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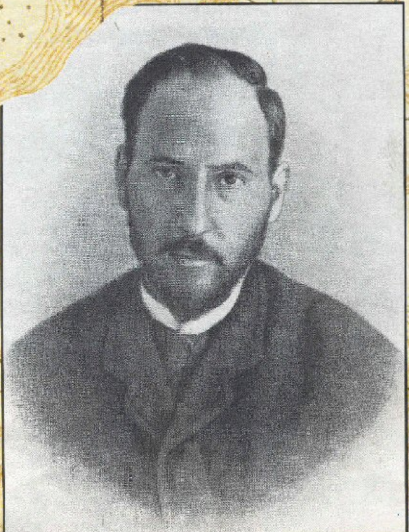

Cajal and the Retina

Cajal and the retina: a 100-year retrospective

Marco Piccolino

One century ago, Cajal published his first studies on the retina using the Golgi method. This work represents a milestone in the birth of the field of neuroscience. From these studies on the retina, Cajal drew important conclusions about the basic principles of the organization of the nervous system, leading to his theories of the neuron doctrine and the 'dynamic polarization' of nerve cells. Drawing largely from his autobiography, I have tried to revive the time in which Cajal started his studies with the Golgi methods, and point out the importance of his theoretical formulations. At the same time, however, I consider some of the difficulties Cajal encountered in interpreting the cellular architecture of the retina and its function. The way in which Cajal dealt with these difficulties reveals interesting aspects of his personality and also sheds light on the mechanisms underlying the progress of understanding in science.

Cajal and the Golgi method

An important event for the development of modern neurobiology took place in Madrid in 1887 in the house of Luis Simarro, a brilliant psychiatrist interested in histological research[1]. Dr Simarro had just returned from Paris, and showed specimens of the nervous system stained with new techniques to a young colleague, Santiago Ramón y Cajal, professor of anatomy at the University of Valencia. In particular, some of these preparations were stained with silver salts, according to the *'reazione nera'* method discovered 14 years earlier by Camillo Golgi in Pavia. At the time, Cajal had only been studying the nervous system for one year, mainly to collect suitable illustrations for a book of histological techniques. His aim in writing this book was to begin a Spanish 'scientific emancipation, following the paths whereby the young Italy succeeded in shaking off the tutelage of German and French science'[2]. While visiting Madrid, he hoped to become acquainted with new histological techniques, since he realized how inadequate the ordinary methods were for studying nervous tissue. The methods he had been using only visualized the perikarya of nerve cells and the initial part of their processes, leaving the intricate forest of arborizations of nerve fibers in between a *terra incognita*. But what a splendor now shone from the microscope! In these Golgi preparations, nerve cells appeared 'coloured brownish black even to their finest branchlets, standing out with unsurpassable clarity upon a transparent yellow background'[1]. Cajal was enraptured.

After returning to Valencia, he began to use the Golgi technique, and started work that was to lay down the foundations of modern neurobiology. In a feverish burst of activity, he first studied the organization of the cerebellum, continuing research he had already begun with previous, unsatisfactory methods. To his surprise, he observed that the axons of nerve cells came in contact with the dendrites and cell bodies of other nerve cells, although each cell remained a separate entity; these findings were difficult to reconcile with the prevailing views on the organization of the nervous system. The 'reticular theory' of Gerlach maintained that the arborizations of nerve cells established anastomoses and were truly continuous with each other, giving rise to a single network linking the entire nervous system (*'Nervenfasernetz'*). Golgi modified Gerlach's initial conception by proposing that dendrites do not take part in nervous conduction, since he had demonstrated that these processes end freely[3] without contributing to the nervous network (his *'rete nervosa diffusa'*), which he considered to be the conductive element. He suggested therefore that perikarya and dendrites have only trophic functions. This is why Cajal was surprised to observe, in the cerebellum, axons that ended in contact with dendrites or cell bodies, implying a conductive function for these structures, and was further surprised to find that nerve cells were not continuous.

