

Guest editorial

Nobel stains

A century ago, Camillo Golgi (figure 1) and Santiago Ramón y Cajal (figure 2) were jointly awarded the Nobel Prize for medicine. Golgi (1873) devised a novel technique for staining nervous tissue; it consisted of hardening the preparation in potassium bichromate and then impregnating it with silver nitrate. The subsequent black reaction (*reazione nera*) exposed the networks of nerves in grey matter in a manner that had not been possible previously.

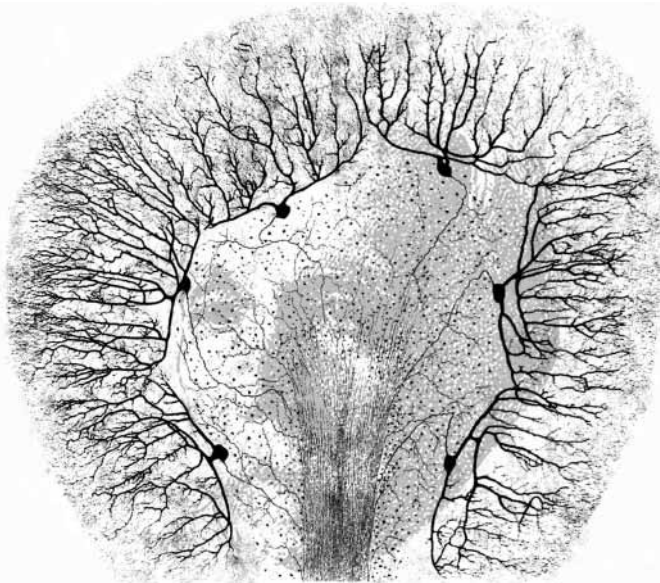


Figure 1. *Golgi stain* by Nicholas Wade. A portrait of Camillo Golgi (1843–1926) embedded in a network of stained Purkinje cells as drawn by Golgi. The original diagram is in colour and can be seen on the *Perception* website at <http://www.perceptionweb.com/misc/p3501ed>.

Golgi was born at Corteno near Brescia in northern Italy and studied medicine at the University of Pavia, where he was appointed Professor of Histology in 1876 and where he passed all his academic life (see Mazzarello 1999). Cajal was born at Petilla a small village in the region of Aragon in Spain. Both Golgi and Cajal had conflicts with their parents in the choice of their career. Golgi's interests were in scientific research but his father encouraged him to become a hospital doctor. Camillo, however, continued histological research in a small home laboratory while serving as chief doctor in a hospital in Abbiategrosso near Milan and it was there that he succeeded in discovering the *reazione nera*. Cajal, on the other hand, displayed skills at drawing from an early age and wished to develop them as an artist but he was persuaded by his father to study medicine at the University of Saragossa. He obtained his degree in medicine from the University of Madrid in 1877 and later occupied chairs of anatomy and histology at the universities of Valencia, Barcelona, and Madrid (Cajal 1923, 1937).

The black reaction transformed histological studies of the nervous system, and was extensively used by Golgi and by his students. After having been first exposed to the process in 1887, Cajal adopted and adapted it, and in the next year he published the results of his initial investigations in the cerebellum and in the retina (see Piccolino 1988).

In the cerebellum he was able to establish the individuality of the cellular elements and, moreover, to recognize the presence of an ordered pattern of connectivity. Of particular relevance for his further research were the connections involving the Purkinje cells, at both their rich dendritic region and at the soma—with the arborizations of climbing fibres and with the basket-like terminations of the cell or the molecular layer.

