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Santiago Ramón Y Cajal, the retina and the neuron theory

MARCO PICCOLINO, ENRICA STRETTOI & ELENA LAURENZI
Istituto di Fisiologia Generale dell'Universita' di Ferrara Italy, and Istituto di Neurofisiologia del C.N.R. di Pisa, Italy

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'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-longlegs; 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' [1].

With these words in 1909 [2], Santiago Ramón y Cajal (Fig. 1, 2) described with a sense of drama and high emotion, the enchantment aroused by preparations of the nervous tissue obtained with the chromo-argentic technique, the famous "*reazione nera*" discovered by Camillo Golgi in 1873 [2]. In his autobiography, Cajal mentions even the address of the laboratory in Madrid where in 1887, for the first time, he saw histological sections prepared according to the method of the celebrated scientist of Pavia (at the '*Calle del Arco de Santa Maria*', number 41). At that time, Cajal was a young professor of anatomy at the university of Valencia (he was born in 1852). He could have spent his life 'vegetating sadly in a provincial university without passing in the scientific order beyond the category of more or less estimable delvers after details' [3]. But suddenly the year 1888 arrived, 'the year of fortune' which was a milestone in an extraordinary scientific adventure whose fruits were to lay firm foundations for neuroanatomy and, more generally, for all studies of the nervous system. Against all expectations, a long-cherished dream was realized, 'the rather chimerical idea of building up histology in Spain in spite of the indifference, when there was not hostility, of the intellectual atmosphere' [3].

One century ago, in 1888, Cajal published the first results of his studies

Fig. 1. Santiago Ramón y Cajal at the beginning of his academic career (1884).

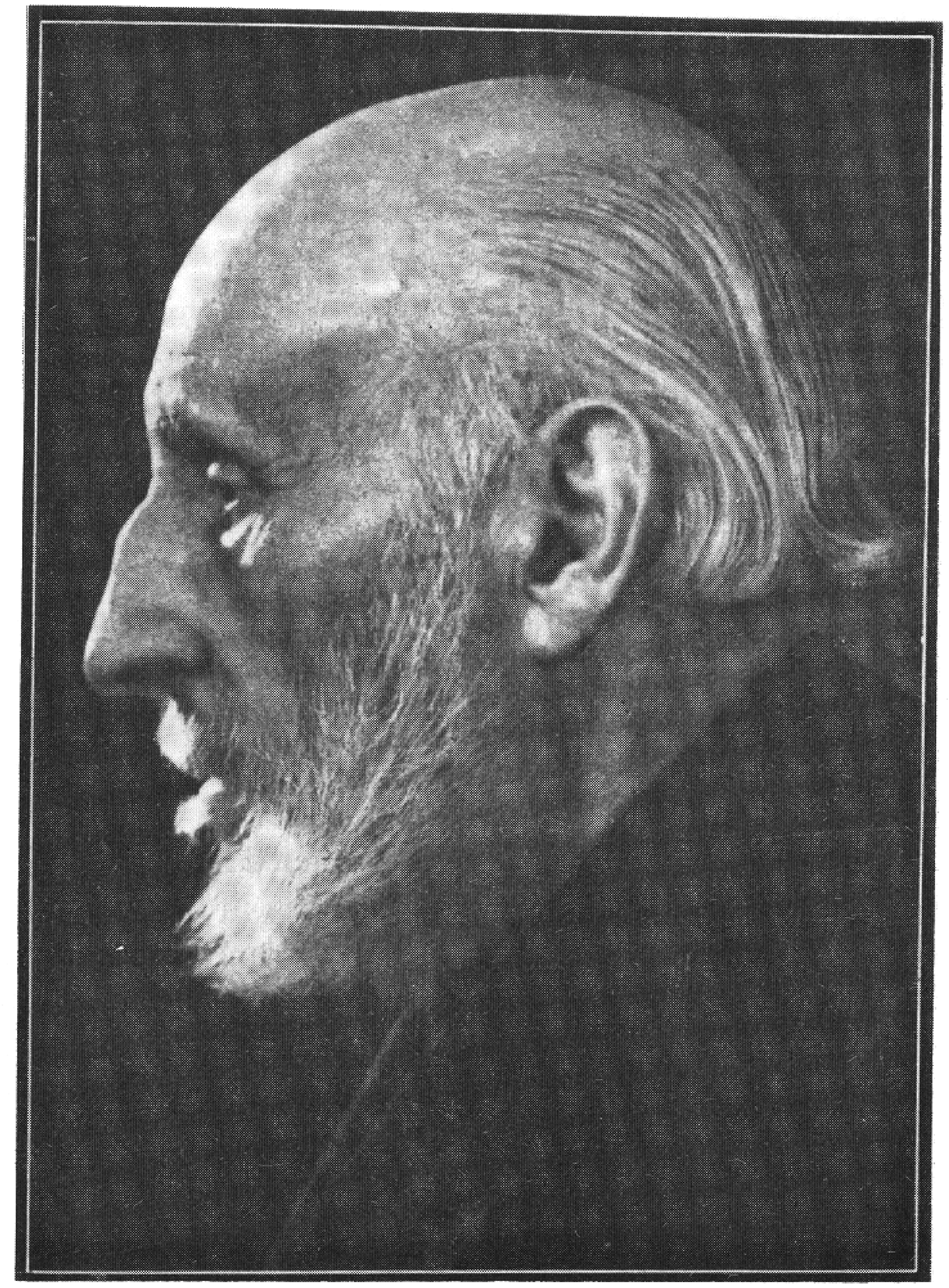


Fig. 2. Santiago Ramón y Cajal in one of the last photos of his life (From Cajal, 1923).

carried out with the Golgi method, studies which he had undertaken in a 'feverish ardour' on his return to Valencia after the short visit to Madrid where he had become acquainted with the new technique [4–7]. With an illuminating intuition, Cajal assumed that the elegant structures appearing most clearly in the Golgi preparations were nothing but the nerve cells, stained in their entirety, 'coloured brownish black even to their finest branchlets' [3]. Interpreting on this base the results of his studies, Cajal soon realized that they contradicted in some fundamental points the prevailing theories on the structural organization of the nervous system. In the cerebellum he observed that the axons of nerve cells came in contact with the dendrites and cell bodies of other nerve cells, but he saw that each cell retained in the contact its individuality ('the fortunate discovery of the *terminal baskets* and of climbing fibers' [3], Fig. 3); the "reticular theory" of Gerlach [8] viewed the arborizations of nerve cells establishing anastomoses with each another in a relation of true continuity, giving rise to a single overlying network permeating the whole nervous system. Golgi, somewhat modifying the initial conception of Gerlach, assumed that the dendritic arborizations did not enter in the constitution of this network, (his "rete nervosa diffusa"). He had shown that the dendrites end in the gray matter with free endings, and he supposed that dendrites and cell bodies were not involved in nervous conduction, but instead had a pure trophic function [9]. In contrast to the views of Gerlach and Golgi, Cajal's observations on the cerebellum showed the absence of any protoplasmic continuity between nervous elements; moreover, the existence of contacts between the axonal terminals on the one side, and the cell bodies or dendrites on the other, implied a conductive role also for dendrites and cell bodies, and contradicted Golgi's hypothesis of their exclusively trophic function.

