pyramidal cells, forming the most direct route for transmission of sensory messages into reflex outputs such as movements. The arc is not made up of single elements but by groups of afferent fibers connecting to groups of pyramidal neurons. This leads to an "avalanche of conduction" that is amplified and extended to other output neurons by the collateral branches of the axons of the pyramidal cells.

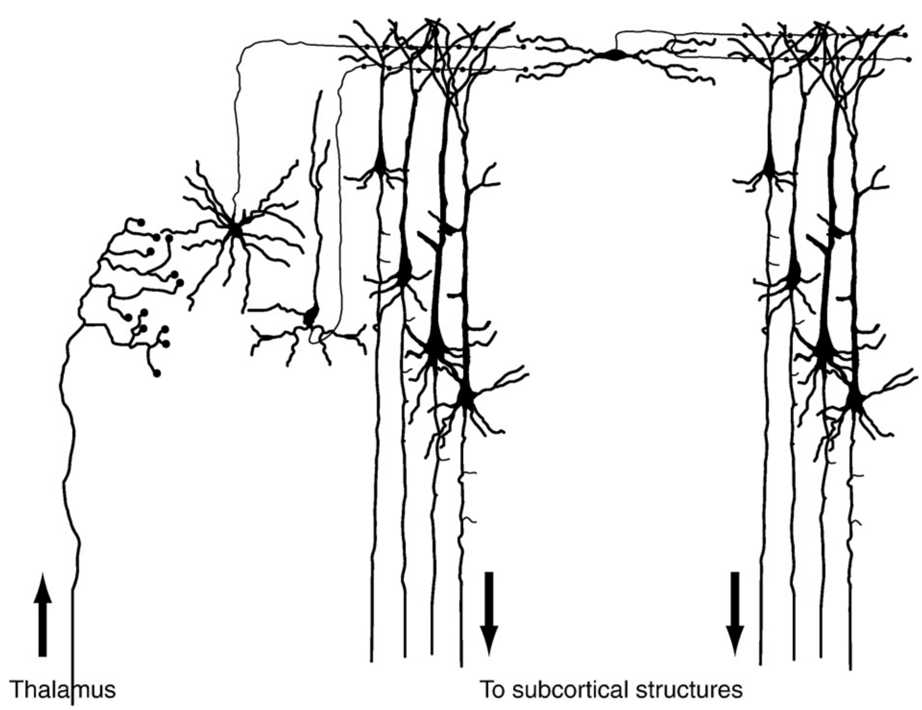

Fig. 9 – The second of Cajal's intracortical and corticocortical "arcs". This "intragriseal arc with intercalation of cells with ascending axons" begins with terminations of thalamic fibers upon small pyramidal and stellate cells whose ascending axons contact the dendritic tufts of layer III and V pyramidal cells. The collateral axons of these cells, as shown in Fig. 8, would carry the activity to other pyramidal cells located in the same gyrus but at a considerable distance from the terminations of the afferent fiber. This activity would be further extended by the intercalation of the axons of horizontal cells located in layer I carrying activity to even more distant pyramidal cells, including those located in other gyri.

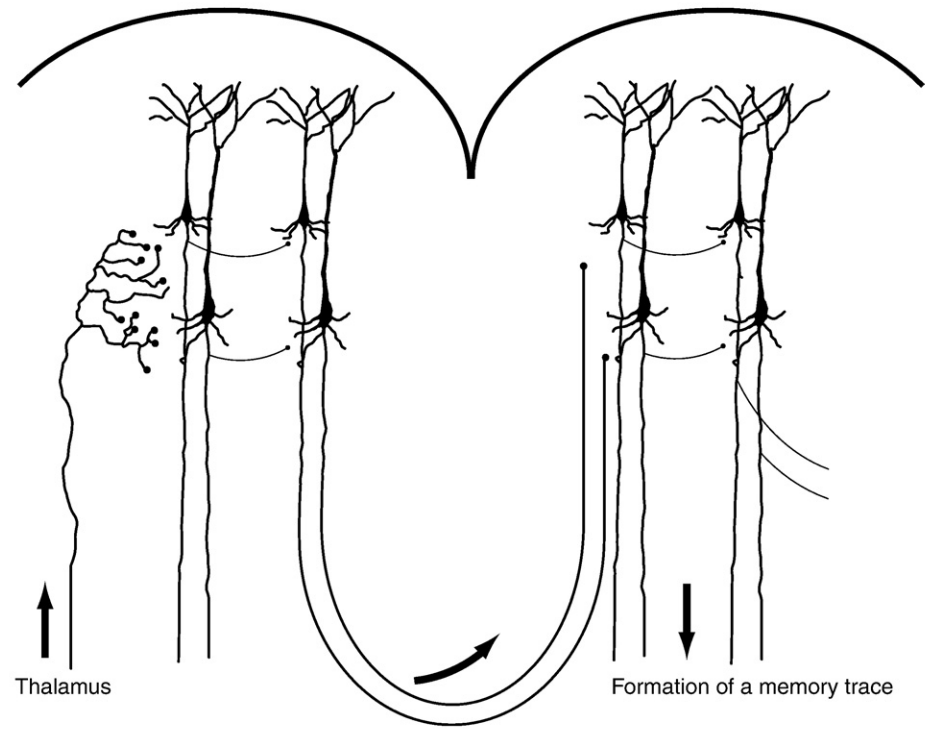

Fig. 10 – The third of Cajal's intracortical and corticocortical "arcs". This "intraareal intragriseal arc" is formed by pyramidal cells whose axons terminate in the association areas of the same hemisphere, extending the influence of an afferent input to areas concerned with higher level cortical function in the same hemisphere. An extension of this arc is the "interhemispheric arc" whereby activity is carried across the corpus callosum by the projecting axons of the pyramidal cells.

system that had only been dimly glimpsed with earlier staining techniques. In the course of his rather brief period of working on the central nervous system, Golgi provided the details about nerve cell structure, dendritic organization and axonal branching that served as the launching platform for Cajal's investigations and the fundamental basis on which Cajal was to build up those patterns of intrinsic cerebral circuitry that have been modified only in minor details to this day.

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