

# On Nerve-End Cells in the Dental Pulp

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VII.—On Nerve-end Cells in the Dental Pulp.

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[Plates 49 and 50.]

In a paper on "The Distribution of the Nerves of the Dental Pulp," published in the 'Philosophical Transactions' for 1912,\* I was able to show that the nerves of the tooth pulp do not terminate at the inner margin of the dentine as had been generally supposed, but that they actually enter the dentinal tubules with the dentinal fibrils or processes of the odontoblast cells, and are distributed within the hard tissue of the dentine.

By the methods of preparation I then used, I was unable to demonstrate the neurofibril as a continuous uninterrupted fibre, although its continuity with the axis cylinder of the medullated nerves of the pulp was evident.

By certain modifications of these methods I have recently succeeded in obtaining preparations in which the neurofibrils are stained throughout their whole length as continuous beaded fibres both in the pulp and in the dentine. In these preparations the nerve-end cells, to be presently described, remain transparent and invisible, and the neuro-fibrils appear to pass direct to the dentine, but their continuity with the medullated fibres of the pulp is clearly seen (Plate 49, fig. 10), (5 b).

The most important point shown in my latest specimens is that the neurofibrils in the plexus beneath the odontoblasts do not pass direct to the dentine but enter small nerve-end cells or corpuscles which lie in a row along the bases of the odontoblast cells (Plate 49, fig. 1).

The peripheral distribution of the nerves in the dentine is thus seen to take place from definite nerve-end bodies situated at the lower margin of the odontoblast layer; each end cell contributing a process to the dentine which would appear to represent the axon of the cell, and numerous other processes apparently corresponding to the dendrons of a nerve cell; these dendrons form a network around the odontoblasts. The individual end cells have mostly a stellate form and are collected into groups slightly separated from one another (Plate 49, figs. 1 and 4). As shown in fig. 1a, short dendritic processes are seen at the inner end of many cells which would appear to form synapses with the fibres of the deep plexus.

The distal process of the end cell is usually unbranched and passes up between the odontoblasts in a wavy course, to enter the dentine with the dentinal fibril.

\* 'Phil. Trans.,' B, vol. 202, pp. 337-349 (1912).

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In the illustrations in the present paper I have chiefly chosen parts of the sections where the dentine is slightly separated from the pulp, since these show the relations of the nerve fibres to the dentine more clearly. In these parts owing to the very extensile nature of the nerve fibres, the latter are more or less drawn out into straight lines and slightly pulled out of the dentinal tubules. In Plate 49, fig. 2, at the very thin margin of a section, a nerve fibre is shown under great tension, in fact, after I had photographed this fibre, it gave way and its portions became widely separated. This great extensibility of the neurofibrils is shown in many preparations; in some they are drawn out in parallel strings of such tenuity that they can only be properly seen with a high power of the microscope, although their continuity is completely maintained.

The nerve-end cells, which as previously stated, are arranged in groups, form a definite layer in the pulp, parallel with the inner border of the dentine, and lie among the odontoblast cells at their proximal ends. They are often seen to communicate by parallel cross branches giving a kind of basket-like appearance to the cluster (fig. 3, Plate 49, and fig. 5, Plate 50).

In many parts of the periphery of the pulp the cells cannot be seen, probably owing to the thickness of the section, or to the plane of the section not being quite parallel to them, and unless the reduction of the gold has been very complete they do not appear at all in these preparations, but apparently remain quite transparent, although the fibres with which they are connected are darkly stained and their connection with the dentine plainly shown. Sometimes a chain of nerve-end cells is met with connected by straight horizontal processes (Plate 49, fig. 12), and the larger cells are seen to form connections with smaller radiating cells (see figs. 11 and 12).

I have examined many preparations with a high power (1/16th and No. 4 ocular) to ascertain if any of the nerve fibres in the deep plexus are distributed to the odontoblast layer without the intervention of the end cells, but I was quite unable to obtain any evidence of this in sections where the end cells were distinctly stained. Sometimes one of the larger end cells is seen with fine processes radiating from it on all sides, and there is no apparent process to the dentine—but these are scarce, and in most places the beaded delicate fibres forming a network around the odontoblasts are distinctly seen to be given off from lateral processes of the end cells. The absence of the distal process in some of the radiating cells may possibly be due to the section not having passed in the plane of this process, for in most places it appears as if each end cell is provided with both sets of processes.

Sometimes the processes to the dentine appear to be wound together in spirals, and there is often an appearance of fibrillation in the cell body.