The reasons why Golgi and other scientists adhered to the reticular theory may be found in part in the great influence exerted in the 19th century by the "non-localist" conception of the nervous function [10] supported by the studies and the authority of the French physiologist Pierre Flourens. In opposition to the "phrenology" of Franz Gall and Johan Spurzheim, who favoured an extreme localization of the cerebral functions, Flourens supported the fundamental unity of the brain.

In the opinion of Flourens, 'from the physiological point of view the brain is a unit carrying out the same functions in its totality as well in its components' [11].

Golgi quoted this sentence in an article published in 1891 in which he discussed his theory of the "Rete nervosa diffusa" [12]. In the same article, he mentioned the famous experiments of Friedrich Goltz, the physiologist of Strasbourg, who in those same years showed how important motor functions persisted in the dog after extensive cerebral ablations [13].

For Golgi, the brain activity involved the existence of an "... intimate relation between the function of the different parts of the nervous system; intimate relation having the character of reciprocity". And his "rete nervosa diffusa" appears to be suited to 'connect functionally the different parts' of the nervous system, and, at the same time, it 'seems in such contrast with the conception of the precise cerebral localizations that we would be brought to reject completely the doctrine of localizations' [12].

Contrary to the expectations of the reticular theory, the results of Cajal's

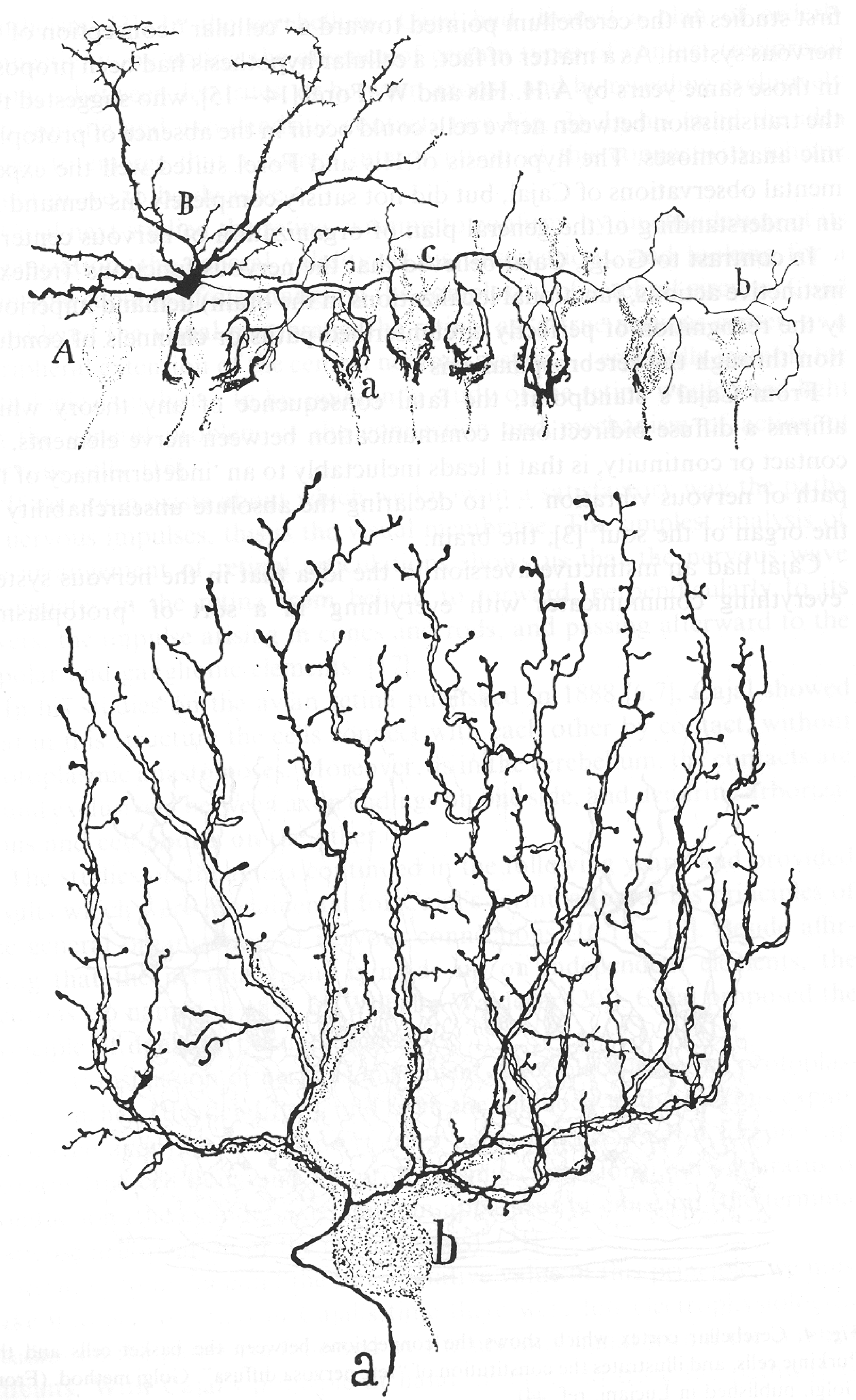


Fig. 3. Basket cell (A) and climbing fiber (B), impregnated with the Golgi method, and their connections with Purkinje cells. (From Cajal, ref. 1).

first studies in the cerebellum pointed toward a "cellular" conception of the nervous system. As a matter of fact, a cellular hypothesis had been proposed in those same years by A.H. His and W. Forel [14–15], who suggested that the transmission between nerve cells could occur in the absence of protoplasmic anastomoses. The hypothesis of His and Forel suited well the experimental observations of Cajal, but did not satisfy completely his demand for an understanding of the general plan of organization of nervous centers.