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The retina and the neuron doctrine

Eager to confirm his observations in other central neural tissues, Cajal decided to work on the retina, which he considered to be particularly suitable for studying the basic organization of the nervous system. As he later stated in his classic article *La Rétine des Vertebrés* published in 1893[4], the retina is an advantageous structure for the neurobiologist because of its accessibility, its orderly organization in alternate layers of cell bodies and intercellular contacts, and the easy identification of the main direction of the nervous message flow. After his initial observations on the avian retina, published just a century ago[5], the pace of his studies on the retina built to an extraordinary crescendo in the following years (see Refs 1, 4). Indeed his interest in the retina would never diminish, being 'the oldest and most persistent of [his] laboratory loves'[1]. These studies were decisive for Cajal's formulation of his laws on the morphological and functional organization of the nervous system. Besides stating that nervous tissue, like any other tissue, consists of individual cells, or 'neurons' (a term introduced by Waldeyer in 1891), Cajal formulated the well-known principle of 'dynamic polarization'. According to this principle, dendrites and cell bodies are the receptive regions of the neurons, conducting the neural signal toward the axon, which in turn transmits it away from cell body toward other nerve cells[6,7]. This principle was of enormous importance for the study of nervous circuits during a time when such studies were based almost exclusively on anatomical evidence. According to Cajal, to trace in the main pathway of signal transmission through a neural circuit, it was sufficient to identify the soma-dendritic region and the axon of the constituent neurons. Besides conforming with experimental observations, the neuron doctrine reflected an intimate intellectual necessity of Cajal for order and precision. He could not admit that 'everything

Fig. 1. *Cajal at the time of his first histological studies.* (Taken from Ref. 28, courtesy of the Instituto Cajal, Madrid, Spain.)

communicates with everything else' in the nervous system, and that 'the paths of nerve impulses are indeterminate'[1]. It is easy then to understand Cajal's interest in the retina, a structure in which, according to his views, cellular connections should be organized with an extreme precision to suit the great analytical power of visual processing.

Cajal's studies were also fundamental for the elucidation of retinal architecture. Until Cajal, the retina had appeared as an inextricable membrane consisting of reticular and granular layers of uncertain significance, and only after his work did it become a true neural structure, in which specific classes of nerve cells convey the visual message toward the encephalic centers along well-defined paths. In particular, among the achievements of these studies were the characterization of several types of bipolar, amacrine and ganglion cells, the identification of different sublaminae in the inner plexiform layer, and the discovery of retinal centrifugal fibers and of interplexiform cells (Cajal's 'small stellate cells' of avian and mammalian retina). His contributions to retinal histogenesis were also of great importance. To emphasize the impact of Cajal's work suffice it to say that his 1893 article is still a point of departure for any anatomical study of retinal circuitry. However, Cajal encountered a series of difficulties in his studies on the retina, which I would like to analyse because they reveal important aspects of his scientific personality.

Difficulties with horizontal cells

Many of the problems he encountered concerned the cellular organization and functional significance of horizontal cells. In most vertebrates, there are two main morphological classes of horizontal cells, depending on the presence or absence of an axon (see Ref. 8). Nevertheless, Cajal only described horizontal cells with an axon, interpreting dubious features such as short, thin filaments as the initial part of axons that had been incompletely impregnated (see Fig. 2). The absence of an axon was accepted as a normal feature of horizontal cells by earlier histologists, but Cajal could easily dispose of the old views on the basis of technical inadequacies. However, the problem became more serious with the introduction of the Golgi technique and, particularly, with neurofibrillar methods, which in mammals preferentially stain axonless horizontal cells (see Ref. 8). It is difficult to avoid the idea that Cajal 'saw' only axon-bearing horizontal cells because these conformed well to the principle of 'dynamic polarization'.