There is great variation in the size of these end cells, but many which appear to be single, are probably groups of cells. The size of the nerve fibre or bundle of neurofibrils passing to the dentine is also very variable, as shown in the illustrations in this paper.

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The bundles are especially large at the crown of the pulp where there is a much larger area of dentine to be supplied. The bundles probably divide and sub-divide within the dentinal tubes and their branches. Small enlargements or nodes are seen upon the fibres distributed around the odontoblasts, but are not present on the distal or dentine process. It is these enlargements that were considered by Huber (1), Guido Fischer (2), and others to form the terminations of the nerve fibres of the pulp, and which are similar to those shown by Retzius in the mouse, and in fish and reptiles (3).

It is these strongly beaded fibres which I first described as the marginal plexus, but although my first preparations appeared to indicate that the neurofibrils which pass into the dentinal tubes arise from this plexus, the later ones show that this is not the case, for these fibres pass direct to the dentine as the distal processes of the nerve-end cell with which they are continuous. What I had formerly described as the marginal plexus is evidently a portion of the network of fibres surrounding the odontoblasts and extending to the dentine margin; it is derived from the dendritic processes of the nerve cells. In Plate 49, fig. 7, I have shown these enveloping fibres of the odontoblast cells with the enlargements above described. The nuclei and a portion of the odontoblast cell are faintly stained.

I found great difficulty in procuring a definite reduction of the gold in the neurofibrils within the tubes deeply in the dentine. The fibres were seen to pass some little way into the tubules as definite dark stained fibrils accompanying the dentinal fibril but soon ceased to be uninterrupted, and were continued as lines of black dots.

In some fortunate preparations, however, the reduction had reached the right stage within the tubes and even close to the cement beneath the granular layer of Tomes, the fibrils are seen as winding black fibres within the tubes (Plate 49, fig. 8). In several places the nerve fibre and the dentinal fibril can be seen in the same tube—the nerve fibre appearing as a delicate black line accompanying the brown stained dentinal fibril. In the very fine terminal branches of the dentinal tubes at the cement margin only a delicate uniform dotting can be seen in these gold preparations, and this appearance is also visible in the canaliculi of the cement with which these fine branches are continuous. But while these appearances would seem to indicate that there is a direct nerve communication between the pulp and the cement we cannot definitely prove this until the fibre in this situation is seen to be clearly continuous; since in all metallic impregnations deceptive appearances may arise from deposits.

It is, however, noticeable that in these finer divisions of the tube the dotting is uniform, and does not show the variation in size of the particles usually exhibited by the irregular deposit which so often occurs in the larger tubes.

After several attempts I was successful in showing the nerve terminations at the enamel margin of the dentine. To make evident the relations of the nerve fibres to the enamel it was necessary to stain ground sections of teeth which had not been

decalcified. Such sections were ground fairly thin, then subjected to the prolonged action of a weak gold chloride solution, and reduced in the same manner as the decalcified preparations. They were then ground as thin as possible on a stone with water.

Owing to the very numerous branchings of the tubes in this situation, and the fact that these in many places take a diffuse brown stain from the gold treatment, the nerve fibres are a good deal obscured, but in places they are very evident, and are seen to terminate in fine arborisations (Plate 49, fig. 9).

Here and there they pass for a short distance into the enamel, apparently in the interprismatic spaces, where they terminate in small bulbous enlargements.

Where the so-called "spindles" are present in the enamel, especially at the apices of the cusps, fine winding fibres are sometimes seen within them, but these spindles do not in my preparations appear to contain definite nerve-end organs as described by Römer (4). It is impossible, however, to speak with certainty on this point, and the spindles being comparatively infrequent, a great many teeth should be prepared in this manner to decide the question. From the preparations I have made, however, one would be inclined to consider the penetration of these bodies by nerve fibres more as due to the fact that nerve fibres in the tubes would be likely to penetrate into any open space with which the tubes communicate than as constituting definite nerve-end organs. The refraction in these spindles greatly interferes with accurate observation even in thin sections. It is considered by many that the spindles are interprismatic spaces which have remained uncalcified, and my own observations on the enamel of marsupials appear to confirm this interpretation of their nature  $(5\alpha)$ .

Minute beads or enlargements are seen upon the neurofibrils in the tubes in many places, and in some, under high magnification, delicate threads are seen to be continued from these enlargements into the finer divisions of the dentinal tubes.

In Plate 49, fig. 6, two dentinal tubes are shown, at a short distance from their origin in the pulp, in which these nodes are of an angular shape.

