Published in final edited form as:

Brain Res Rev. 2011 January 7; 66(0): 92–105. doi:10.1016/j.brainresrev.2010.09.009.

The first images of nerve cells: Golgi on the olfactory bulb 1875

Gordon M. Shepherd^{a,*}, Charles A. Greer^a, Paolo Mazzarello^b, and Marco Sassoè-Pognetto^c

Gordon M. Shepherd: gordon.shepherd@yale.edu

^aDepartment of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

^bMuseum for the History of the University of Pavia and Department of Experimental Pathology, University of Pavia, Pavia, Italy

^cDepartment of Anatomy, Pharmacology and Forensic Medicine and National Institute of Neuroscience, University of Torino, Torino, Italy

Abstract

The third paper by Camillo Golgi on his new method was on the olfactory bulb. This paper has never been translated into English, but is of special interest both for its pioneering description of olfactory bulb cells and for containing the first illustration by Golgi of cells stained with his new method. A translation into English is provided in this paper, together with commentaries on the significant points in his descriptions. These results are placed in the perspective of Cajal's subsequent first publication on the olfactory bulb and brief mention of the work of other early histologists. This perspective allows one to see more clearly Golgi's fundamental contributions to the olfactory bulb in particular and to the description of the neuronal architecture of the brain in general.

Keywords

Golgi stain; Olfactory bulb; Ramón y Cajal; Mitral cell; Granule cell; Olfactory glomerulus

The dramatic breakthrough by Camillo Golgi in staining entire nerve cells with his new method of silver impregnation of nerve tissue is usually and rightly associated with his first two concise papers on the grey substance of the brain (Golgi, 1873) and on the human cerebellum (Golgi, 1874). Less well known is that his next paper (Golgi, 1875) was on the olfactory bulb. This paper was critical for the development of the method and for laying the foundation of modern neuroscience for several reasons. First, this paper contained in one magnificent drawing the first illustration of nerve cells impregnated with the new method. Second, it was the first publication in which Golgi provided a detailed description of the method. And third, the results in the olfactory bulb contained a difficult challenge to his reticularist interpretation of the relations between nerve cells, though this was not acknowledged in his discussion.

Given the importance of this paper for the development of the Golgi method and for the development of ideas on nervous organization, a wider knowledge of it is long overdue. The meeting in Pavia on “Camillo Golgi and Modern Neuroscience”, and this volume honoring Golgi shortly after the centenary of his Nobel Prize, provide a timely opportunity to publish a translation into English of the paper with commentaries on the fundamental descriptions of olfactory bulb cells made by Golgi. In addition to the three points mentioned above, we will see that a fourth reason for interest in this paper is how future work by Ramón y Cajal and others on the olfactory bulb built on the foundation established by Golgi to a larger extent than is traditionally appreciated.

In brief summary, in this 1875 paper Golgi describes for the first time the morphology of the cells of the olfactory bulb (his examples are from the dog). The extraordinary plate of his drawings of the bulbar cells is shown in Fig. 1. It is the first complete view of a brain region stained by the Golgi method. The different types of cells are shown with their dendrites, and are arranged in relation to each other and to the main histological layers. This is the format used by all subsequent work in portraying the cells of a brain region. The main histological layers are clearly seen. His labeling combines the glomerular, external plexiform, and mitral cell layers (B on the right) as distinct from what he calls the fiber layer (granule cell layer) (C). The large cells with tufted dendrites in the middle are clearly what we now recognize as mitral cells; the two smaller horizontal cells below the glomeruli appear to be what we now call tufted cells. There are two populations of small cells: what are now known as periglomerular cells around the glomeruli, and granule cells in the deeper “fibrous” layer. Within the latter are two larger cells which are now called short-axon cells.

The translation of the original Italian article (Golgi, 1875) follows. The original paper was published in the journal “Rivista Sperimentale di Freniatria e Medicina Legale”. However, Golgi’s works on the structure of the nervous system had their first major impact on the histologists of his time through his collected works in Italian (Golgi, 1886), which however did not include the work on the olfactory bulb, and even more through the German translations (Golgi, 1894). His articles were also re-published in Italian in his “Opera Omnia” (Golgi, 1903).

1. The plate

A striking contribution of this paper is the illustration drawn by Golgi. There is a somewhat complicated story about this single magnificent plate of the cells in the olfactory bulb. Golgi published the drawings in the journal “Rivista Sperimentale di Freniatria e Medicina Legale” in black and white. But the 1875 reprints of the same article were in color. One of us (PM) has ascertained these differences by checking the 1875 volume of “Rivista Sperimentale” kept in the University Library of the University of Pavia and in the old Golgi’s Institute of General Pathology and by checking two reprints (one of which is kept at the Golgi’s Institute) of this 1875 paper. A third reprint (sent by Golgi to Cajal, probably in the second half of 1889; see later) is kept in the Library of the Cajal Institute in Madrid and the color plate is published by DeFelipe (2010). The color image of the olfactory bulb published only in the reprints was re-published in the German edition of Golgi’s work (Golgi 1894) and in the Opera Omnia (Golgi, 1903). The only difference was that in the “Rivista

Sperimentale di Freniatria” the table was indicated as “Tav. VII” (it means the seventh table of that volume of the “Rivista Sperimentale”) instead in the German edition was indicated as “Taf. (i.e. Tafel) 2” and in the Opera Omnia as “Tav. 3”.

The evolution of the diagram from black and white to color is further described in Mazzarello (2010). Here we wish to keep the focus on the anatomy of the cells, and therefore have elected to show the color version published in the reprints of 1875, in the German edition (1894) and in the Opera Omnia (Golgi, 1903). As will be seen, Golgi primarily used color to differentiate the cellular elements of the plate (for example the glial cells are colored red).

2. The text

In general we have attempted to retain the use of language of that period, in order the better to assess how Golgi presented his data and his arguments for his interpretations. Thus, protoplasmic prolongation was the term for what came to be called “dendrite”, and axis-cylinder for “axon”. “Ganglion cell” was a general term in that era for any large cell in a region of the brain; Golgi also uses it more specifically for a cell with a long axon leaving the region of the cell of origin. We have also retained the grammatical form of presentation, with its attempt to describe as precisely as possible these new forms that he was seeing for the first time.

Superscripts indicate points for further commentary following the translation. Editor's notes in brackets clarify when Golgi is referring to axons or dendrites. In several cases a statement in the text is bolded that represents a point of special significance to modern readers. The authors' assessments of this important paper draw on their experimental and computational studies of olfactory bulb structure and function.

3. On the finer structure of the olfactory bulb by Dr. Camillo Golgi

In spite of the fact that the olfactory bulbs have been studied with the help of the newer methods of microscopical technique, those who, aware of the latest results of studies on the central nervous organs, refer to the latest works published on that argument (1), can't avoid noting that not only some parts of this subject have been left unexplored, but also many of the reported facts were not the result of strict observation but the fruit of preconceived opinions.

In fact, one could not admit so easily now the numerous direct transitions of nerve fibers coming out of the tractus olfactorius [lateral olfactory tract: LOT] into the separate ganglion cells of the olfactory bulb, or the direct continuation of the separate prolongations of these cells into a nerve fiber; transitions that are said to have been observed with ease by Walter, Owsjannikow, and Clarke and were also admitted by Koelliker. One will think even less accurate the numerous anastomoses between the prolongations of the ganglion cells described by Walter, and certain connections of the peripheral prolongations of the large ganglion cells of the gray matter with the fibers which leave from the supposedly small bipolar nerve cells, as Walter and Clarke described them. The same can be said about Owsjannikow's claim, confirmed by Walter, that the nerve fibers coming out of the *tractus*,

after having on one side attached themselves to the cells, appear on the other side at the periphery in order to form the olfactory fibrils. (1) Finally, with regard to the shape of the cellular elements that form the organ in question, no one will now consider as satisfactory the terms *granules* or *nuclei* that have been used for a great many of these even by the latest scientists who have treated this subject (Henle, Meynert, and Huguenin).¹

While these simple examples are more than enough to prove the necessity of new studies to bring new, clearer light onto what we know about the finer structure of this organ, to which the peripheral nervous olfactory fibrils attach, directly and after a very short trajectory, at the same time they also reveal many problems, the solving of which would be of great importance also to physiology.