In contrast to Golgi, Cajal believed that the nervous functions '(reflexes, instinctive actions, functional localizations in the brain) demand imperiously the recognition of perfectly circumscribed paths or channels of conduction through the cerebrospinal axis'.

From Cajal's standpoint, the fatal consequence of any theory which affirms a diffuse bidirectional communication between nerve elements, by contact or continuity, is that it leads ineluctably to an 'indeterminacy of the path of nervous vibration ..., to declaring the absolute unsearchability of the organ of the soul' [3], the brain.

Cajal had an instinctive aversion to the idea that in the nervous system 'everything communicates with everything' in a sort of 'protoplasmic

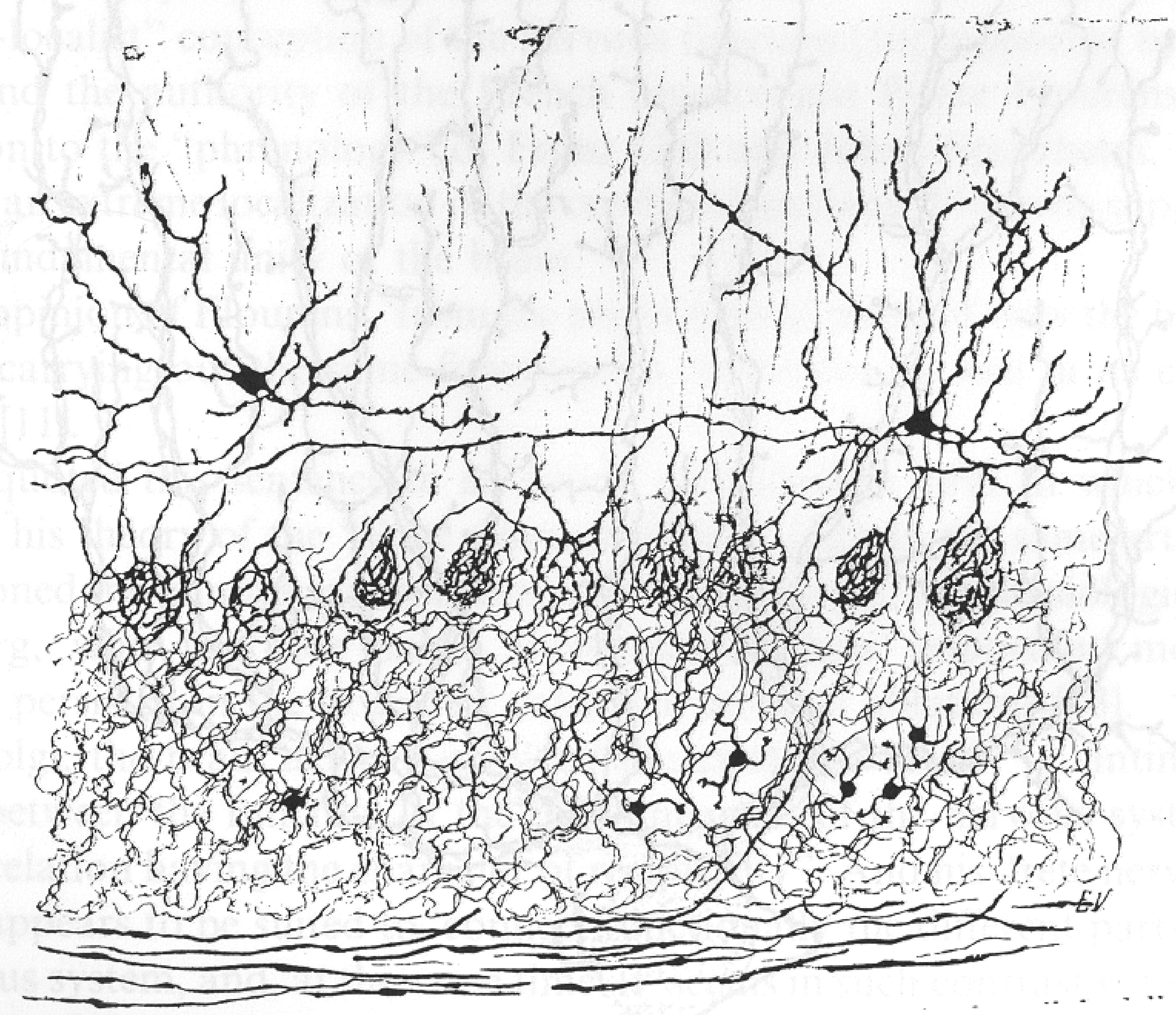


Fig. 4. Cerebellar cortex which shows the connections between the basket cells and the Purkinje cells, and illustrates the constitution of "rete nervosa diffusa". Golgi method. (From Golgi, published in Luciani, ref. 44).

pantheism' [3]. In the cerebellum, Cajal had divined a plan of orderly connectivity, by noting the absence of certain types of contact (reciprocal contacts between dendrites, or between axons), and by revealing exclusively axo-somatic and axo-dendritic contacts (see Fig. 3). In his mind the idea began to emerge that a correct interpretation of this connectivity scheme might prove to be decisive.

Cajal next studied the retina, wishing to confirm, by an elucidation of its organization, the general validity of his observations, and looking for a principle capable of disentangling the complexity of cerebral circuits. Cajal considered the visual membrane, the retina, as 'a true nervous center, as a peripheral extension of the central nervous system ... especially suitable for histological analysis'. In his opinion 'a study of the retina would shed light on the general problem of the connection and mechanism of action of nervous cells' [16].

'If an organ exists about which we know in a satisfactory way the paths of nervous impulses, this is the visual membrane. The simplest analysis of the arrangement of retinal articulations shows us that the nervous wave propagates in the retina from behind to forward, perpendicularly to its layers, the impulse arising in cones and rods, and passing afterward to the bipolar and ganglionic elements' [17].

In his studies on the avian retina published in 1888 [6,7], Cajal showed that in this structure the cells connect with each other by contact, without protoplasmic anastomoses. Moreover, as in the cerebellum, the contacts are found exclusively between axon endings on one side, and dendritic arborizations and cell bodies on the other.