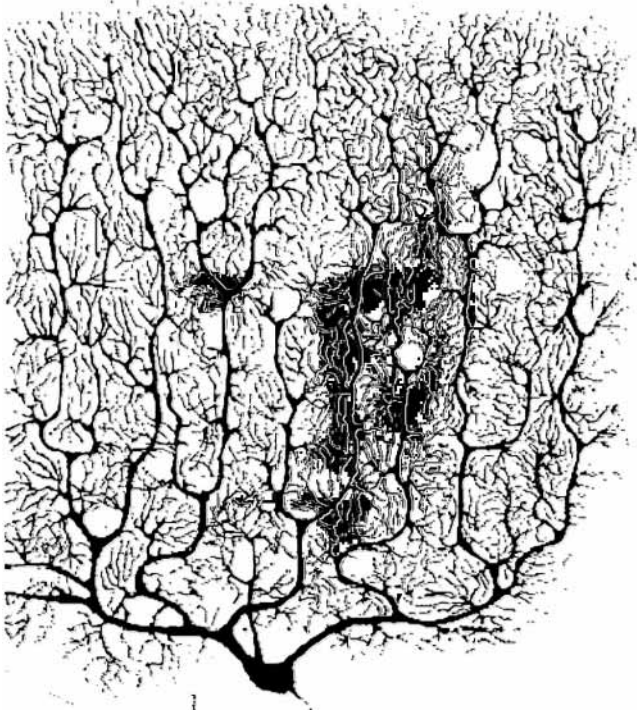


Figure 2. *Purkinje cell* by Nicholas Wade. A portrait of Santiago Ramón y Cajal (1852–1834) embedded in his diagram of a Purkinje cell.

It is a strange irony of science that Jan Evangelista Purkinje or Purkyně. (figure 3) did not experience the beauty of the cells with which his name is so closely linked. He was able to distinguish them principally on the basis of their large cell bodies, and he did not see the tree-like structure that was exposed to the gaze of both Golgi and Cajal with the aid of the silver staining method.

Purkinje cells, and the cerebellar structure, played an important part in the fierce debate that was raging about the structure of the nervous system. On the one hand, the ‘reticularists’ argued that the nervous system was a single interconnected and continuous network. This was the general position supported by Golgi who, however, proposed a personal view of the reticular theory, in which the connections among nerve elements was represented by an intricate network made up of fine arborisations stemming exclusively from the axons (see figure 4). On the other hand, the nerve cells (or neurons, as Wilhelm Waldeyer called them in 1891) were considered to be independent units which communicated by electrical signals along well defined paths and in a ‘dynamically polarized’ way: the signals passed from the dendro-somatic region of one cell via its axon terminal to the dendro-somatic region of the next cell. Cajal started elaborating this view after his first studies applying the Golgi technique to the cerebellum, and had to defend it for much of his scientific life.

Although using the same staining method, and in spite of their extreme skillfulness as observers, Cajal and Golgi interpreted the images of cerebellar structure as evidence

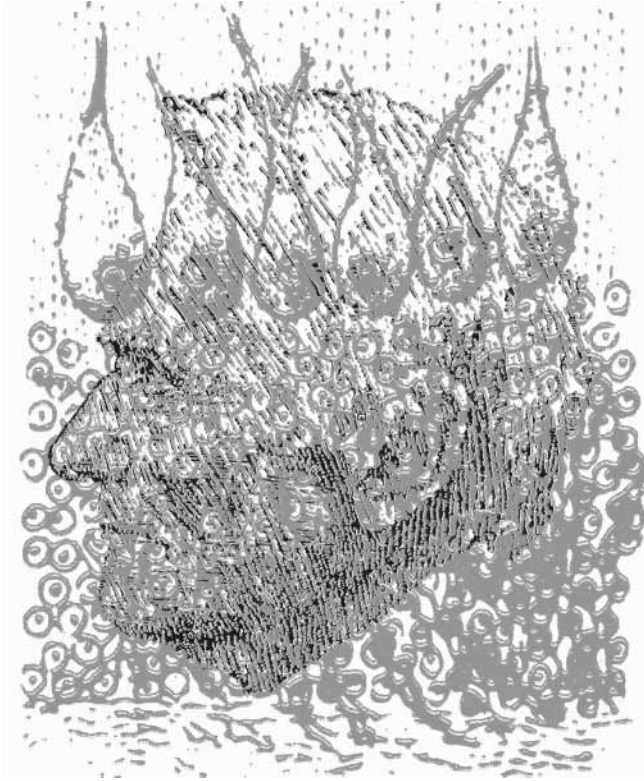


Figure 3. *Purkinje cells* by Nicholas Wade. A portrait of Jan Evangelista Purkinje or Purkyně. (1787–1869) embedded in his initial representation of the cells (in 1837) that bear his name.

of opposite views of the basic organization of the nervous system. Where Golgi saw an intricate network connecting the axonal arborizations of a variety of nerve cells, Cajal identified specific and well organized intercellular contacts between individual cells allowing for a circumscribed and unidirectional flow of the nervous signal (compare figures 4 and 5).