It is actually to make a small contribution toward the solving of these problems that I shall report briefly the results of some of my studies which I have made by the methods that have proven most efficient in the study of the central nervous system. In spite of the fact that I have taken as objects for my studies the olfactory bulbs of humans as well as of a series of animals (dog, cat, rabbit, hare, guinea pig, pig, calf, etc.), I shall at the present give a cursory survey of my results in which I touch briefly on some differences among the animals which possess a more or less strongly developed smell-organ. However, I intend to give a separate, comparative, description as soon as I have finished with a series of recently started meticulous examinations.

Coming now to the point of elucidation of what I was able to find during the examination of the organs in question, I shall as others before me, in order to facilitate the description, treat separately the different layers of which they consist. But in doing so I shall not follow the example of Clarke, Henle or Meynert, who identified six, seven or even eight layers; such a differentiation is, in my opinion, based more on the superficial appearance of the tissue than on histological differences of real value; respecting these histological differences, I think that it is only possible to discern three layers in the olfactory bulb, namely:

- I. A thin outer layer of white-grayish color consisting of bundles of peripheral olfactory nerve fibers, which, issuing from the lamina cribrosa, enter into the parenchyma of the bulbs.

Referring to the outer layer, I shall only briefly mention the fact that by the method of preparation I have used, it is possible to discover with surprising clarity, and at first glance, the nerve bundles' course by which it is formed. This is very easy to understand when one thinks of the fact that, under the influence of silver nitrate, the separate fibrils take on a perfectly black coloring for a long and uninterrupted journey, while the interstitial matter retains its normal color and transparency.

¹F. Leydig, Lehrbuch der Histologie, Frankfurt a.M. 1857. Vom Geruchsorgan der Tiere, S. 216. – Lockhart Clarke, Ueber den feineren Bau des Bulbus olfactorius. Zeitschrift fuer wiss. Zool. XI. – Ph. Owsjanikow, Ueber die feinere Structur der Lobi olfactorii der Saeugetiere. Muellers Archiv fuer Anat., Physiol. Und wss. Med. 1860, S. 469. – Georg Walter, Ueber den feineren Bau des Bulbus olfactorius. Virchows Archiv fuer path. Anat. und Physiol., Bd. 22, 1861, S. 241. – Th. Meynert, Der Bau der Grosshirnrinde und seine oertlichen Verschiedenheiten u. s. W., Vierteljahrsschr. Fuer Psychiatrie, Lepz. 1872. – Th. Meynert, Vom Gehirne der Saeugetiere, Strickers Handbuch der Lehre von den Geweben, 1872, S. 74. – Henle, Handbuch der systematischen Anatomie, Bd. 3S. 343, Braunschweig 1873. – Huguenin, Die allgemeine Pathologie des Nervensystems, Bd. 1 Anatomischer Teil, Zuerich 1873.

Thus it is possible to recognize how the fibril bundles, progressing from the outside toward the inside, divide, diverge and then cross again in the most complicated manner. Above all it is interesting to see how these bundles, as well as the individual fibrils found scattered here and there, always head toward the apparently granulated matter, the so-called olfactory glomeruli, which are found in the most peripheral zone of the middle layer, into which they penetrate in order to spread and to subdivide in a way we shall mention later on. I shall only point out here that under certain circumstances it is possible to observe the nerve bundles separated from each other and themselves divided by numerous wide and thin connective tissue cells [glia] with long prolongations, partly thread-like, partly flat, but rarely ramified, which are absolutely similar to those found in the white matter of the cerebrum and spinal cord (2).

- II. A middle layer of gray matter, similar to the cerebral gray matter, mainly consisting of widely ramifying ganglion cells and, in minimal part, of an interstitial stroma of connective [glial] tissue.

Much more complicated, and demanding a new detailed examination, is the middle layer, or layer of gray matter. Inwardly, its border with the white matter is very clearly marked by a row of large nerve cells which are located in the border zone as clearly as the large cells of Purkinje in the border zone of the molecular layer and granular layer of the cerebellum's convolutions. Toward the periphery, the border is not as sharply marked, because the separation of the layers corresponds to the entrance of the peripheral nervous fiber bundles in to the glomeruli, which do not form a regular row in one line, but are placed at different heights, and at some points form a double row. When studying this layer, one has to consider separately:

1. the large ganglion cells which form the just-mentioned regular row; 2. the elements that crowd the gray matter between this row and the area around the glomeruli; 3. the structure of these glomeruli.

1. The cells which, placed with surprising regularity, mark the border between the gray and the white matter belong to the category of large nerve cells, many of them having the same diameter as the largest cortical cells; their shape is normally an irregular triangle, and they are placed in such a way that, without exception, the steepest angle of the triangle is turned toward the inner part of the bulb. This point ends generally in a continuous prolongation which shows all the characteristics proper of the so-called *axis-cylinder-prolongation* [axon] of the cerebral nerve cells, while other prolongations, similar to the so-called protoplasmic prolongations [dendrites], leave from the two corners and the side facing the periphery, and at times even from the lateral surfaces.

The first prolongation I do not hesitate to call the *nervous prolongation* [axon], each cell possessing only one, because without a doubt it is meant to form one or more nerve fibers; it appears smooth and evenly shaped and, shortly after its start, it shows a restricted area of diminished dimension and a slight bending. It runs vertically toward the center of the bulb where it has

been possible for me to follow it many times for the considerable distance of more than 200 μ , without seeing it emit any ramifications [axon collaterals]. I do not dare to pretend that it does not obey the law valid for the equivalent prolongation of the nerve cells of the cerebral cortex, that is that it forms directly the axis-cylinder [axon] of a nerve fiber without emitting any secondary fibrils [collaterals], in spite of the fact that I always observed it that way. I am rather inclined to believe that not having seen any ramifications thus far depends on the imperfect result of the reaction (3).

The protoplasmic prolongations (this somewhat inadequate designation is justified by the fact that it is now nearly generally used), which originally start as thick prolongations of the cell body, appear three, four or several more in number; they always turn towards the bulbs' periphery and, in this direction, head in a more or less oblique manner toward the olfactory glomeruli, into which they enter (4). During their passage through the gray layer they undergo some slight ramification; but as soon as they have entered the glomeruli, they develop rapidly into very entangled, fine and delicate weavings which are attached at the extremity of a very strong branch, which gives them a rather extraordinary appearance. A great part of the protoplasmic prolongations [dendrites] of 1, 2 or 3 grade run in a very oblique course towards the periphery. The plate belonging to this present work (see the row of cells along the long inner margin of layer B) will give, better than any description, an accurate idea of the course of the prolongations [dendrites] of the cells forming the regular layer we are speaking about here.

About these prolongations it has to be mentioned especially that, during their course toward the glomeruli, they cross and lie over each other in a most complicated manner, but never enter into any real connections or anastomoses. The same is true for the prolongations of the nerve cells which will be described later on.

In animals with a poorly developed smell-organ, these cells are disposed, as I said, in a very simple row, in much the same position as those of the middle layer of the pes hippocampi, also lying at a certain distance from each other. In contrast, in the case of animals with highly developed olfactory bulbs (dog, horse, ox, etc.), the cells not only form a much denser row, but also a more or less important number of them lie in an outer line, thus forming a double row (5); as the nervous prolongations [axons] always run in a direct line toward the center of the bulb, the result is that they form a sort of fence, remarkable for its regularity, at the periphery of the white matter.