The studies on the retina continued in the following years, and provided results which were fundamental for Cajal's formulation of his principles of the general organization of nervous connections [16,17–19]. Beside affirming that the nervous tissue is made up on independent elements, the neurons (so named in 1891 by Wilhelm Waldeyer [20]), Cajal proposed the principle of dynamic polarization:

'The transmission of nervous movement takes place from the protoplasmic branches, (the dendrites), and from the cell body to the nervous expansion, (the axon). In this respect, every nerve cell possesses a receptor apparatus (the cell body and the protoplasmic expansions), an apparatus of conduction (the cylinder-axis), and an apparatus of emission (the terminal varicose arborization of the cylinder-axis)' [21].

In order to understand the interpretative value of this principle, we must take into account that in Cajal's time there were few electrophysiological studies on the pathways followed by the nervous signal within the cerebral circuits. With Cajal's principle at hand, in order to trace the pathway of signal transmission along the central circuits, it sufficed to identify the somato-dendritic region and the axon of the constituent neurons and the arrangement of their contacts.

His interest in the retina never abandoned Cajal, as he recognized in his memoirs '... the retina has always shown to be generous with me ... the retina the oldest and most tenacious of my laboratory loves ...' [3].

The scheme of general organization of the retina proposed by Cajal is still valid in its fundamental outline, and in several aspects Cajal studies remain 'the most outstanding and comprehensive descriptions of retinal structure' [22]. The retina, previously regarded as an inextricable membrane consisting of reticular and granular layers of uncertain significance, with Cajal became a true nervous structure where specific classes of nerve cells connect with other nerve cells, in order to convey the visual message toward the encephalic centers, along well defined pathways (Fig. 5).

The specific contributions of Cajal to the study of the retina are numerous. Among others there were the description of several morphological types of bipolar, amacrine and ganglion cells, the identification of different sublaminae in the inner plexiform layer, the discovery of the centrifugal fibers in the optic nerve, the identification of the circuit of efferent control in the retina, the description of the interplexiform cells (his small stellate cells of teleosts and mammals), in addition to the detailed description of Müller cells (see in particular ref. 16 and 19); moreover, Cajal's contributions to retinal histogenesis were of fundamental importance.

Although the work on the retina was so fruitful and important in the scientific life of Cajal, we should not think that it developed along a pathway devoid of obstacles. In fact, the difficulties and the polemics that Cajal encountered were many. Moreover, in some cases his observations and conclusions do not appear to be well supported by solid experimental evidence.

The difficulties and uncertainties of Cajal do not cast a shadow on the work of the great Spanish scientist. In our opinion they constitute an element of great interest because their analysis offers an insight not only into Cajal's personality, but also into the psychology of the investigative process in science.

Clear difficulties in Cajal's work on the retina concern the general plan of the connection between nerve cells. In Cajal's view, the retina is organized according to a scheme of point-to-point transmission of the visual message. That message, which originates in photoreceptors, should be conveyed by the most direct anatomical pathway to the brain so as to create a faithful neural copy of the optical image. Any excessive convergence in nervous pathways, and any lateral propagation of retinal circuits, appear to be

potentially dangerous, since they could lead to a weakening or to a loss of the power of spatial discrimination. In many of his diagrams on the flow of visual messages in the retina, Cajal only showed the chain formed by photoreceptors – bipolar cells – ganglion cells, thus excluding systematically other retinal neurons (Fig. 5).

In the retina there are at least two classes of neurons which, by their anatomical arrangement in a tangential plane, seem to be organized expressly for a lateral flow of information: the horizontal and the amacrine cells; moreover, the wide dendritic arborisations of some classes of bipolar and ganglion cells are suggestive of great functional convergence.

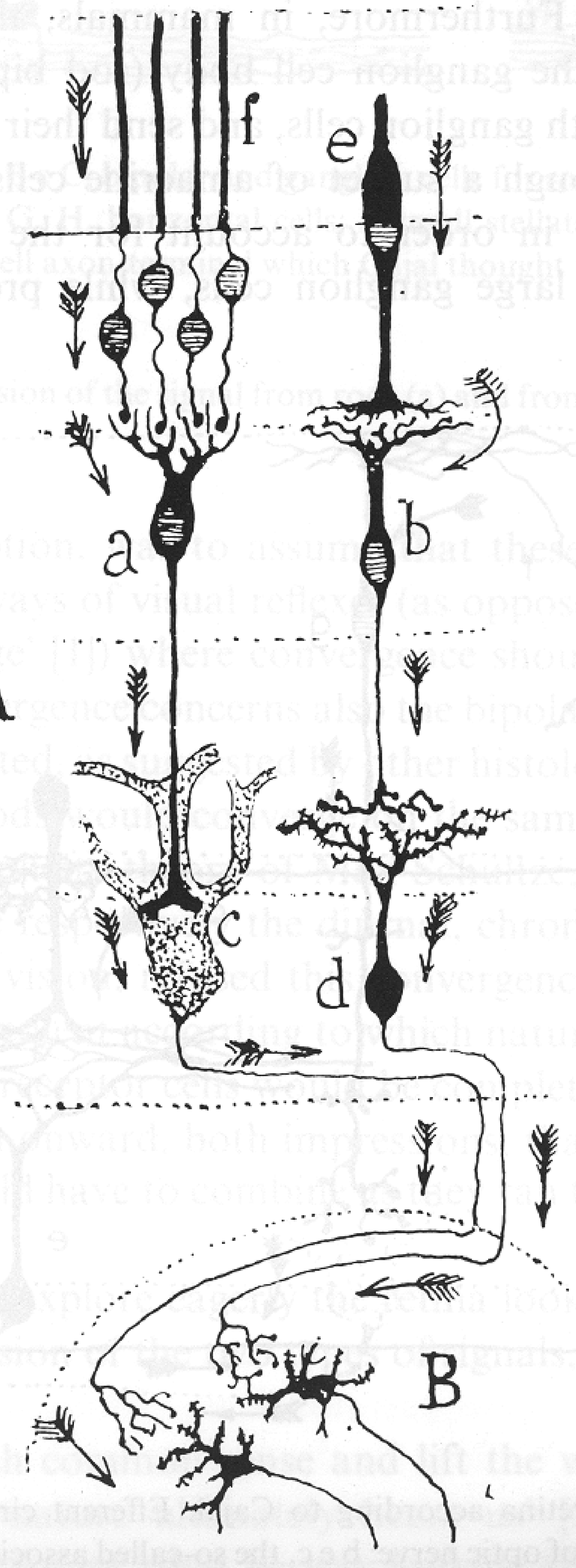


Fig. 5A. Scheme of the retina according to Cajal: Pathways of the nervous impulse in the vertebrate retina up to the geniculate body.