The retina, the second structure studied by Cajal, would be seen by him as providing strong evidence in favour of the neuron doctrine. In particular it gave him important clues for the identification of the direction of signal transmission at the interneuronal contacts. This was because of its relatively simple structure and of the evident direction of visual signal flow from photoreceptors toward the ganglion cells. However, it was one of Golgi's students, an ophthalmologist, Ferruccio Tartuferi (1852–1925), who first applied the silver staining technique to the retina (Tartuferi 1887), and his beautiful illustration is shown in figure 6. The horizontal and amacrine cells are clearly represented, as are the rods, cones, and bipolar cells. Despite the clear representation of the constituent parts of the retina, Tartuferi retained his teacher's view that the nervous system was a connected and continuous unit by assuming that nerve signals circulated laterally in the retina through two netlike structures—the '*rete sottoepiteliale*' (subepithelial net) below the photoreceptor endings, and the '*strato reticolare interno*' (internal reticular layer) situated at a more vitreal level.

When Cajal viewed the same structure he did not represent it in the same way (figure 7). He took the principal distinction between the rods and cones to support Schultze's duplicity theory. He also sought to retain the distinction for subsequent connections with the receptors. In this regard he did find different bipolars for rods and cones in some species. The presence of lateral connections from the horizontal and

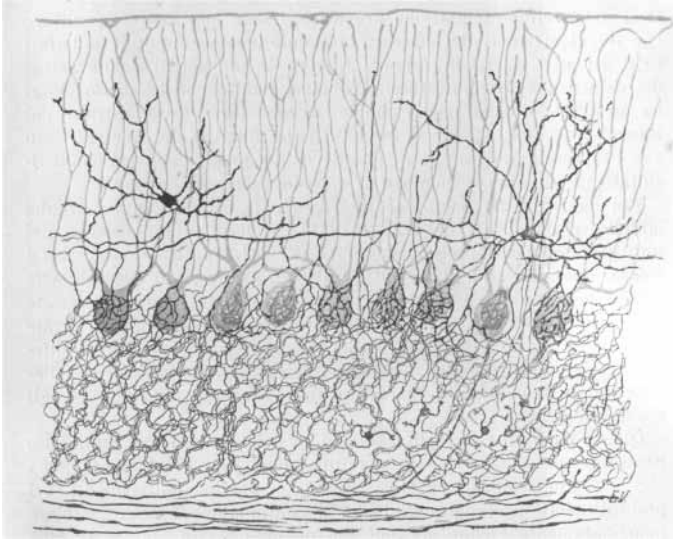


Figure 4. Image of the cerebellar cortex impregnated with the black reaction which shows the relations between the basket cells and the Purkinje cells, and illustrates the constitution of Golgi's '*rete nervosa diffusa*' (This figure was sent in 1902 by Golgi to Luigi Luciani who published it, together with Golgi comments, in his famous textbook of physiology (Luciani 1905). The original was in colour and it can be seen on the *Perception* website at <http://www.perceptionweb.com/misc/p3501ed>).



Figure 5. Cajal's image of a basket cell (on the left) and of a climbing fibre (on the right), impregnated with the Golgi method, and their respective relations with Purkinje cells (from Cajal 1909–1911).

amacrine cells presented a problem for Cajal's conception of the manner in which vision operated, and he tended to exclude these two classes of cells from the operative visual network of the retina, by assigning them 'energetic' or modulator roles. He considered that there was a point-by-point analysis of the retinal image, with the punctate pattern being retained at central sites. Lateral connections in the visual pathways could disturb this pattern of projection, and Cajal sought to see in his histology the evidence for such a sequence. It would seem that he saw what he sought by matching the microscopic appearances to his operational conception of retinal function (see Piccolino 1988; Piccolino et al 1989).