Another important controversy, dating from the pre-Golgi era, concerned the presence of anastomoses between horizontal cells (and also between other retinal neurons). Previous authors had supposed that horizontal cells form syncytia. Krause, for example, considered the different layers of horizontal cells to be continuous laminae with holes for the passage of fibers of other retinal elements[9]. Tartuferi, the first to apply the Golgi method to the retina[10], claimed that horizontal cells form extensive anastomoses, thus creating a network beneath the photoreceptors (*'rete sottoepiteliale'*). Another strong supporter of continuity between horizontal cells was Dogiel, who used the methylene blue staining of Ehrlich. Cajal was opposed to these views. Within the framework of the neuron theory, he considered horizontal cells as individual elements, and regions of contiguity as contacts[4,11]. The polemic was again revived with the introduction of neurofibrillar methods, which gave the appearance of continuity between certain classes of horizontal cells[12,13]. Cajal's explanation for this was that the images were due to overstaining[13]. Of course, a definitive answer to this problem was beyond the reach of light microscopic techniques, which could not resolve the narrow space between adjacent neurons. In this case, it is difficult to see how Cajal concluded that horizontal cells were distinct entities based on such ambiguous morphological appearances. Although the 'cellular' nature of horizontal cells is now beyond doubt, it is interesting that modern studies have pointed out some syncytial characteristics of the organization of horizontal cells (see Ref. 14). Adjacent horizontal cells are connected by gap junctions[15,16], whose basic components are transcellular hydrophilic channels that establish a real continuity between the cytoplasm of communicating cells. In some types of horizontal cells, the functional unit does not correspond to the individual cell visible in Golgi preparations, but would be better represented by the syncytium stained with the neurofibrillar methods[13,17,18] (see Fig. 2) or visualized following intracellular injection of small fluorescent dyes, such as Lucifer Yellow, which permeate gap junction channels[19] (Fig. 3).

In the retinae of lower vertebrates, this syncytial nature is especially evident in the axon terminal expansions of axon-bearing horizontal cells. Cajal's attitude in describing and interpreting the nature of the intricate plexus formed by the axon terminals of the horizontal cells of the fish retina is particularly revealing of his 'neuron doctrine' bias. These axon terminals have a fusiform shape and are joined to the perikarya, situated at a more external (scleral) layer, through a very thin and rather long axon fiber, as first shown by Stell in 1975[20]. In most cases it is impossible, in Golgi preparations, to trace the entire course of the axon. Worried by the 'reticular' appearance of the network formed by these anucleate structures, Cajal claimed that these axon terminals contained cell nuclei, and considered them as a further type of horizontal cell ('internal horizontal cells', see Fig. 4). He even discussed[4] why other authors, such as Schiefferdecker, had not seen nuclei, and had referred to such formations as *'kernlose concentrische Zellen'* ('anucleate concentric cells'). Cajal actually admitted that fusiform formations without a clear appearance of a cell body were sometimes seen, but he concluded that they were the result of imperfect impregnation.

Another series of difficulties in Cajal's work concerns the general plan of connectivity of retinal neurons. Cajal assumed that the retina must be organized according to a scheme of point-to-point centripetal transmission of message generated in the mosaic of photoreceptors[7], without lateral flow of signals, and with a minimum of convergence (at least for the cone pathway). Several problems arise when one tries to fit the experimental data into this scheme. At least two classes of neurons in the retina, horizontal and amacrine cells, are organized expressly for a lateral flow of information. The arborizations of these cells spread mainly in a tangential plane and are thus perpendicular to the main flow of visual message. With regard to horizontal cells, it was difficult for Cajal to escape the conclusion that they connect distant photoreceptors. However, Cajal had an instinctive aversion to these associative connections which 'could lead to a weakening or a loss of the spatial discrimination power' of visual processing. So he suggested that horizontal cells might serve as a 'depot of nervous

Fig. 2. *The morphological appearance, in a tangential section, of horizontal cells of the rabbit retina stained by Cajal with his reduced silver method.* a *indicates what Cajal supposed was the axon. In fact, the cells stained with this method in rabbits are exclusively of the axonless type. Note the syncytial aspect of adjacent cells, which Cajal denied.* (Taken from Ref. 13, courtesy of the Instituto Cajal, Madrid, Spain.)