Insofar as amacrine cells are concerned, Cajal seems to exclude that these neurons receive the visual input from the bipolar cells, and he assumes that they are involved uniquely in a circuit of efferent control (centrifugal fibers of optic nerve – amacrine cells – ganglion cells, Fig. 5b). In this way, Cajal can account also for the wide dendritic arborisations of some ganglion cells, by supposing that these structures serve to receive the signal conveyed by amacrine cells along the efferent circuit. The large ganglion cells would receive the "visual" message mainly in the region of the soma, from a small number of bipolar cells (Fig. 5c and b). Such an arrangement has not been confirmed by modern studies, which, on the contrary, underline the scarcity, or even the absence, of any synaptic input from bipolar cells onto the ganglion cell body. Furthermore, in mammals, the bipolars that Cajal assumes to end on the ganglion cell body (rod bipolars) actually do not make any contact with ganglion cells, and send their visual message to these cells exclusively through a subset of amacrine cells. A second possibility considered by Cajal in order to account for the existence of extensive convergence on the large ganglion cells, while preserving the analytical

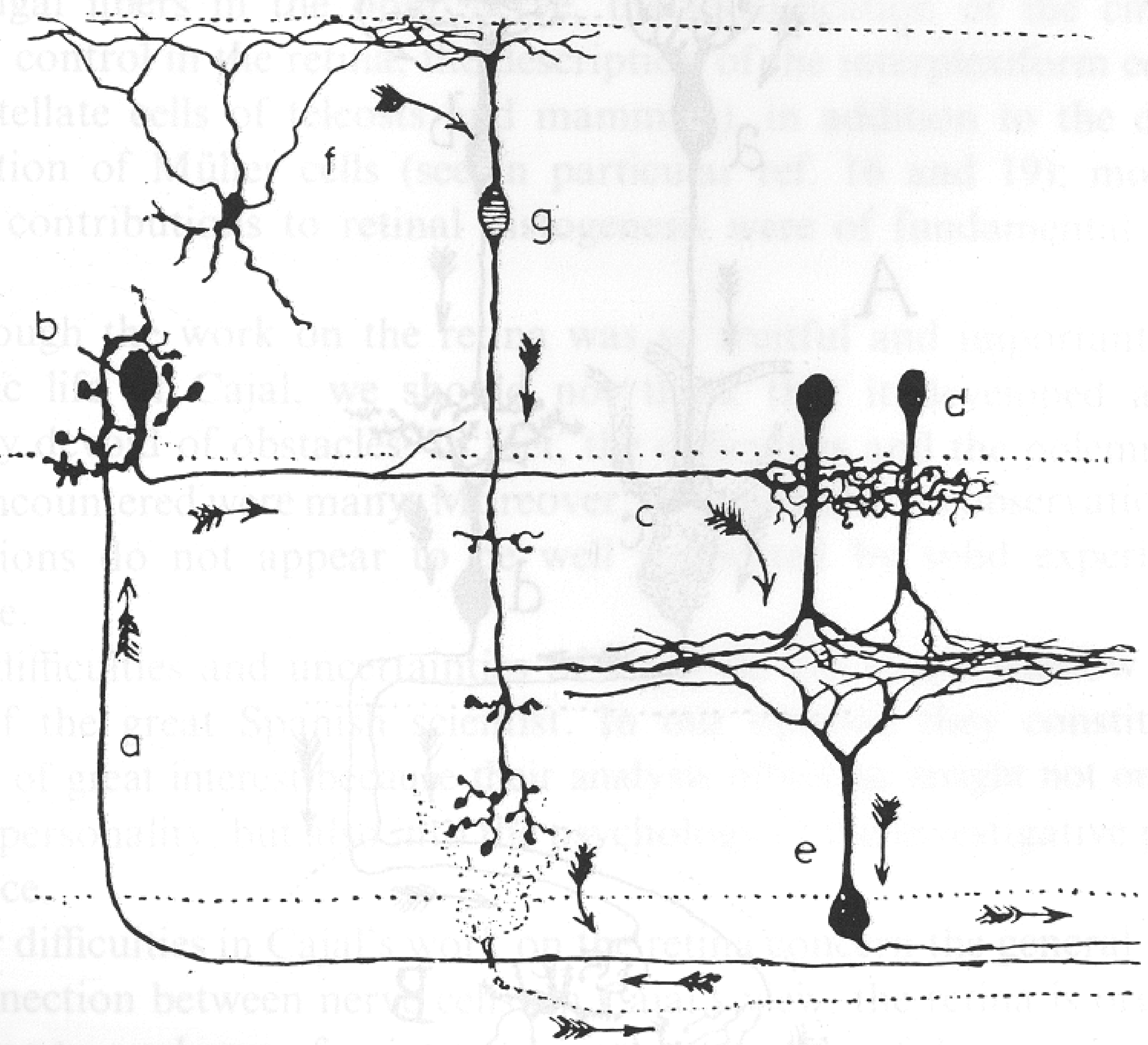
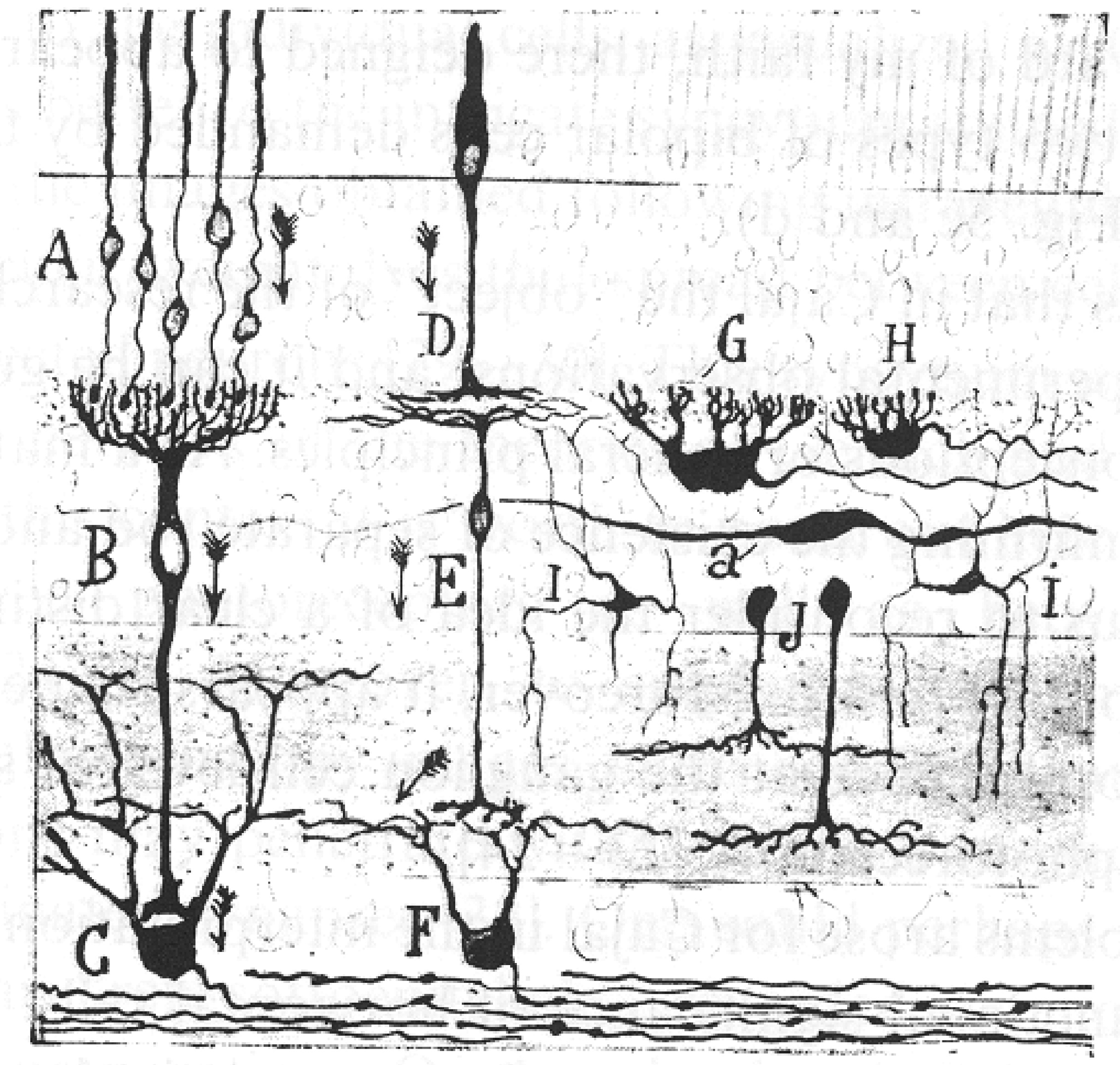