2. The region of the gray matter between the just described cell layer and the glomeruli is populated by two kinds of nerve cells: a) large cells, scattered irregularly [presumably tufted cells], and b) small cells gathered around the glomeruli [presumably periglomerular cells].

- a. The large, scattered cells are mostly spindle-shaped, arranged parallel to the surface of the organ and at a considerable distance from one another. Like the cells forming a regular row, they are provided with several powerful prolongations belonging to the protoplasmic category [dendrite] and with only one of the kind I described as essentially nervous [axon].

The protoplasmic prolongation [dendrites] course similarly to the protoplasmic prolongations [dendrites] of the cells described earlier. After a more or less long course in a horizontal or oblique, upward direction, and after a few ramifications, they also penetrate into the glomeruli, where they ramify finely and form an entangled weaving. We shall see later if we have enough reasons to come to a conclusion about the ultimate destiny of all these ramifications, or whether the problem has to be left still unsolved (6).

The prolongation with the characteristics of the axis-cylinder [axon] turns vertically towards the center of the bulb, and, in this direction, I have been able to follow it, in the olfactory bulb of the rabbit, for a distance of more than 400 μ , that is, right into the deepest layers of the white matter.

In connection with this prolongation I have been able to establish that, just like the equivalent prolongation of a cerebral nerve cell, it sends out, at a distance of 20–30 μ from its start from the cell body, a large number of fibrils, which in their turn form new ones equally ramified. It has only been possible for me to observe for a short distance the fibers growing out of the nervous prolongation [axon] during its course through the gray layer. Therefore I cannot say, even by inference, what their final destiny is. But, with regard to the fibrils which this same prolongation emits during its course through the white matter, I have observed that many of them quite easily connect with the horizontal nerve fiber bundles at hand (fibers coming out of the *tractus olfactorius* [LOT]), and are perfectly similar to them in what concerns their course and manner of ramification (7).

- b. Touching here on the second category of the gray matter's nervous elements, that is, the small cells [periglomerular cells] which are grouped around the glomeruli, I shall only remark that in structure and size they are similar to those forming the middle layer of the pes hippocampi; their body has an oval shape, the one pole ending by a very thin prolongation which shows the same qualities as all nervous prolongations [axon], while the other pole sends out two or three prolongations which correspond to the protoplasmic ones [dendrites]. This pole, whatever the location of the cell body, at the interior, on the side, or even at the exterior of the glomeruli, is always turned towards the glomeruli, into which the protoplasmic prolongations

[dendrites] penetrate and ramify in a way similar to that of the thick prolongations of the large nerve cells which lie at the border of the gray layer. The thin nervous prolongation [axon] ramifies into fine branches within a short distance of its origin, quite similarly to the cerebral nervous fibrils. One part of the threads thus formed bends backwards, running in a contrary direction to the original fibers, and enters the glomeruli along with the nerve fiber bundles coming from inside the bulb.

3. Finally, in order to complete the description of the gray matter, we have to concentrate especially on these little round or oval islands of finely granulated appearance and gray color (*stratum glomerulosum* of Meynert), or the transition-zone toward the olfactory nerve fibers.

These little islands appear very clearly delimited toward the exterior and on the sides, where they are surrounded by peripheral fiber bundles, whereas at the interior they usually have a gradual transition into the gray matter. I have already mentioned that the bundles of peripheral olfactory fibrils, after having divided up and mixed repeatedly, always run toward the glomeruli into which they penetrate and where they spread. If we now go a step further, we notice that individual fibrils, while moving away from each other at the interior of the little islands, divide up at very short intervals, usually at a right angle, and in a very complicated way; and how the thusly formed tiny fibers of immeasurable fineness progress in entangled and bizarre convolutions and finally connect, forming a dainty and fine network contained within the borders of the glomerulus. **In some cases it is possible to see, joining this network, numerous fibrils coming from the gray matter and probably also from the white matter (ed. bold) (8).**

In order to complicate and obscure even more the structure of these small islands, we have to add other elements, connective tissue cells [glia], that by their shape and relationships are very much like the cells forming the interstitial stroma of the cerebrum's, cerebellum's and spinal cord's gray matter. They have an irregularly round cell body that sends out in all directions uncountable finely ramified prolongations. One usually finds these cells attached closely to the walls of the capillaries traversing the glomeruli. But, even when not clinging so closely to the vessels, they are intimately linked to them by their prolongations, some of which resemble wide expansions, nearly like the capillaries themselves. The ramification of these cells is so rich that by itself it forms a wide complicated weaving from one side of the glomeruli to the other.

After having described the different elements taking part in the formation of the glomeruli and rendering its structure so complicated, it would be of interest to decide if and how they relate to each other. In fact, this is the most important problem that I would like to solve, but for the moment I have to declare it unsolved.

As on the one hand the protoplasmic prolongations [dendrites] of the large and small cells populating the gray matter, and on the other hand, the nerve fibers coming from the olfactory region, both enter the glomeruli and ramify very finely there, the conclusion accepted by Walter and Owsjanikow (without nearly possessing the exact knowledge that I have exposed) that they should connect with each other, seems obvious (9).

As natural as this conclusion may seem, I am not inclined to support it. I merely observe the fact that the olfactory glomeruli are a kind of assembling point for the elements forming the olfactory bulb: blood vessels and plasmatic connective tissue cells [glia], endings of the protoplasmic prolongations [dendrites], olfactory nerve fibers coming from the periphery, and nerve fibers coming out of the *tractus* [LOT]. These different elements are without a doubt closely linked here; however whether they merely lie beside each other, or which parts connect with each other, apart from the vessels and the plasmatic cells, cannot be decided from the experiments until now. Anyone daring to say anything more exact, would, in my opinion, only state hypotheses. **It would be especially hypothetical to pretend that the final ramifications of the protoplasmic prolongations [dendrites] of the bulb's large and small cells each continues in as many peripheral olfactory nerve fibers [ed. bold] (10).** Inconnection with this I shall make here some observations adequately motivating the doubts just described.

It is one of the advantages of the method of preparation I have used, to be able, when dealing with certain modifications of the treatment of the pieces, to highlight in black color at one point the cellular elements with the finest ramifications of their prolongations, or, at another time, one or the other kind of nerve fibers or the interstitial elements of connective tissue, or one part of both classes of elements, with predominance of either. These different stages of the reaction also appear in the special study of the glomeruli. When the reaction is at its best, it can happen that the interweaving within the glomeruli appears so dense and complicated that it is possible to believe that one row of fibers goes over into the other. But if, as often happens, the reaction appears separately in the nerve fibrils or in the last ramifications of the protoplasmic prolongations, one is then able to compare the different weavings and see quite clearly that they are very different from each other, and most importantly it appears then clearly that the kind of ramification of the protoplasmic prolongations' [dendrites'] terminal branches differs essentially from that of the nerve fibrils. Despite the fact that both (the protoplasmic prolongations and the nerve fibrils) have very fine subdivisions, one never sees a progressive connection from one type of filament to the other) (11).

Alone the fact, that the chemical reaction which colors the nerve fibrils [axons] and the protoplasmic prolongations [dendrites] in black appears in general separately and at different times, is a good enough reason to admit that important chemical differences exist between the two kinds of fibers;

there should be no reason for such a chemical difference, if they really are connected with each other (12). We should also add that all the mentioned differences, shape, way of ramification and course, and the outcome of the chemical reaction, do not appear when one compares the peripheral nerve fibrils [olfactory axons], not with the final ramifications of the protoplasmic prolongations [dendrites], but with the embranchments of the unique prolongation, the one we call *nervous* [axon].