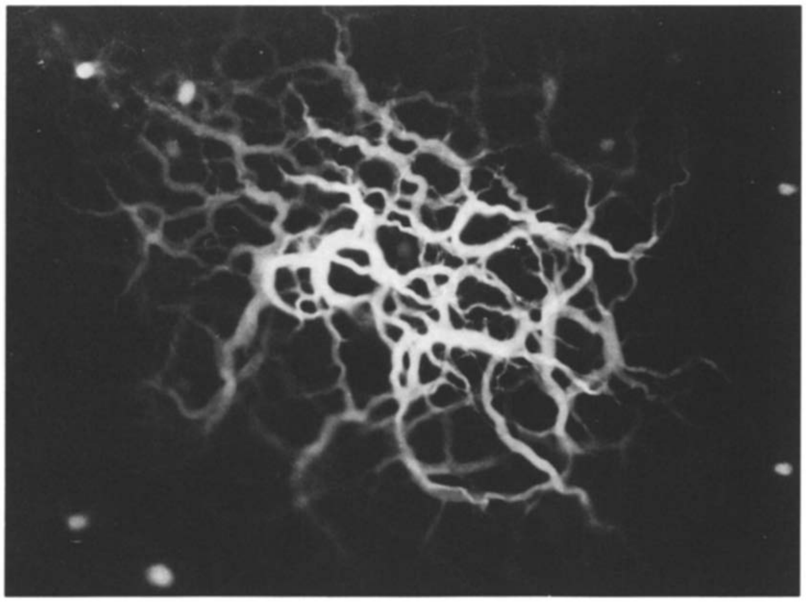

Fig. 3. *Syncytial nature of the axon-bearing horizontal cells of the turtle* Pseudemys scripta elegans *stained with Lucifer Yellow, as they appear in flat-mounted whole retina. The stain, injected intracellularly into one axon terminal in the live retina with a microelectrode, diffuses to adjacent cells through the gap junctions. Scale bar is 100 μm.* (Kindly provided by G. Demontis and M. Piccolino.)

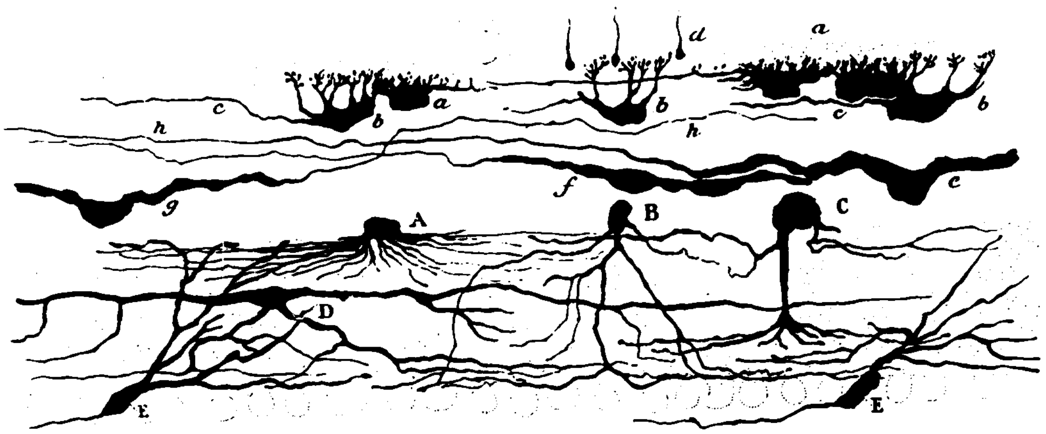

Fig. 4. *Cajal's camera lucida drawing of retinal cells of the carp as they appear in vertical section.* e, f *and* g *indicate his spindle-shaped 'internal horizontal cells', and* h *a fiber looking like an axon. Note the presence in these structures of expansions thought to be perikarya. Actually, Cajal's 'internal horizontal cells' are the axon terminals of more external horizontal cells (of the type indicated by* a *or* b *in this figure), and they lack a nucleus.* (Taken from Ref. 4, courtesy of the Instituto Cajal, Madrid, Spain.)

energy aimed to reinforce and give to the visual excitation a tension sufficient to bring it up to the centers'[7]. This was a function he ascribed to all short-axon cells found in nervous centres, and he attributed it to horizontal cells since he was unable to accept the possibility that the precise connectivity of brain structures could be wasted by the flow of neural message in local circuits[21].