The question arises whether these are all sensory fibres or whether some of them are trophic and are supplied to the secreting cells of the odontoblast layer.

The mode of termination in the pulp which I have described above, from peripheral nerve-end organs or cells, is not a usual one for sensory nerves, and does not appear to be met with elsewhere in the body.

That healthy dentine is sensitive does not admit of doubt. A fractured tooth, when the surface of the dentine is exposed and the pulp not opened, is intensely sensitive, and in the operation of "coning" or cutting down the surface of a tooth with a living pulp for the adjustment of a gold crown, as soon as the enamel has been removed and the dentine is reached, the grinding of the latter is acutely felt.

In the excavation of a carious tooth, little or no pain is felt during the excavation of the diseased portion, but when the last layer of carious substance is raised from the healthy dentine beneath, the pain is usually very acute.

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These observations lead to the conclusion that the processes prolonged to the dentine from the nerve cells in the pulp are principally sensory fibres.

We know also that the pulp of the tooth when accidentally exposed is acutely sensitive. We should therefore imagine that both sensory and trophic fibres would be distributed to the pulp and also to the dentine, which contains within the tube a protoplasmic prolongation of the odontoblast cell, the maintenance of the functions of which would probably necessitate a nervous supply.

Since the publication of my paper in 1912, the late Prof. Dependent of Leipzig has published the results of his researches on the same subject (8). He employed many different staining and impregnation methods—usually staining small pieces in bulk, and he corroborated my own observations that the nerve fibres of the pulp enter the dentinal tubes. He did not describe nerve cells in the pulp, but only the small enlargements on the nerve fibres above described. Certain statements of this author as to the passage of nerve fibres across the tubes on the surface of the sectioned dentine, and figured as quite independent of the tubes, are difficult to understand.

## Methods of Preparation.

These specimens showing the nerve-end cells in the pulp were all treated by the gold method of Beckwith with some modifications of the process.

This method, which was adopted from FREUD's gold process, consists chiefly in the use of alkalis for the reduction of the gold and is strongly recommended by Prof. Sims-Woodhead for the demonstration of neurofibrils (7).

In treating teeth by this method I find it absolutely necessary to carry out the following procedure:—

Fixation.—Teeth, immediately after extraction are placed in solution of formol and water or formol and normal salt solution, preferably 4 per cent. of formol (the commercial 40 per cent. solution of formaldehyde). This is after a few days changed to a 10 per cent. solution, and the teeth kept in this for at least a fortnight; specimens which have been kept in this solution, even for many months, have given excellent results.

Teeth with unfinished roots may be placed in the fixing solution without being cut, but those which have completed roots should be previously sawn in two at the neck.

Decalcification.—This is always carried out with formic acid—I have never procured any good nerve preparations of teeth which have been decalcified in the mineral acids; Prof. Dependorf had the same experience.

The decalcifying solution used is that recommended by Guido Fischer (2) and consists of a 33.3 per cent. solution of formic acid in distilled water—Fischer adds 5 per cent. of formol to the solution. This is not a very rapid decalcifying agent, but teeth are generally completely softened in a fortnight.

Washing.—The teeth must be washed in running water for 24 hours.

They are then cut into pieces of the required size and form, and transferred to distilled water for a few minutes.

Impregnation.—The pieces are taken from the distilled water and suspended by threads in a large quantity of a weak solution of gold chloride (1 in 5000). Each piece should be suspended in at least 100 c.c. of the solution in which it is left in the dark for from four days to one week, according to its size. On removal from the gold solution it is washed for a few moments only in distilled water before employing the reducing agents.

Reduction.—The pieces are placed in a 20 per cent. solution of caustic soda for four minutes—on removal from this solution they are rinsed in water and placed in a 10 per cent. solution of potassium carbonate for from half-an-hour to an hour. This is then drained off and the pieces are placed in a 10 per cent. solution of potassium iodide for a short time—usually five to ten minutes. As soon as they are seen to darken they should be removed from this solution to water, placed in gum for 12 hours and cut on the freezing microtome.

The sections are then dehydrated and mounted at once in camsal (propylic) balsam. This mounting medium gives very clear definition and clearing agents are dispensed with as they can be mounted direct from absolute alcohol.

I have for some time employed a 1 per cent. gold chloride solution in distilled water, which has not been kept in the dark but exposed for several hours to direct sunlight in a tightly stoppered bottle.