Actually we believe that the anatomical connection between the net of fibers present in the glomeruli and the ganglion cells of the bulb, as well as the nerve fibers of the tractus [LOT], which we can admit primarily for functional reasons, can also take place *indirectly* with the first through the numerous filaments leaving the nervous prolongation [axon] (in fact one part of these threads penetrates the glomeruli) and directly with the second by means of the fibril bundles which we have seen drawn through the gray matter, equally on their way into the glomeruli (13).

Finally I should like to recall that I have only revealed these observations in order to underline how far we still are from the point where we shall be able to declare the problem of the connection between the olfactory fibrils and the ganglion cells of the bulb as solved.

III. An inner layer of white matter, consisting mainly of bundles of nerve fibers that come out of the tractus, and spread in the parenchyma of the bulbs in a direction toward the gray matter.

I also have some observations to add to the account of the white matter. This layer is, as already known, only macroscopically similar to the white matter of the other parts of the nervous system; studied under the microscope, it looks quite different; it is formed of numerous, transverse, alternate layers of nerve fibers and cellular elements. It would be more adequate to say that the fiber bundles which have a very bent, nearly spiral-like course, criss-cross each other, leaving open elongated spaces disposed in parallel with the bulb's surface and filled with cellular elements.

As the anatomists' opinions on this point vary greatly, it is especially on the shape and structure of these elements that I should make some observations.

Henle contents himself to call these elements by the unsuitable name of *granules*. Meynert also describes them as very similar to the granules of the cerebellum and retina, but thinks that they are of a nervous nature. Walter collects all the elements scattered in the white matter under the name of bipolar nerve cells, and he pretends having seen them in direct connection with the prolongations [axons?, dendrites?] of the larger multipolar nerve cells [mitral cells?], as well as with the nerve fibers extending out of the *tractus* [LOT], etc.

What I can say with the greatest certainty on this argument is that (apart from the radiating connective tissue cells [glia] which are common on this layer, the distribution of which I shall describe later) the largest part of the elements in the mentioned, horizontal empty spaces have to be declared as being characteristically,

even though very small, cells of probably nervous nature [presumably granule cells]. They mostly show a pyramidal shape, the base of which is always turned toward the bulb's center, whereas the apex points toward the periphery (14); they are provided with several prolongations [dendrites; see below], which can be distinguished as the prolongations coming from the apex and the prolongations coming from the base; therefore we can say that these cells are absolutely similar in form and disposition to the pyramidal cells of the middle of the cerebral cortex. The prolongation [dendrite] which is like a continuation of the apex of the pyramid throws very sparse lateral branches on its way towards the periphery and generally runs through the entire white layer, sometimes penetrating into the gray layer as a thin thread, running along the regular row of large cells. The basal prolongations mostly spring from the angles of the pyramid, they also dichotomize soon and turn toward the bulb's center, where, because of their extreme fineness, they soon cannot be traced any further. **Both the prolongations show the characteristics of protoplasmatic prolongations [dendrites]. I cannot say with confidence if another, a nervous prolongation, exists [ed. bold].** It is true that in several cases I have seen that one of the prolongations leaving the central part of the pyramid looks fairly more homogeneous; and I also saw at times this separate prolongation bending horizontally to take its course along with the fiber bundles, emitting a few fine filaments as other nervous prolongations [axons] do. However, because of the extreme fineness of the filament, its differences from the so-called protoplasmic prolongations [dendrites] do not appear clear enough, I do not think that we can say that the small cells discussed here correspond in every way to the pyramidal cells of the cortex (15).

Among these small cells it is possible to find larger ones, similar to the ganglion cells, scattered here and there in the white matter. But they are rather rare, appearing only occasionally in some sections. They are multipolar, oval in shape, spindle-like or irregularly polygonal. Their protoplasmic prolongations [dendrites] proceed in a peripheral direction, but also to the sides and toward the interior; they ramify, as usual, by dichotomizing; they have no connections with nerve fibers (16), and do not anastomose with the corresponding prolongations of other ganglion cells. It is not yet possible for me to comment with certainty about their terminals.

The nervous prolongation [axon] usually springs from the side of the cell turned towards its center, sometimes also laterally, in order then to bend toward the interior. At a distance of 15–20 μ it emits filaments [axon collaterals?] which connect with the horizontal bundles of nerve fibers (17). It would seem that these occasional cells scattered in the white matter can be compared to those which are found scattered similarly in the gray matter (18).

As to the nerve fibers which play a chief part in the structure of this layer, I shall only briefly call attention to their course and ramifications. We particularly note the strange entangled twisting of their course.

The familiar aspect of a fan-like spreading, which is best given by the gold chloride method, is only presented by a few bundles running straight for some distance and then parting gradually. But if one makes a precise analysis with the method of black coloring, it is very easy to notice that the course of most of the fibers is much more complicated than it seems in preparations obtained with other methods. When observing the course of only a few fibers, one will see that, starting from the parallel bundles in the region of the central ventricle, they run for a short distance radially toward the periphery, then bend in order to run horizontally for some time, later taking another step forward in oblique direction, in order to curve again several times; as they gradually advance, they continuously send out filaments which again follow the same course and produce fibrils, thus forming a network so fine and complicated that it cannot be described.

This kind of ramification is typical for the nervous fibers [axon], as well as for the axis-cylinder cerebral prolongations [axon].

Finally I have to mention briefly the stroma of connective tissue [glia], though the only notable observation about this is that it shows no nominal difference from that of other parts of the central nervous system and that especially the radiating elements of connective tissue [glia] of the cerebellum's granule layer are absolutely similar to those of the bulb. I should only like to point out the extraordinary quantity in which these elements appear in the white matter; there is not one loop of the capillary-net (here very fine) in which one does not meet a whole group of these elements, placed with a certain regularity, so that the ramifications of their numerous prolongations form an uninterrupted network, in the interstitial spaces of which lie the nervous elements.

Regarding the connective stroma [glia] in this layer, it is also remarkable to see how the intimate relations between the separate radiating cells and the walls of the blood vessels show clearly; the cells are applied directly to the walls or send out several prolongations towards them. The connection is brought about by a fine lamelliform enlargement that merges with the same walls. Actually the stroma formed by these cells in the innermost layers of the bulb, near the central cavity, appears denser than elsewhere; it somewhat diminishes in the outer layers of the white matter; becomes more abundant in the neighborhood of the regular layer of ganglion cells; diminishes again greatly in the gray matter, in order to appear again more abundantly, as we saw, in the glomeruli and the superficial layer of nerve fibers.

At the end of this survey I would like to draw some physiological conclusions from these anatomical facts. But these facts are not yet so well coordinated as to produce conclusions of adequately based on evidence. That is why I shall, instead of launching myself into the field of hypotheses, be content with this bare, loose enumeration of the facts, wishing that very soon further studies will be able to fill the still existing gaps.

The single basic deduction, which I shall allow myself, is the anatomical explanation of a well known physiological law.

The fact that the fibers coming out of the *tractus* [LOT], at least in major part, form, before connecting with the nerve cells, a most intricate network because of their complicated ramifications, and furthermore the other fact that the nervous prolongations of a large part of the bulb's ganglion cells, with their complicated branching, produce not one, but several nerve fibers, and allow the conclusion that each fiber of the *tractus*, [LOT] coming out of the brain, connects not only with one cell, but with several (19). This anatomical observation leads of course to the physiological conclusion that the functional connection between the cells of the bulb (sensitive center) and the cells of the cerebrum (center of ideation) is brought about by the fibers of the *tractus* [LOT] in a holistic way, meaning that not an isolated conduction of separate elements takes place, but a conduction on the whole (20). It is perfectly clear that such a conclusion based on anatomical facts harmonizes perfectly with what we really know about the olfactory functions and their relations to the centers of ideation (21).