Fig. 5B. Scheme of the retina according to Cajal. Efferent circuit in the avian retina: (a, efferent, retinopetal, fiber of optic nerve: b e c, the so-called association amacrine with its axon; d, ordinary amacrine; e, ganglion cell; f, small stellate cell (now denoted as interplexiform); g, bipolar cell.



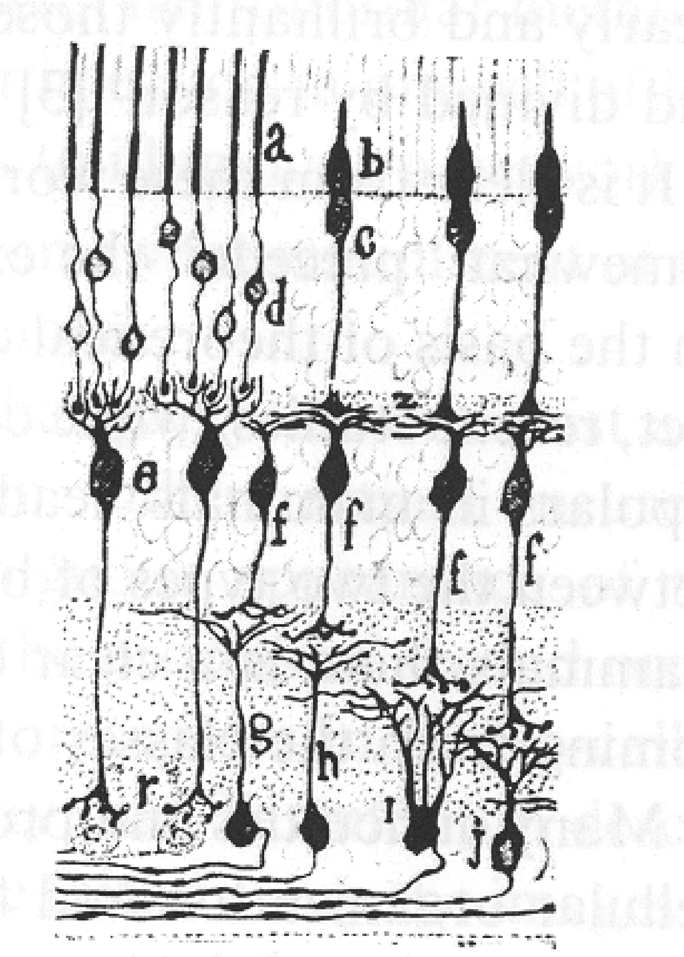


Fig. 5C. Fish retina: A, rods; B e C, bipolar and ganglion cells for rods; D, cone; E e F, bipolar and ganglion cell for cones; G, H, horizontal cells; I, small stellate cells (interplexiform); J, amacrine cell; a, horizontal cell axon-terminal which Cajal thought to be a subtype horizontal cells.

Fig. 5D. Channel of transmission of the signal from rods (a) and from cones (b) in the mammal retina (from Cajal, ref. 3).

power of visual perception, was to assume that these cells were involved exclusively in the pathways of visual reflexes (as opposed to the pathway of the 'mental visual image' [1]) where convergence should be minimal.

The problem of convergence concerns also the bipolar cells. And for these cells, the possibility existed, as suggested by other histologists, that the signal from both cones and rods would converge on the same bipolar cell. Cajal, who adhered to the duplicity theory of Max Schultze, according to which cones and rods mediate respectively the diurnal, chromatic, vision and the nocturnal, achromatic, vision, refused this convergence because:

'… the ingenious expedient according to which nature has organized two classes of specific photoreceptor cells would be completely frustrated: since, from the second neuron onward, both impressions, that of colour and that of black and white, would have to combine as they ran together through the same channels' [3].