The principle of projection featured strongly in Cajal's analysis of vision. As a schoolboy, he thought he had discovered the 'camera obscura': 'There, in the darkness of the school prison, with no other light than that which filtered faintly through the cracks of the rickety window shutter, it fell to my lot to make a tremendous discovery in physics, which, in my utter ignorance, I supposed entirely new' (Cajal 1937, page 45).

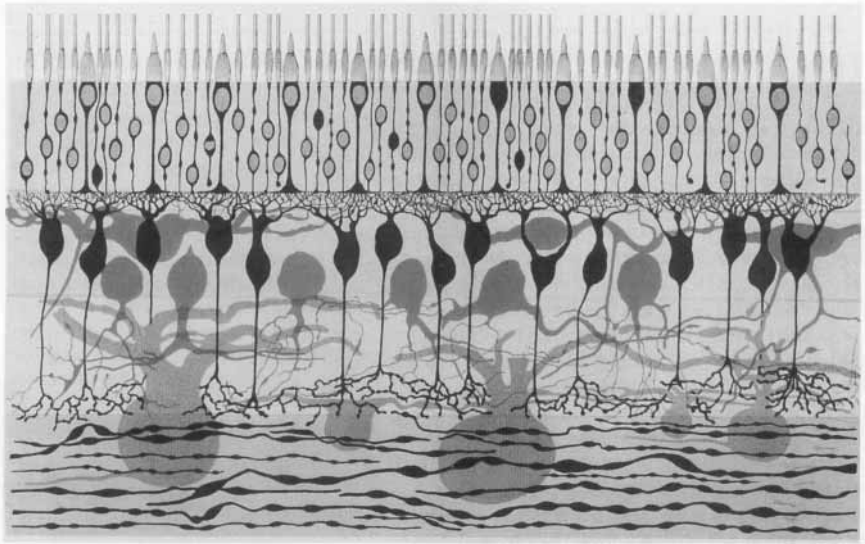


Figure 6. Tartuferi's (1887) illustration of retinal structure. The original was in colour and it can be seen on the *Perception* website at <http://www.perceptionweb.com/misc/p3501ed>.

Optical projection is also a fundamental feature of photography, with which Cajal was involved for much of his life. Indeed he developed fast-acting emulsions and he wrote a book on colour photography (Cajal 1912). His initial contacts with photographic techniques, and particularly with the development process, are poetically described in his autobiography:

“... the *revelation* of the latent image, by means of pyrogallic acid, positively stupefied me. The thing appeared to me simply absurd. I could not explain how one could imagine that in the yellow film, recently exposed in the camera, resided the germ of the wonderful drawing, capable of appearing through the action of the reducing agent. And then the prodigious exactitude, the richness of details of the plate appears as a sort of analytical exhibition whereby the sun is pleased to reproduce the most difficult and complicated things, from the inextricable entanglement of the forest up to the simplest geometrical shapes, without overlooking a leaf, a splinter, a cobble-stone or a hair!” (From Cajal's *Recollections* pages 140–141, translation from Spanish revised by M Piccolino.)

The functional paradigm of retinal and visual physiology adopted by Cajal was clearly inspired by the process of photography. Not only did he develop great skills as a photographer but he also applied them to stereoscopic vision (see Bergua and Skrandies 2000). The technique he devised (figure 8) is not unlike the principle of random-dot stereograms and had the intention of encrypting messages. Cajal (1901) described it thus:

“During my stereoscopic honeymoon, that is to say, long ago between the years 70 and 72, I was absorbed in imagining new fancies and recreations of this genre. My aim was to achieve a mysterious writing, which could only be deciphered with the stereoscope and usable for those people who don't want to divulge their own matters The game consists of making a proof [a print on glass] only with dots, lines and scribbles, or also of letters, crossed and entangles in a thousand ways. A proof in which, with the naked eye, you cannot read anything at all. And, nevertheless, as soon as you see the double image of this background in the stereoscope, a perfect legible sentence or text suddenly appears, standing out on the foreground and clearly detaching itself from the chaos of the lines or dots.” (Translated by Bergua and Skrandies 2000, page 71.)