One could just as well apply this same reasoning to the kind of connection present between the peripheral olfactory fibers and the bulb's cells, and, maybe also the fibers of the *tractus* [LOT], being that we saw that the indirect connection with the cells and the probable connection with the fibers are brought about by the network placed in the glomeruli. (22).

We cannot yet absolutely deny the fact that perhaps a certain number of cells operate in a manner different from what we just describe here, meaning that they possess an isolated transmission (23). Perhaps one would have to consider as such the large cells which are placed with regularity along the inner border of the gray layer, being that I have not yet been able to trace any ramifications of the nervous prolongation of these cells, corresponding to those that I demonstrated for the nervous prolongations [axons] of the ganglion cells of the cerebral and cerebellar cortex and of the dorsal (sensitive) horns of the spinal cord (24). If this negative observation should be confirmed, one would have to admit that this prolongation fuses directly into the axis-cylinder of a nervous marrow-fiber, as not long ago it was believed to be the case for the cerebrum's and cerebellum's nervous prolongation [axon], as well as for the spinal cord-marrow. But I like to repeat that we shall have to wait for the results of further studies before we can admit such an important difference between the different categories of ganglion cells.

4. Method (25)

Concerning the methods employed in this anatomical study, I have to say that I used all those which have been of greatest help in the study of the central nervous system, ranging from the simple teasing-apart of fresh material or after slight hardening with bichromate, to the method of the combined action of bichromate and silver nitrate which I discovered, without forgetting to test also the other modern methods such as those of gold chloride or osmic acid.

Because of all of these methods the best results were obtained by mine (which makes it possible to obtain, by means of specific modifications, the black coloring of cells or nerve

fibers, or of connective tissue elements, or the simultaneous coloring of one part of the former and one part of the latter), I shall in this survey state briefly a few rules in order that it may be used, making it clear however that I am not yet in a position to give with absolute accuracy the ways of procedure which are required in order to obtain the best results. These still depend very much on chance.

The method I have followed consists mainly of two different procedures: 1. Hardening of the tissue pieces in a solution of potassium bichromate or ammonia. 2. Immersion of these pieces in a solution of silver nitrate.

The most appropriate solution for the hardening [fixation] is prepared following Mueller's formula (potassium bichromic acid 2 g and sodium sulfate 1 g) (26); but it is appropriate to increase gradually every eight days the dose of bichromate so that one reaches a dose of 3 1/2–4 g. The fresher the pieces that have to be examined, the better are the results; it is therefore convenient to bring the bulb into the solution as soon as the animal has been killed. Bulbs of a certain size, like those of a dog or an ox, etc., first have to be cut into pieces so that the action of the bichromate on the parenchyma emerges uniformly. The length of the immersion necessary to obtain an appropriate hardening or in order best to prepare the pieces for the desired special reaction which should appear in the elements during their passage through the nitrate solution, depends not only on the concentration of the hardening liquid but also, and much more, on the surrounding temperature. In a cold environment the immersion must last longer; conversely in a warm place there can be large differences; e.g.: the reaction, that during the warm season can be obtained within 30–40 days of immersion in the bichromate, often requires 3, 4 or more months during the cold season. You see now that no definite rules can be made, and good results often depend, as I have already said, on chance.

The most difficult and at the same time the most important part of my method is therefore to determine the length of time after which the transition of the tissue pieces from the bichromate into the silver nitrate has to take place. One has to replace the lack of precise rules by numerous tests made from time to time; besides the following few points will help.

During the cold season it is best to start the first test only three months after the immersion in the bichromate, and repeat the tests every tenth day. During the gradual transition from a cold to a warmer season, one should start the tests proportionally earlier and shorten the period of the repetitions, and in the warm season one could start the tests with the silver salts after 30–40 days and repeat them every fourth or fifth day. I also have to admit that I have not yet been able to find out the exact rule concerning the effects on the nerve cells, fibers and elements of connective tissue [glia], etc., because, frequently, under apparently similar conditions, the results have been very different. But I can say that the most common succession of reactions in the different tissue elements happens as follows.

1. Bundles of nerve fibers forming the most superficial [olfactory nerve] layer of the bulbs and entering the glomeruli; 2. large nerve cells [mitral cells] lying in a regular row at the inner border of the gray layer; 3. bundles of nerve fibers from the white matter [granule cell layer]; almost at the same time, or only a little later, appear the small pyramidal cells

[granule cells] which lie in the empty spaces between the nerve bundles in the same layer; 4. elements of connective tissue [glia] and blood vessels. (27). These are usually partly colored at earlier stages, together with the cells and nerve fibers, being more abundant in one or the other layer: but their most complete, widest and most delicate coloring appears only at an advanced hardening stage; 5. the large isolated cells scattered in the middle, inner layer [tufted and/or short-axon cells?] color without set rule, sometimes together with the elements of connective tissue [glia], at other times together with the nerve fibers.

I think it unnecessary to add that these different stages of the reaction follow each other rapidly or slowly, always depending on the surrounding temperature, just as it has been mentioned to be the case for the time of immersion in the bichromate; for the succession of the different stages, this means nearly as many gradings and transitions as there are between the lowest and highest temperatures of our climate. It is easy to understand that one only rarely is able to bring about a definite succession of the different stages, because of this transition grade by grade; it is not rare either, that because of the uneven effect of the bichromate, a reaction takes place in a given series of elements at one part of the tissue, while at another part quite another reaction predominates.

The silver solution I generally use is 1:100. It is also possible to obtain good results with weaker solutions, for example with 0.50 or 0.75 per 100, but they have to be renewed every 12, 15 or 20 hours, or even more often, when several pieces lie in a comparatively small quantity of liquid. The liquid needs renewing when it starts to show a yellowish color.

As to what concerns the immersion of the tissue pieces in the silver solution, no strict period of time has to be observed, as it may last for weeks or months without causing damage; but it has to be observed that the reaction of the silver nitrate also takes place much more rapidly in the warm than in the cold. A few hours are usually enough to show the first signs of the reaction on the superficial layers, but in order to let the silver solution penetrate into the depth, 24 hours is necessary in the summer, and in the winter 48 hours or more is required. The time given here is the minimum in each case and it may of course be prolonged for weeks and months, especially because while they are in the silver solution the pieces get a certain consistency which favors the thinner sections. But it will be better to transfer into alcohol the pieces meant to keep for several years. (28).

For microscopical examinations the sections are plunged into damarfirmiss or into Canada balsam, after having been dehydrated in absolute alcohol and made transparent with creosote.

Time and light always damage the microscopic preparations made by my method, therefore it is wise to keep a few pieces in alcohol, from which new sections may be obtained for demonstration in a short time. Careful handling, especially complete dehydration of the separate sections with alcohol, and storing in a dark place, can postpone deterioration a long time, keeping the preparations quite valuable for over one year; but then deterioration appears soon in the form of at least a diffuse yellow coloring.

Lastly I shall add about my method, that some experiments have proved that by artificially increasing the temperature of the place in which the pieces are located it is possible to cut

down considerably the immersion time in the bichromate, which is normally quite long; but I am not able to give any precise details on this argument, as I have not had enough opportunities to make sufficient experiments.

5. Explanation of the figure (Plate VII)

All the different elements shown on this plate have been drawn point by point and with the greatest possible exactness by Oberhauser's camera clara [camera lucida].

The drawing shows in a semischematic manner, at approximately 250 diameters magnification, a piece of a vertical section through olfactory bulb of a dog. The three different layers of this organ are marked on the side by the letters A, B, C.