This procedure is strongly recommended by Dr. Lindsay Johnson (9) who considers that the many failures in gold impregnation are largely due to the neglect of this precaution, the ripened and sunned solutions reducing much more completely than fresh solutions. If kept tightly stopped no precipitation occurs in the gold solution, as it is, as he points out, not the light, but particles of dust which cause the deterioration of the gold solution.

This was first drawn attention to by APATHY (10). The original method of BECKWITH was employed with sections—but although I procured very good preparations of the nerve fibres by this means—I have never been able to demonstrate the nerve-end cells by staining sections, but only by the method of staining in bulk above described.

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#### DESCRIPTION OF PLATES.

Lettering applicable to all the figures.

- d. Dentine.
- n. Nerve cells.
- f. Dentinal fibril.
- a. Nerve process to the dentine.
- e. Enamel.
- l. Lateral processes of the nerve cells.
- b. Blood vessels.
- m. Medullated fibre.
- o. Odontoblast nuclei.
- r. Plexus of Raschkow.

#### PLATE 49.

- Fig. 1.—From a transverse section of a human premolar. The pulp is slightly separated from the dentine—the faintly stained dentinal fibril is seen passing across the space accompanied by the nerve fibre. A row of nerveend cells is seen at the base of the odontoblast layer communicating with the deep plexus or plexus of Raschkow beneath them. Drawing from photograph × 800.
- Fig. 1A.—A single end cell from the same preparation showing the dendritic processes which appear to form synaptic communication with the fibres of the deep plexus.
- Fig. 2.—A portion of the same preparation at the thin margin of the section. The neurofibrils are very much stretched and pulled out of the tubes owing to the separation of the pulp. Drawing from a photograph × 1400.

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- Fig. 3.—A group of end cells in a similar section showing the basket-like appearance produced by the parallel lateral processes. From a photograph × 1400.
- Fig. 4.—Nerve-end cells in the thin margin of a section. From a photograph × 1400.
- Fig. 5.—A group of small end cells from the same preparation. From a photograph × 1400.
- Fig. 6.—Two dentinal tubes near the dentine showing strongly stained neurofibrils with nodular enlargements. Drawing  $\times$  1000.
- Fig. 7.—The plexus of nerve fibres surrounding the odontoblasts, showing the nodular enlargements. The nuclei of the odontoblasts only are stained. Abbé Prism drawing. × 1000.
- Fig. 8.—Neurofibrils within the tubes of the dentine near the cement margin, beneath the granular layer of Tomes. Drawing from photograph × 1000.
- Fig. 9.—Terminations of neurofibrils at the enamel margin. Drawing  $\times$  1000.
- Fig. 10.—Neurofibrils at the cornu of the pulp, showing their connection with the medullated fibres of the pulp. Reduced one half from an Abbé Prism drawing which was magnified 1000 diameters.
- Fig. 11.—Showing grouping of the nerve-end cells. Drawing  $\times$  1000.
- Fig. 12.—A similar group of end cells with parallel strands between them. Drawing × 1000.

#### PLATE 50.

## (Photographs.)

- Fig. 1.—From a transverse section of a human premolar tooth. Showing under low magnification, the row of nerve-end cells at the base of the odontoblast layer. Neurofibrils and dentinal fibrils crossing the space where the pulp is slightly separated from the dentine. × 375.
- Fig. 2.—From a similar section. Showing the nerve-end cells with their nuclei. × 1000.
- Fig. 3.—From the thin end of a similar preparation.  $\times 1400$ .
- Fig. 4.—From a similar section, showing the axon passing to the dentine and the dendrons at the lower and lateral margins of the nerve cells. × 750.
- Fig. 5.—Groups of nerve-end cells with lateral processes.  $\times$  1400.
- Fig. 6.—Another part of the same preparation.  $\times$  1400.
- Fig. 7.—Showing the axon of the end cells passing to the dentine in company with the dentinal fibril.  $\times$  1000.
- Fig. 8.—Nerve-end cells in the pulp.  $\times$  1000.
- Fig. 9.—At the thin margin of the section showing the tension of the neurofibrils passing to the dentine. × 1000.
- Fig. 10.—Neurofibrils in the dentinal tubes near the junction of dentine and cement. × 800.

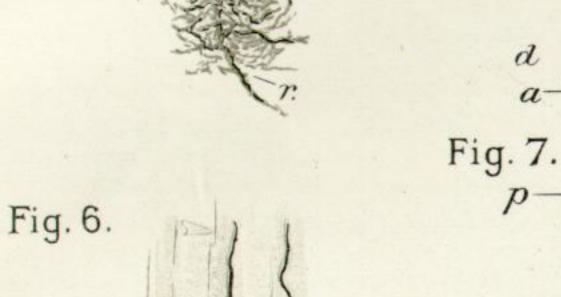


Fig. 3.