And thus he began to explore eagerly the retina looking for bipolar cells specific for the transmission of the two types of signals, profoundly convinced that:

'When we reason with common sense and lift the war club determined upon vigorous action, nature ultimately hears us' [3]; and in fish and mammals eventually he found what he was looking for, bipolar for cones distinct from bipolar for rods:

'... and finally, as the reward of my faith, there deigned to appear most clearly and brilliantly those two types of bipolar cells demanded by theory and divined by reason' [3] (Fig. 5c and d).

It is clear from these words that in Cajal the "object" of the research may somewhat "precede" the experimental observations, and it can be guessed on the basis of theoretical conceptions or general principles. As a matter of fact, recent studies, while confirming the existence of separate rod and cone bipolars in mammals, lead us to reconsider the idea of a clear distinction between the two types of bipolars in fish. Moreover, it appears that even in mammals there is a clear convergence at the ganglion cell level of signals coming from the classes of photoreceptors [23–24].

Many difficulties and problems arose for Cajal in the interpretation of the cellular organization and functional significance of horizontal cells.

Surprisingly, Cajal ignored in a systematic way the presence, in the retinas of most species, of an axonless type of horizontal cell [16], in spite of the fact that the existence of such neurons had been reported by other authors. And this even when histological research could take advantage of neurofibrillar methods, which, in the retina of many mammals, mainly stain axonless cells [17]. One is led to conceive that Cajal saw exclusively axon-bearing horizontal cells because these conformed well to the principle of dynamic polarization.

As for horizontal cells (and other retinal neurons), another series of problems concerned the possible existence of intercellular, protoplasmic anastomoses. These had been reported by other authors (Krause, Dogiel), but Cajal refuted them with vigour [1,16–17,19]. In particular Tartuferi, the first to apply the Golgi method to the retina [25], claimed the existence of extensive anastomoses between horizontal cells, as well as between photoreceptor endings, thus creating an overspread network ("rete sottoepiteliale"), beneath photoreceptors. Anastomoses were also reported by other histologists using neurofibrillar methods [26]. Although in this case Cajal guessed correctly the discrete character of horizontal cells, we must take into account that the histological appearance was not unequivocal in that respect. As a matter of fact, only electron microscopy could give a definite answer to this problem, due to the narrow space existing between neighbouring neurons.

Moreover, in the case of horizontal cells, recent studies have pointed the syncytial character of their organization. Adjacent cells may be interconnected by wide and numerous gap junctions. These junctions consist of hydrophilic channels which allow for the transcellular passage of ions and small molecules between connected cells [27–28]. In the case of some horizontal cell types, because of these junctions, the functional unit does not correspond to the individual cells, as visualized by Golgi preparations; it corresponds better to the intricate syncytium stained with neurofibrillar methods, or to the images obtained following intracellular injection in one cell of low molecular-weight dyes that spread between cells to illuminate a network of connected neurons [29–30]. This syncytium is somewhat reminiscent of the nerve network of the theories of Gerlach and Golgi.

In this context it is worth to point out here that gap junctions exist also between photoreceptors as well as between other retinal neurons [23–24,31]. Moreover, to emphasize the ambiguous appearance of the optical microscopy images, let us consider that the process of second order neurons may penetrate within the base of photoreceptors in the so-called invaginated synapses [32]. One could perhaps say that Tartuferi, in describing his "rete sottoepiteliale", recorded in a more faithful way the images of Golgi preparations. On the other hand, Cajal interpreted these images more correctly, since, supported by more adequate theoretical principles, he was able to see beyond the pure objective datum.

Coming back to horizontal cells, it is worth mentioning here that in the retinas of lower vertebrates the syncytial aspect of the terminal expansions of the axon-bearing horizontal cells is particularly remarkable. In the fish retina, such expansions are spindle-shaped and they form a continuous plexus layer lying internal to the horizontal cell bodies [33]. In preparations stained with the Golgi method it is extremely difficult to trace the entire course of the axon. Cajal was disconcerted by these anucleate structures. He described then the presence of nuclei in the axonal expansions, and considered them as a specific subset of horizontal cells ("internal horizontal cells", ref. 16, Fig. 5d). He even wondered why other authors were not able to see the presence of nuclei in these structures. For example, Schiefferdecker denoted them as "anucleate concentric cells ('kernlose concentrische Zellen') [34]. It is difficult to escape the conclusion that Cajal, in "seeing" the nucleus in the axonal expansions of fish horizontal cells may have corrected in a "cellular" sense an image that appeared to be in contrast with the postulates of neuronal theory.

With respect to the comprehension of the role of horizontal cells, many problems arose for Cajal, which were somewhat analogous to those regarding the amacrine cells. On the basis of his experimental observations, Cajal was brought to conclude that horizontal cells did not participate in the pathway of the "vertical" transmission of the visual message, and that they might establish connections between photoreceptors separated by long distances [1,16]. But then:

"we are obliged to admit that the visual signal seized by these tangential neurons flows back, toward the visual corpuscles of other visual radiations (the photoreceptors of other retinal regions), somewhat far away, such as to constitute a kind of vicious circle' [35].

A similar possibility was contrary to the conception of a plan of orderly and economic connectivity that Cajal supposed the structural scheme of organization of the nervous system in general, and of the retina in particular.

'May one admit that this would be a real association, in a transverse plane, of visual corpuscles. For a sensitive apparatus endowed with such an analytical power, is it ever possible to admit that nature has established an arrangement which implies the destruction of the differentiation power of cones and rods and which is capable of weakening or even suppressing in certain regions their spatial sign [1]?'

To solve what would remain for his entire life the 'paradox of horizontal cells' [35], Cajal assumes that the function of these cells was not to transmit the visual message from one point to another of the retina, but instead, that horizontal cells would serve as 'depot of nervous energy aimed at reinforcing visual excitation and giving it a tension sufficient to bring it up to the centers [1].' He applied to horizontal cells an a priori view that he had formulated to explain the presence and the functional role of neurons with short axons in many regions of the nervous system [36]. The study of horizontal cells had been rather decisive in the elaboration of this conception. Due to their anatomical characteristics, the short-axon neurons appeared to Cajal unsuited to "project" the electrical signal from one region to another. They seemed to be in many cases a redundant, or even functionally dangerous, element in the structural plan of the nervous tissue. For example, in the cerebellum, to assume that short-axon neurons are all involved in conduction pathways would lead to a variety of parallel or recurrent pathways that Cajal considered a 'superfluous complication' [36]. And in the retina, 'if horizontal cells were constantly interspersed between the two factors of nervous articulation (photoreceptors and bipolar cells), the physiological effect would be to disturb, or to hinder, the spatial function of every retinal point' [36].