A indicates the superficial layer of the bulb, the one of the peripheral [olfactory] nerve fibers. It consists mainly of nerve fiber bundles coming from the olfactory mucosa. These bundles criss-cross, run toward the glomeruli, enter them (at the points a,a,a), and divide into fine branches. In the midst of these bundles one also sees a blood vessel which sends out several branches vertically toward the interior.

B indicates the middle layer, the gray matter. On its peripheral border lie the glomeruli; on its inner border lie the large nerve cells [mitral cells], arranged in a regular series. The prolongation essentially nervous (b,b,b), (axis-cylinder prolongation [axon]) of these cells is, without exception, turned vertically toward the inner layers of the bulb; the protoplasmic prolongations [dendrites] (b',b',b') go toward the glomeruli instead, enter them and ramify there in a most complicated manner. This kind of distribution is shown in our drawing only by one of the prolongations [dendrites] (b'').

Toward the middle of layer B also drawn two isolated large fusiform nerve cells, the blue-colored *nervous prolongation* [axon] of which descends ramifying in the layer of nerve fibers.

Around the glomeruli one can see a few small nerve cells. Their prolongations [dendrites] turned toward the glomeruli look protoplasmic, whereas the only prolongation going in the opposite direction (the one colored blue) shows all the characteristics of nervous prolongations [axon].

C in the end marks the inner layer, the layer of nerve fibers coming out of the *tractus* [LOT]. In the spaces left empty by the criss-crossing fibers, one can see the small element of chiefly pyramidal shape and probably nervous nature [granule cell]. In the middle of the layer one can also see two unmistakably nervous cells, characteristic by their shape and size, as well as by the presence of a prolongation (the one colored blue) which obviously belongs to the category of nervous prolongations [axon]. The fibrils created by the subdivisions of this prolongation connect with the bundles coming from the *tractus* [LOT] (29).

In order not to obscure the drawing too much, we have left aside here the complicated ramifications of the nerve fibers; the course and manner of ramification of these fibers can be seen approximately on the periphery of layer C. At this point some of the fibers detach

from the bundles, ramify in a complicated way, and cross the border of the white matter in the course of their twisted route; then they penetrate into the gray matter, also pass through it, and one is able to follow many of them as the finest, tiniest fibers, right into the glomeruli (30).

The stroma of connective [glia] cells was reproduced as exactly as possible, concerning their number and relation with the blood vessels, only in the deeper parts of layer C, where in fact the radiating connective cells (31) tend to be most abundant. The other parts are only indicated at the border zone between the white and the gray matter, in the glomeruli, and in the layer of peripheral nerve fibers. (See the parts of the drawing colored in red).

6. Commentaries

The reader will note, as indicated above, that we have retained in general the terminology of Golgi's time. Thus, protoplasmic prolongation was the term for what came to be called "dendrite", and axis-cylinder for "axon". "Ganglion cell" was general term in that era for any large cell in a region of the brain; Golgi also uses it more specifically for a cell with a long axon leaving the region of the cell of origin (Golgi type I neuron).

Specific comments follow on the points noted in the text:

1. This claim by Owsjannikow and Walter that tractus fibers (i.e. LOT axons) connect to olfactory fibers is of particular interest to Golgi; on the one hand, he is looking for evidence of reticular continuity between axons, on the other, he is against continuity between axons and dendrites a la Gerlach.
2. These thin connective tissue cells appear to be the special type of glia around olfactory nerve axons that are now called Olfactory Ensheathing Cells (OEC).
3. Golgi is disappointed not to have seen axon collaterals, one of his great discoveries in the cerebellum and hippocampus.
4. It is not clear whether he is seeing primary or secondary dendrites.
5. This is a novel observation, associating a double row of mitral cell bodies with a macrosmatic species. In assessing these and other observations it would help to know the age of the animals.
6. He refers to the critical question of the connection between the olfactory axons and ultimately the lateral olfactory tract.
7. Golgi appears to infer that the tufted cell axon gives off collaterals (unlike the mitral cell earlier described), and that these connect with the fibers of the LOT.
8. This is a critical point; he is making a case for a special example of a network; it is not clear how this relates to the type of network Golgi imagined formed by axon collaterals in the cerebellum and hippocampus.
9. Perhaps nowhere else in his studies was Golgi so tantalizingly close to the principle of transmission by contact, but he had already in the cerebellum paper of 1874 committed himself to the interpretation of continuity by axon collateral reticulum.

His skepticism about whether the intermingling of fibers means transmission between them was of course justified.

10. He accepts the belief of Walter and Owsjanikow that the LOT sends fibers that penetrate the glomeruli and form a network with the dendrites of the mitral, tufted and PG cells. Some of these fibers could be centrifugal fibers originating in the brainstem, some of which are now known to reach the olfactory glomeruli.
11. Golgi here is analyzing differences between interwoven fibers that argue against a continuous network formed by axons onto dendrites. This difference provided the basis for his clear distinction of free endings of the dendrites, a principle that was supported by nearly all the subsequent classical histologists and was a firm basis of the neuron doctrine.
12. This may or may not be a strong argument regarding continuity; for example, arterioles and venules are continuously connected by capillaries. One may add that neurons also have separate subcompartments that are in continuity with each other but are “chemically” and physiologically very different (e.g. axon and dendrites).
13. This appears to support his view that axons can form networks with axons, but not axons with dendrites. There were no axon collaterals of the mitral and tufted cells to form a network with the incoming olfactory axons, so Golgi here posits other axons coming from the depths of the olfactory bulb, possibly from the lateral olfactory tract.. As noted by Mazzarello (2010) the question of how transmission occurs between olfactory nerve terminals and mitral cell dendrites (and similarly between basket cells and Purkinje cells in cerebellum) was critical to the controversy over neuronal vs reticular transmission. The absence of axon collaterals to provide for this transmission was to bother Golgi; and he assigned students to the problem to discover axons from the olfactory bulb to support the reticular axon-to-axon pathway required by his reticular theory, but such fibers were never found (see Mazzarello, 2010).
14. Note this distinction in the orientation of the granule cell's inner and outer dendrites; the outer longer radial dendrite arises from the apex toward the periphery.
15. They certainly do not correspond.
16. This is evidence against Golgi's concept of networks formed by axon collaterals, though he doesn't count it as significant in that respect.
17. In other words, he believes that these axon collaterals connect to fibers coming from the LOT without a clear axon, but it raises the question of whether the granule cell peripheral dendrite has a basic similarity to the apical dendrite of a cortical pyramidal neuron, though adapted for presynaptic as well as postsynaptic connections and functions; this has been implied by the similar fine structures.
18. These appear to be the short-axon cells in the granule layer which were called Blanes cells in subsequent work from Cajal's laboratory (Blanes, 1897); see also Pressler and Strowbridge (2006).