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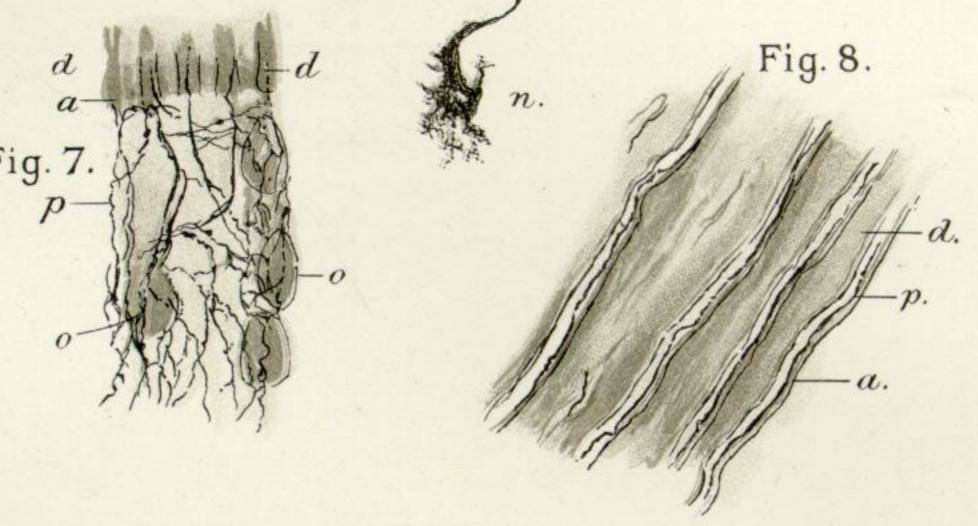
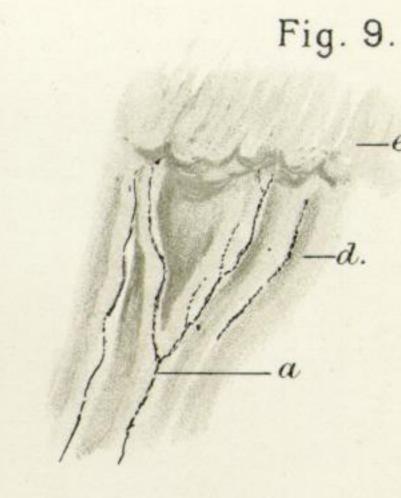
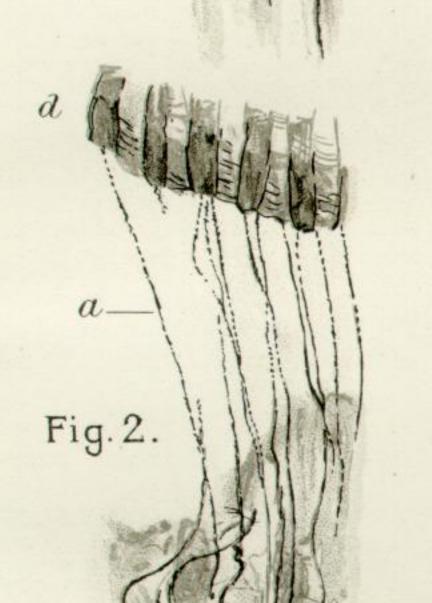