Amacrine cells: 'non-visual' neurons

For the same reasons, Cajal had difficulty in interpreting the functional meaning of amacrine cells. To circumvent this problem, however, he assumed the special arrangement in the avian retina as a general scheme of amacrine cell connectivity (see Fig. 5 and Ref. 7). In the optic nerve of birds, Cajal discovered centrifugal fibers[5] ending on the cell body of a special class of amacrine cells (association amacrines), which in turn establishes contacts, through a long process, with the cell body or main process of ordinary amacrines. These amacrines would then contact the dendritic arborizations of ganglion cells. In this way, Cajal avoided the conclusion that the principal input to amacrines was from bipolar cells and that this impinged mainly on amacrine dendrites. He also avoided the idea that amacrine cells lack an obvious dynamic polarization. The processes of these cells would be functionally polarized like multiple axons in a direction away from the cell body ('cellulifugal'), conforming to Cajal's view that the shape of a neuron can be modified to suit special functional necessities[7,22].

By 'depriving' amacrine cells of a 'visual input' from bipolars, Cajal avoided what he saw to be the potential degradation of visual message resulting from lateral communication between retinal neurons. A centrifugal action having a regulatory effect on retinal transmission was easier to accept. Moreover, this line of reasoning enabled Cajal to account for the presence of large dendritic arborizations of some ganglion cell types. These arborizations could match the arborizations of amacrine cells and thus serve to receive the influence from the encephalic centers. The main 'visual' input from bipolar cells would arrive on the cell body or central dendrites of ganglion cells. This would avoid the unwanted convergence along the main line of signal flow, and thus preserve the 'differentiative power' and 'spatial sign' of retinal transmission[7].

Although many of the difficulties in Cajal's work on the retina could be partly due to the limits of his techniques, some of his interpretations described above suggest that there were other more conceptual reasons. In fact, although many features of retinal architecture corresponded well to Cajal's theoretical framework, other characteristics were difficult to reconcile with his views. Cajal seems to have lacked an unprejudiced mind when considering aspects of retinal organization that contradicted his two main conceptual principles, the neuron doctrine and the orderly connectivity plan. Cajal was