19. Golgi appears not to understand the LOT as arising from mitral/tufted cell axons; he appears to imagine that the LOT provides axons to interconnect with multiple axons and their branches coming from the mitral/tufted cells.
20. This is Golgi's favorite theme, of the nervous system working through a network as a whole rather than through isolated pathways.
21. This is a rather big conceptual jump from apparent facts to fact-based conclusions!
22. This sudden collection of all observations presented as an overall conceptual scheme seems rather forced.
23. This admission of the possibility of "isolated conduction" seems a big concession, though remember that this is 1875, more than a decade before Cajal and his specific theory of transmission by contact. It means that Golgi is here debating this issue with himself, not with Cajal! It would be interesting to examine more closely whether the fact that Golgi conceived of the possibility of "isolated conduction" as an alternative to network conduction planted the idea of isolated conduction linked to transmission by contact in Cajal's mind.
24. Again, he cites his inability to visualize collaterals of the mitral cell axons.
25. There was always a complaint that Golgi in his first papers did not adequately describe his method, so this is the first detailed description. It seems to be a relatively in-depth description of his method; it should be compared with other descriptions.
26. No volumes are indicated.
27. This is an interesting sequence of impregnation, which should be compared with Cajal's paper on the olfactory bulb in 1890–91.
28. No information is given on the thickness of the sections.
29. Here is an unambiguous statement that the axon collaterals of the short-axon cells in the granule layer connect with bundles of fibers coming from the LOT, an erroneous interpretation.
30. This seems to suggest that the nerve fibers of the granule cell layer also enter the glomeruli, another erroneous interpretation.
31. Note the observation of radially-oriented connective cells. The history of these cells is covered by Bentivoglio and Mazzarello (1999). Radially-oriented glia were subsequently described in the spinal cord by Golgi and published in Golgi (1886), and subsequently were described in the cerebral cortex by Giuseppe Magini in 1887–88 (following the suggestions of Golgi) applying the Golgi technique. These observations anticipated the fundamental discoveries by modern methods of radial glia and the structure of the cerebral cortex by Pasko Rakic beginning in the 1960s (summarized in Rakic (2007).

In summary, these observations constitute a remarkable scientific and artistic achievement, showing how Golgi working alone was wresting from his impregnated specimens the first

forms of cells in this part of the brain. The plate is one of the most complete representations of all of the elements – different types of nerve cells, glial cells, fiber tracts and blood vessels -by any histologist working on the nervous system. It shows how aware Golgi was of the potential relevance of all of the elements and their relations to understanding the cellular basis of nervous function. Later histologists, and continuing to the present day, conceive of nervous function primarily in terms of the nerve cells; Golgi however thought much more broadly.

The accuracy and amount of detail are astonishing. To recapitulate, what we call mitral, tufted, granule and periglomerular cells are seen within their appropriate histological layers. The lamination and cell types are there, the images clearly represent the modern versions of these cell types, although the forms of the cells are still not completely represented. It was however a solid foundation for all subsequent studies.

An additional point regards the presentation of the cells. Before Golgi, the only nerve cells that had been visualized were independent entities, teased out from their poorly fixed, in some cases unfixed, tissue. Deiters' (1865) magnificent view of a spinal motor neuron is a dramatic instance. In contrast, Golgi could observe the individual neuron types in full and in relation to the histological laminae. The illustration thus shows for the first time that the nerve cells composing a specific part of the nervous system are differentiated into several distinct types. Furthermore, they are shown organized in relation to the layers in which they are found.

Since the Golgi method only stains individual cells, their relations with each other had to be inferred from observing the different types individually in their different layers. This in itself was a monumental achievement; the first concept not only of the different cell types that carry out the functions of a given brain region, but also how they might be related to, and interact with, each other. The olfactory bulb served this purpose well, as did the hippocampus (see Golgi et al., 2001) and cerebellum (Golgi, 1874). Golgi's first diagrams thus provided the blueprint for all subsequent representations of the multiple cell types in a given brain region, starting with Cajal when he first took up the Golgi method in 1887–88. In summary this was the first paper in which a modern drawing of the structure of a portion of the central nervous system was outlined.

From the perspective of later controversies, a significant aspect of this first view by Golgi of a nerve center is that the cells and their processes are represented as bounded entities. This is seen most clearly in the glomeruli, where all of the dendrites – of mitral, tufted, and periglomerular cells – terminate abruptly. (What we call secondary dendrites in the external plexiform layer have obviously been cut in the sectioning.) He was unable to resolve the question of the termination of the olfactory axons in the glomerulus, but at least at this early stage he was willing to leave the matter undetermined, or perhaps, indeterminate.

7. Foundation for subsequent studies

We are now in a position to compare in detail for the first time this original study of olfactory bulb neurons by Golgi with the studies that came after. This is particularly relevant

because the later studies, particularly by Cajal, have been the ones that have come down to us as representing the work of the classical histologists.

As is well known, Golgi's first publications of his new method aroused little interest among anatomists. It was not until Santiago Ramón y Cajal in Spain stumbled across a demonstration of the method in the home of Simarro that the potential of the method began to be exploited. Cajal's first publication with the method in 1888 was on the cerebellum, but the olfactory bulb followed soon after in 1890. It will be instructive therefore to consider Cajal's very first representation of the cells of the olfactory bulb, keeping in mind that he had undoubtedly studied Golgi carefully and surely knew the details of Plate I by heart! In fact Golgi sent a reprint of the paper to Cajal, probably in the second half of 1889; (see DeFelipe 2010; Mazzarello 2010 and the extract of a letter from Cajal to Golgi reproduced in this volume: Sassoè-Pognetto, 2011).

The title of Cajal's paper was "Origin and termination of the olfactory nerve fibers", and his first diagram (Fig. 2) shows just that: the origin of the olfactory nerves in the cells of the olfactory epithelium, their gathering into bundles that traverse the cribriform plate, their criss-crossing as they meet the olfactory bulb, and their termination in the glomeruli of the olfactory bulb. The entire trajectory of the population of fibers is there, though we can't distinguish the trajectory for an individual fiber from epithelial cell to glomerulus — even Cajal couldn't manage that!).

Within the olfactory bulb the mitral, granule and periglomerular cells are clearly seen. The mitral secondary dendrites are now more obvious. In this paper he bestows the name "mitral" on the large tufted cells. The fibrous nature of the inner (internal) and outer (external) plexiform layers is emphasized. However, it is essentially a confirmation of Golgi's concept of the neural types and their laminar organization in the olfactory bulb.

But the Devil is in the details, and there were several in the olfactory bulb that Cajal took advantage of. Perhaps the most important is shown in his second diagram (Fig. 3), where he carefully draws the terminations of the olfactory nerve fibers in the interior of the glomerulus. This was surely a composite of many terminals in many glomeruli in many preparations, with a clear message: the axonal branches all have free endings. There is no hesitancy as with Golgi in making this claim.

With regard to the neuron vs reticulum controversy (see full discussion elsewhere in this volume: Mazzarello; Sotelo; Raviola; Verkhratsky et al.), the issue was not whether there was continuity between the axon terminals and the dendrites. That was Gerlach's idea of a continuum, which Golgi vigorously rejected. Golgi's idea was continuity between axons and axon collaterals, forming a reticulum which carried out the "nervous" functions of the nervous system, while the dendrites provided the nourishment. Although the main battle grounds were the cerebellum and hippocampus, the olfactory bulb can in fact be seen to have provided the clearest evidence that a set of axons — the olfactory axons — terminates at one site — the glomerulus — where it makes contact with dendrites — of mitral, tufted and periglomerular cells. The only hope for Golgi for an axonal reticulum in this pathway was for the olfactory axons to be continuous with axon collaterals of the mitral, tufted and/or

periglomerular cells, not seen clearly, or with fibers from the lateral olfactory tract and/or centrifugal fibers from elsewhere in the brain. As is evident in Golgi's account, these were difficult to demonstrate.

Another important insight made by Cajal was to see that the contact of the olfactory axons on the distal tufts of the mitral and tufted cell dendrites meant that the dendrites were the only pathway to transfer the response into an output in the axons arising from the cell bodies. This was a priori proof of the nervous function of the dendrites. Since Golgi had pinned his interpretation of nervous function on axons and axon collaterals, dendrites were denied a nervous function, and therefore assigned a nourishing function.

8. Concluding remarks

In assessing the contributions of Golgi and Cajal to the foundation of our understanding of the olfactory bulb, there is plenty of credit to go around. Golgi was the pioneer. He met the cells for the first time, saw how they were different, gave them individual names, put them in their laminar homes, and arranged them as a coherent population of interspersed elements along with the glia and the blood vessels. He oriented his comprehensive diagram with the input coming in from the top and the output going out from the bottom, a general orientation used in most modern representations of the olfactory bulb and indeed of most other brain regions (see Shepherd and Grillner, 2010).