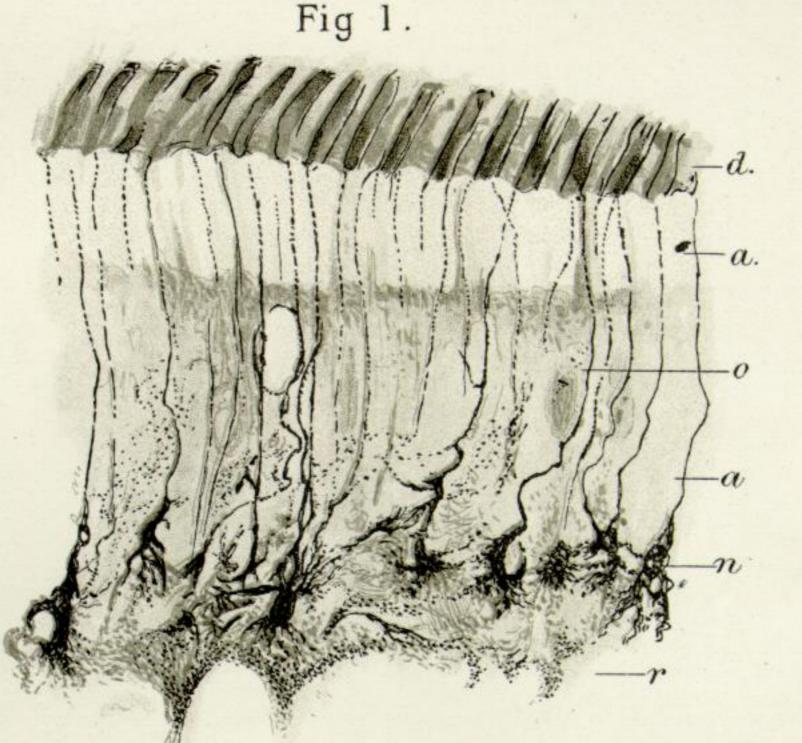
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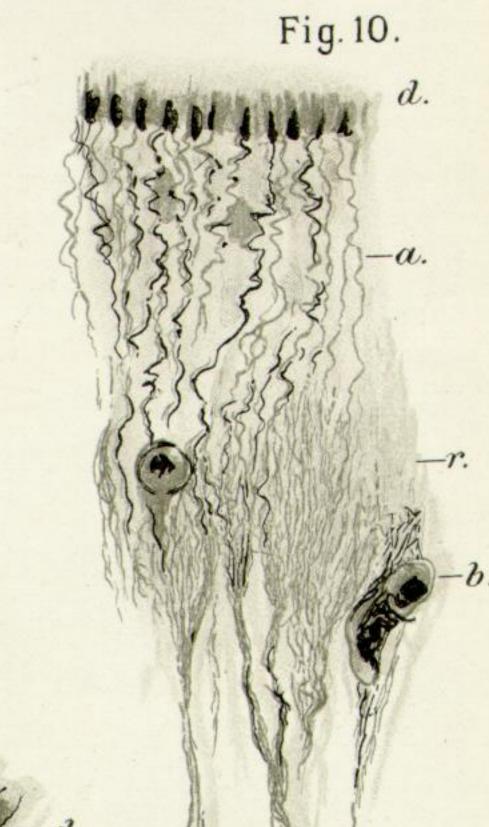
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Fig. 4.

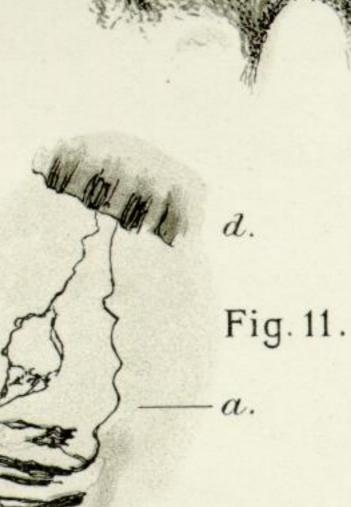


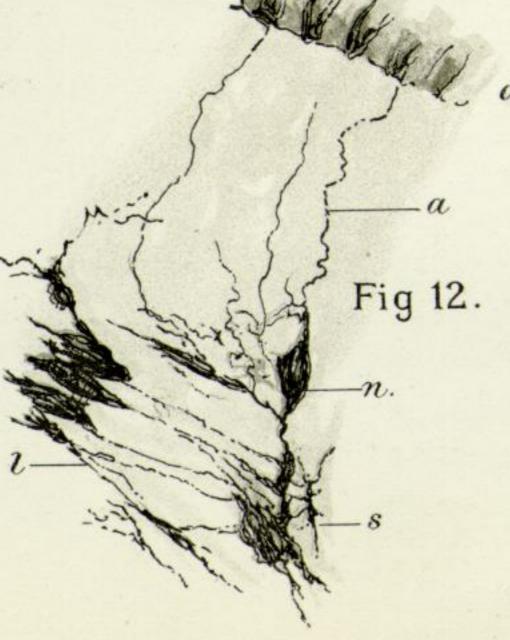


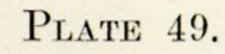




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  - Fig. 2.—From a similar section. Showing the nerve-end cells with their nuclei.  $\times$  1000.
  - Fig. 3.—From the thin end of a similar preparation.  $\times 1400$ .
  - Fig. 4.—From a similar section, showing the axon passing to the dentine and the dendrons at the lower and lateral margins of the nerve cells.  $\times$  750.
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