It is mainly due to Cajal's study of retinal architecture (culminating in 1893 with his monumental memoir *La rétine des vertébrés*), that the photographic paradigm dominated physiological investigations of the retina for the first half of the 20th century. This was so despite the clear demonstration of lateral interactions within visual channels

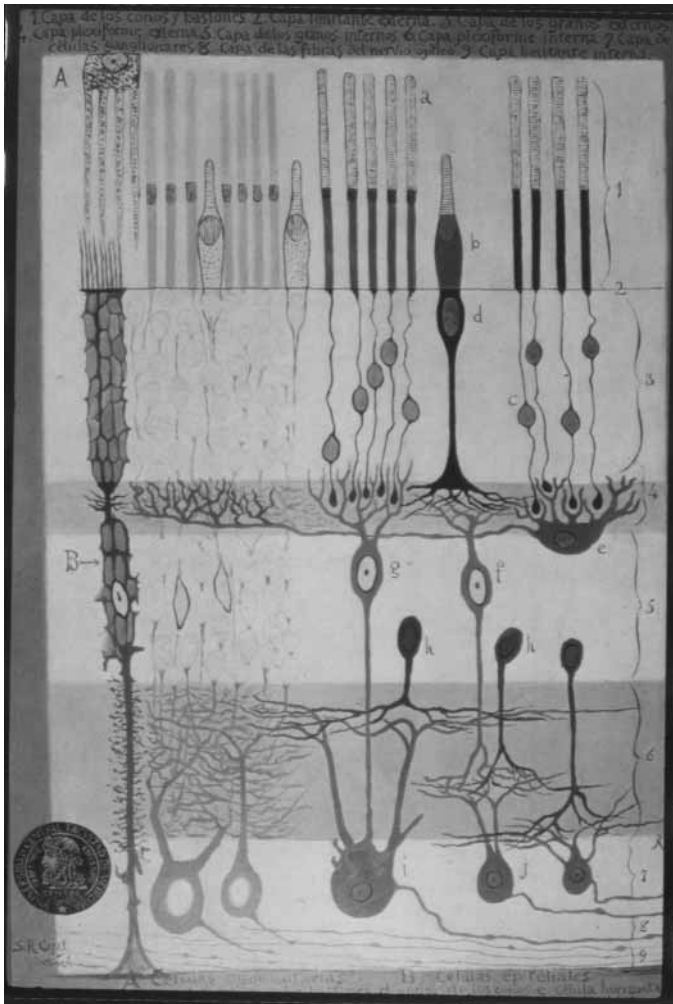


Figure 7. The structure of the retina according to Cajal. The original illustration was in colour and it can be seen on the *Perception* website at <http://www.perceptionweb.com/misc/p3501ed>.

provided psychophysically by Mach; it was only since the pioneering physiological studies of Hartline in the *Limulus* and of Kuffler and of Barlow in mammalian eye that a new conception of retinal processing slowly emerged, whereby lateral and recurrent interactions, occurring in a complex network of neuronal circuits, were considered fundamental mechanisms for visual information processing (see Ratliff 1965). Within this framework, which still dominates visual physiology, the retina seems to correspond more closely, both in its organization and function, to its name ('retina' meaning 'small net'). One might even say that nowadays the conception of retinal function seems to correspond more closely to the reticularist scheme advocated by Tartuferi (under Golgi's influence) than to Cajal's photographic paradigm!

Without any doubt Golgi and Cajal differed very strongly in their personalities and in their scientific explorations; they clearly adopted contrasting views of the microscopic organization of nervous system. These differences were stressed in a rather undiplomatic way by Golgi, who took the occasion of his Nobel lecture (delivered on 12 December 1906) to attack the neuron doctrine. On the following day, Cajal used his own lecture to provide passionate support for neuron theory. In his autobiography,