On the other hand, Cajal believed that short-axon neurons played an important role since they are particularly abundant in nervous structures subserving higher functions (such as the cerebral and cerebellar cortices, the striatum), and their number increases along the phylogenetic axis, going from lower vertebrates to mammals and humans. And then he supposed that they would act as 'condensers or accumulators of nervous energy', whose discharge 'would contribute to increase the tension of the impulses flowing along the chain of the corpuscles with long axons' [1]. And, in a following passage, he concludes:

'In every action which takes place a long time after an excitation of internal origin (memory, ideation, judgement, etc.), the aforementioned cells would go on, giving up their dynamic reserve until, exhausted, tiredness would eventually arrive' [1].

The difficulties of Cajal in interpreting the physiological significance of short-axon neurons, and of some aspects of retinal organization, to a great extent were due to the absence, in his general principles, of an integrative and operational conception of nervous function. In the plan of orderly connectivity formulated by Cajal, it seems that the only function of nervous cells is to transmit visual signals from a site to another along well defined pathways, with a minimal number of neurons. It is interesting to note in this context that, according to Cajal, only two neurons (one sensory and the other motor) are sufficient to account for most of the spinal reflexes [1].

In particular, Cajal lacked the notion of inhibitory interaction between nerve cells as a fundamental operative mechanism in the central circuits. He was surprised by the phenomenon of inhibition in spinal reflexes which may occur in several functional circumstances (e.g., intense emotions, electrical stimulations of the contralateral side):

'As a matter of fact it appears rather strange that a powerful excitation results in a motor inhibition instead of eliciting extensive, coordinated reflexes and conscious reactions' [1].

And he assumed that this phenomenon was not a consequence of a true, active inhibition exerted by specific nervous pathways, but depended instead on an intrinsic incapacity of the spinal neuron to respond to excessive stimuli:

'In our opinion, the motor neuron is tuned to respond to a limited scale of stimulus intensities; if this scale is exceeded it shows itself unexcitable both for stimulation coming from the pyramidal tract, and for impulses arriving from sensitive and sensorial nerves' [1].

This absence of the notions of integration and inhibition in the interpretative paradigms of Cajal explains, for instance, his difficulty in accepting the presence of complex local circuits in the cerebellum.

This is also the reason why Cajal could not accept the possibility of a lateral flow of the retinal signal, a flow that, as we have already seen, he considered deleterious for the analytical requirements of the visual process.

The existence of lateral inhibition in the retina was first recognized by Hartline in *Limulus* in the late 1940s, and confirmed some year later by Barlow and Kuffler in vertebrates (see ref. 37). The studies that followed demonstrated that, through processes of lateral interaction, the retina carries out a complex analysis of the visual message. It does not simply generate and transmit a photographic replica of the optical stimulus [23,28,30].

If Cajal had supposed that horizontal cells, by connecting distant photoreceptors through recurrent, inhibitory pathways, could serve, not to deteriorate, but instead to *increase* the analytical power of visual process, certainly he would have had fewer problems in interpreting the structural plan of the retina (and of other nervous centers).

On the other hand, we must take into account that a great merit of Cajal was to recognize, within the apparent complexity of the nervous system, a basic structure accessible to anatomical and functional stuedy; this structure consists of nerve cells, which establish specialized contacts whereby nervous messages proceed along well-defined and circumscribed pathways. And Cajal did this in a period dominated by "anti-localicistic" and "holistic" conceptions of the nervous function. As a matter of fact, in spite of Cajal's statement that in Golgi preparations there was no more need for interpretation, but only to see and to take note of an unambiguous reality, the interpretation is always a fundamental phase in the acquisition of knowledge in science. The same experimental data can lead to conclusions which are diametrically opposed in the hands of two scientists, both endowed with an exceptional talent (Fig. 3A and Fig. 4). Where Cajal sees the axon of basket cells terminating with free endings that embrace the body of Purkinje cells, Golgi sees the axon terminals continuing in a "rete nervosa diffusa".

Integration and inhibition are physiological concepts which could not have emerged primarily from morphological studies. Their formulation in a modern sense is based on the notion of the synapse, which could develop only within the framework of a cellular theory of the nervous function. At the beginning of this century, the notion of the synapse entered the realm of neurobiology mainly due to the work of Sherrington, the great English physiologist, a devoted admirer of Cajal, who recognized the debt on the part of physiologists, to the studies of the great Spanish anatomist [39–40].

At the beginning of 20th century the neuron theory, and with it neurobiology at its inception, was faced by a violent attack delivered by new "reticularists". In particular the zoologist S. Apathy, and the physiologist A. Bethe, supported the notion that the neurofibrils, revealed in nervous cells by recently discovered methods, were the basic structure of the functional organization of the nervous system. By anastomosing with each another, and independently from nerve cells, which were relegated to secondary functions, the neurofibrils would serve to conduct the nervous signal, acting as tiny electrical wires [41–42].

Cajal was obliged to take sides in this polemic against a theory which was really dangerous, and he intervened with a vigour which was criticized. The polemics persisted until Cajal's death, at which time his final defense against old and new antineuronists was in press [43].

Without Cajal's propensity to simplify and to catch the essential in the observation and interpretation of histological images, no doubt the progress of neurobiology would have been delayed. We are now able to understand some minor limitations in his theories and conclusions without losing our faith in his fundamental achievements: we can now accept the idea that, in some cases, neurons form syncytia due to the presence of intercellular bridges; that the nervous signal can circulate along pathways in contrast with the doctrine of dynamic polarization, and that there exist dendro-dendritic or axo-axonic synapses; that in nervous centers there are complex local circuits. We can now accept, and try to understand, the complex and variable nature of neuronal circuits having learned from Cajal what is simple and constant therein.

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Address for correspondence: M. Piccolino, Istituto di Neurofisiologia del C.N.R., via S. Zeno 51, I-56100, Pisa, Italy.