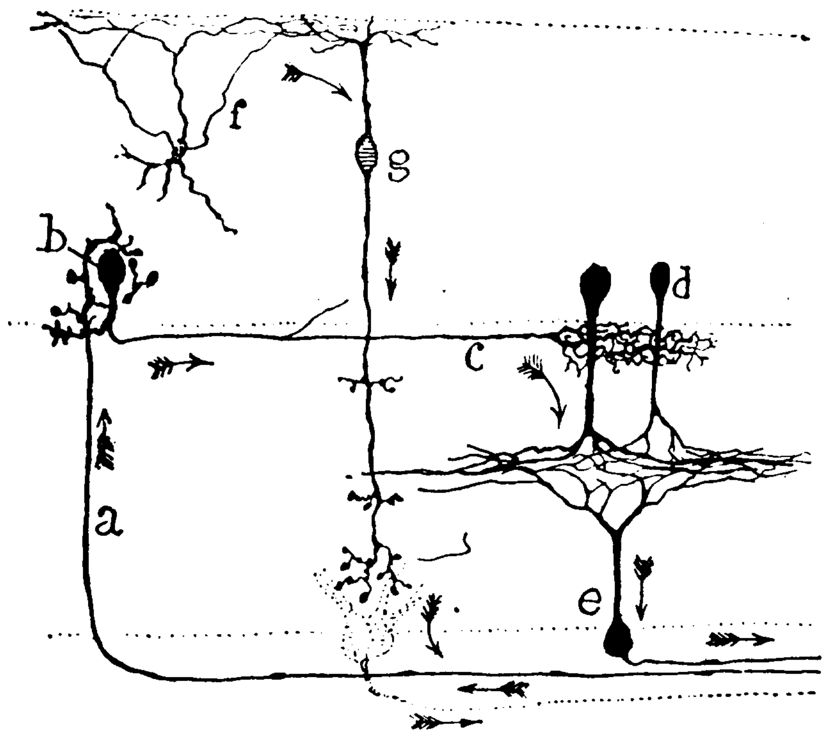

Fig. 5. *Cajal's schema of the path of nervous conduction through avian centrifugal fibers and amacrine cells (arrows from* a *to* e*).* a, *centrifugal fiber;* b, c, *association amacrine with its short axon;* d, ordinary amacrines; e, *ganglion cell.* f *and* g *indicate another path of centrifugal influence, not discussed in this article, capable of influencing neurons situated in the external layer of the retina, i.e. bipolar cells* (g) *and horizontal cells (not shown).* f *is now designated an interplexiform cell. This pathway has received great attention in recent times.* (Taken from Ref. 7, courtesy of the Instituto Cajal, Madrid, Spain.)

driven by a strong intellectual passion, quite understandable for a man who had found a way to disentangle the intricate web of the nervous system. This passion may have somewhat deviated his spirit of objective observation and criticism. From a historical analysis of the development of his thought, Cajal appears to become less open toward possible exceptions to his interpretations as confidence in his theoretical formulations grew stronger. So in 1891[6] he admitted the possibility of dendrodendritic synapses (which are inconsistent with 'dynamic polarization'), although afterwards he denied this possibility[11,22]. A similar evolution is evident in his views of retinal connectivity. However, to the merit of his profound intellectual honesty, he recognized the limits of his interpretations on the retina in an article published in his eighties, one year before his death[23]. There he discussed the 'paradox of retinal horizontal cells' and the 'enigma of amacrine cells'.

In neurobiology, as in all fields of science, progress of understanding often results from dialectic tensions. The acceptance of the neuron doctrine during the epoch of Cajal was a fundamental achievement, even if this doctrine, in its original formulation, could not account for all experimental observations. We are now in a position to recognize some minor limitations in Cajal's theories, without rejecting their fundamental value. We can now accept, for example, that neurons can form syncytia through specialized intercellular bridges (gap junctions), without losing our faith in the discrete nature of nerve cells, as Cajal 'prophetically' anticipated himself in 1896: 'Even if, in certain instances, it will be possible to demonstrate the existence of intercellular protoplasmic bridges, this shall not alter in an important way our conception of the morphology and functioning of nerve cells'[11].

Cajal lacked the operational and integrative view of the organization of the nervous system we have today, particularly since he was not aware of the existence of inhibitory interactions in neural pathways. This is why he could not account for the existence of lateral flow of signals in retinal circuits. The possibility of lateral inhibition in the retina was first recognized by Hartline in *Limulus* in the late 1940s, and some years later in vertebrates by Barlow and Kuffler (see Ref. 24). The idea that the retina, far from transmitting a precise 'photographic' replica of the optic image, operates a complex analysis of the visual message, is now universally accepted. It is generally agreed that the processes whereby the retina carries out such analysis depend largely on the existence of lateral communications between visual channels (see Ref. 25).

Integration and inhibition are physiological concepts that could not have emerged primarily from anatomical studies. Their formulation in a modern sense is based on the notion of a 'synapse', a notion whose natural ground is the neuron theory. In the first decades of this century the development of the concept of a synapse was due mainly to the work of Sherrington, a devoted admirer of Cajal. 'Nowhere in physiology does the cell-theory reveal its presence more frequently in the very framework of the argument than at the present time in the study of nervous reaction', says Sherrington opening his *Integrative Action of the Nervous System*, published in 1906[26]. As acutely pointed out by Granit[27], the phenomenological studies of nervous reactions and behavior, characteristic of the nineteenth century, could not have evolved into modern functional neurobiology without the neuron doctrine, the masterpiece of the extraordinary scientific life of Santiago Ramón y Cajal.

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