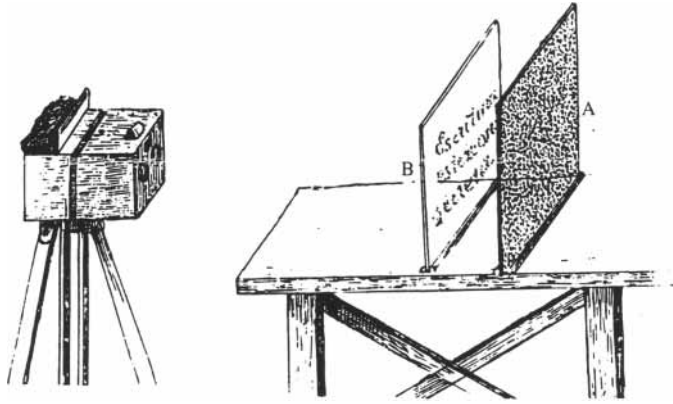


Figure 8. Cajal's (1901) technique for encoding messages stereoscopically: "Two things are necessary to perform this fantasy: the background with dots, lines, letters or entangled scribbles; and a big clean glass, where you write what you want to stand out with the stereoscope. For the illusion to be perfect, it is necessary that the thickness of the lines or the dots of what you write, be the same when you look through the scraped glass as the one of the lines, letters or dots drawn on the background." (Translated by Bergua and Skrandies, 2000, page 71.)

Cajal (1923) reflected on the deep personal and scientific differences between Golgi and himself that appeared so manifest on that Nobel prize occasion; Cajal commented on the strange irony of destiny that linked two scientific adversaries of such antithetic character as "two Siamese brothers united by the shoulder".

In spite of their obvious differences, Golgi and Cajal were similar in that both shared a deep conviction that an understanding of nervous (and psychic) function would be based on accurate and detailed investigations of nervous structure. This is the fundamental legacy that they left for the development of neurosciences in the 20th century. Moreover, Golgi and Cajal belonged to countries on the fringes of mainstream scientific activity in the 19th century. With the successes of their studies, acknowledged by the award of a Nobel prize, they showed that great scientific achievements could be pursued away from the main centres of science. Cajal wrote that at the beginning of his academic career he started his experimental investigation with the aim of initiating for Spain 'a scientific emancipation following the paths whereby the young Italy did succeed in shaking off the tutelage of French and German science' (1889, page 8). It could be said that the 1906 Nobel prize realized Cajal's juvenile dream beyond his wildest expectations.

The scientific importance of both Golgi's and Cajal's work has been immense, and, even though Cajal's views of the nervous system did largely prevail over those of his adversary, it would be difficult to decide which was the more prominent scientific personality. This is particularly so in view of the importance of Golgi's researches in other fields (we could just point to Golgi's discovery of one of the fundamental cell structures, the Golgi apparatus as well as the Golgi tendon organ, not to mention anything of his fundamental study of malaria cycle).

However, there is one aspect in which Cajal was clearly superior to Golgi: this was in writing, where the Spaniard showed a superior literary talent, that led to the production of a variety of books of personal reflections more or less connected to science, that can still be read with interest. An example of this talent, which is evident in his scientific writing, is the manner in which Cajal narrates (in the French edition of his histology textbook) the way Golgi might have invented his *reazione nera*:

"A small block of nervous tissue left from several days, hardening in Müller fluid alone or mixed with osmic acid. Because the histologist was distracted, or because of a scientist's curiosity, it was immersed in a bath of silver nitrate. One sections the block, dehydrates the sections, clears them, and examines them. Surprising sight! Against a perfectly translucent,

yellow background, appear, thinly dispersed, the black filaments, either smooth and delicate or spiny and thick; the black cell bodies, triangular, stellate, fusiform. They might be drawings done with India ink on transparent Japanese vellum. One is taken aback; the eye is accustomed to the inextricable tangles seen in sections stained with carmine or hematoxylin, where the mind strains in prodigies of criticism and interpretation, always in doubt. Here everything is simple, clear without confusion. Nothing more to interpret. One only needs to see and to record this cell with multiple ramified branches, covered with a fuzz like hoarfrost and encompassing with their undulations an astonishingly large space; or this smooth and uniform fiber, which arises from the cell, extending for enormous distances, and suddenly bursting into a spray of budding fibers; or that cell body confined to the ventricular wall and sending a process to ramify at the very surface of the brain; or other stellate cells, resembling feather starfish or the Daddy long legs; amazed, the eye cannot break off from looking! The dream technique is a reality! The metallic impregnation has produced a subtle dissection, more than one dared hope for. This is the Golgi method." (Ramón y Cajal as translated by Sanford Palay in Piccolino et al 1989, page 123.)

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