Cajal therefore had a solid foundation to build on when he brought his restless energy to bear on the cellular elements in this and many other brain regions. He even had the advantage of Golgi's debate with himself over the question of "isolated conduction" vs network conduction. His drawings of Golgi impregnated neurons, in his initial paper and in subsequent publications, give a very modern sense of the observed entities. He had the good fortune to understand from the start that axons as well as dendrites end freely, which was the key to an accurate representation of the relations between cells. He utilized the olfactory bulb brilliantly in this way to provide perhaps the clearest evidence of how axons contact dendrites, and how dendrites must be essential in transmitting nervous information from inputs into outputs. In contrast to Golgi, his general representation of the olfactory bulb was with the input coming from below. Our modern views of the organization of the olfactory bulb thus combine the best that each had to offer.

It remains to note that the 1890s were the golden era of classical histology of the nervous system, and studies of the olfactory bulb by other leading histologists quickly followed. These included Kölliker (1890, 1892), van Gehuchten and Martin (1891), Retzius (1892), Blanes (from Cajal's laboratory) (1897), as well as Hill (1897a, b) and Manouélian (1899). All of this work, and more, was summarized by Cajal in his great compendium of the histology of the nervous system (Cajal, 1911). Although this means that we tend to cite Cajal when referring to this classical work, our present excursion into history shows how Golgi led the way.

Acknowledgments

We thank Dr. Javier DeFelipe for the reproduction of Fig. 1.

References

- Bentivoglio M, Mazzarello P. The history of radial glia. *Brain Res Bull.* 1999; 49:305–315. [PubMed: 10452351]
- Blanes T. Sobre algunos puntos dudosos de la estructura del bulbo olfatorio. (On some doubtful points concerning the structure of the olfactory bulb). *Rev Trim Micrograf.* 1897; 3:99–127.
- Cajal S, Ramon y. Origen y terminación de las fibras nerviosas olfatorias. (Origin and termination of the olfactory nerve fibers). *Gaceta Sanitaria de Barcelona.* 1890–91; 3:133–139.
- Cajal, S.; Ramon, y. *Histologie du système nerveux de l'homme et des vertébrés.* Azoulay, L., translator. Vol. 2. Paris: Maloine; 1911.
- DeFelipe, J. *Cajal's Butterflies of the Soul.* Oxford University Press; New York: 2010.
- Deiters, OFK. *Untersuchungen über Gehirn und Rückenmark des Menschen und der Säugethiere.* Vieweg; Braunschweig: 1865.
- Golgi, C. Sulla strutura della sostanza grigia del cervello. In: Santini, M., translator. *Gazzeta Medica Italiana, Lombardia.* Vol. 33. New York: Oxford University Press; 1873. p. 244-246. reproduced in Shepherd GM 1991 *Foundations of the Neuron Doctrine*
- Golgi, C. *Archivio Italiano per le Malatie Nervose.* Istituto Lombardo di Scienze e Lettere; 1874. Sulla fina anatomia del cervelletto umano. *Rendiconti del R; p.* 90-107. Serie 2: 7: 69–72 Anno 11
- Golgi C. Sulla fina struttura dei bulbi olfactorii. (On the fine structure of the olfactory bulb). *Rivista Sperimentale di Freniatria e Medicina Legale.* 1875; 1:405–425.
- Golgi, C. Sulla fina anatomia degli organi centrali del sistema nervoso (On the fine anatomy of the central organs of the nervous system). Milano: Hoepli; 1886. First published as a series of papers in *Riv. Sper. Freniat.* 1882–1885, volumes VIII–XI; a first partial edition was published in 1884 by Calderini di Reggio Emilia, then followed a new enlarged edition in 1885 by the same publisher which was reprinted in 1886 by the publisher Hoepli of Milan. This work is mainly known from the German translation included in Golgi 1894
- Golgi, C. *Untersuchungen über den feineren Bau des centralen und peripherischen Nervensystems.* Gustav Fischer; Jena: 1894.
- Golgi, C. *Opera Omnia.* Vol. I, II, III. Hoepli; Milano: 1903.
- Golgi C, Bentivoglio M, Swanson L. On the fine structure of the pes hippocampi major (with plates XIII–XXIII). 1886. *Brain Res Bull.* 2001; 54:461–483. [PubMed: 11397537]
- Hill A. Note on “thorns” and a history of the constitution of grey matter. *Brain.* 1897a; 20:131–137.
- Hill A. Notes on granules. *Brain.* 1897b; 20:125–130.
- Kölliker A. Ueber die erste eutwicklung der nervi olfactorii (On the first development of the olfactory nerves). *Sitzungsberichte der physikal-medicin Gesellschaft.* 1890; 9:127–129.
- Kölliker A. Ueber den feineren ban des bulbus olfactorius (On the finer structure of the olfactory bulb). *Sitzungsberichte der physikal-medicin Gesellschaft.* 1892; 1:1–5.
- Manouélian Y. Les fibres centrifuges du bulbe olfactif et les neurones olfactifs centraux (The centrifugal fibres of the olfactory bulb and the central olfactory neurons). *CR Soc Biol Paris.* 1899; 51:530–532.
- Mazzarello, P. *Golgi: A Biography of the Founder of Modern Neuroscience.* Oxford University Press; New York: 2010.
- Pressler RT, Strowbridge BW. Blanes cells mediate persistent feedforward inhibition onto granule cells in the olfactory bulb. *Neuron.* 2006; 49:889–904. [PubMed: 16543136]
- Rakic P. The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. *Brain Res Rev.* 2007; 55:204–219. [PubMed: 17467805]
- Retzius G. Die endigungsweise der riechnerven (The olfactory nerve's manner of ending). *Biol Unters Neue Folge.* 1892; 3:17–24.
- Sassoé-Pognetto M. Molecular and functional heterogeneity of neural circuits: an example from the olfactory bulb. *Brains Res Rev.* 2011; 66:35–42.
- Shepherd, GM.; Grillner, S., editors. *Handbook of Brain Microcircuits.* Oxford University Press; New York: 2010.

Van Gehuchten A, Martin I. Le bulbe olfactif chez quelques mammiferes (The olfactory bulb of some mammals). *Cellule*. 1891; 5:205–237.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1. Olfactory bulb of the dog, impregnated by the Golgi method. Golgi, 1875. For full description, see text.

Fig. 2.

Antero-posterior section of the olfactory bulb and nasal mucosa of the newborn rat. A: Olfactory epithelium situated below the cribriform plate; a, bipolar cell; b, epithelial or supporting cell. B: Dermis of the mucosa and bundles of olfactory fibers; C, ethmoidal cartilage [cribriform plate]; D, olfactory nerve layer; E: olfactory glomerular layer; F, inferior molecular layer [external plexiform layer]; G, mitral cell body layer; H, superior molecular layer [internal plexiform layer]; I: granule layer. c, cartilage, e, olfactory nerves; f, arborization of olfactory fibrils [axons] in the glomeruli; g, central axis-cylinder [axon] of an inferior tufted cell; h, mitral cell; i, granules; j, epithelial cells; n, more inferior granule; m, terminal filament of epithelial cells; o, large stellate cell. Translated from Cajal (1890–91).

Fig. 3. Antero-posterior section of the olfactory bulb of the duck. A, arborization of olfactory fibrils within the glomeruli; B, descending trunks of the large tufted cells, terminating in a granulous meshwork in the glomeruli; C, large superior tufted cells. Translated from Cajal (1890–91).