Mammalian Retina: Associational Nerve Cells in Ganglion Cell Layer

Abstract. *A new type of cell has been found in the ganglion cell layer of the retinas of dogs and humans. Its axon and its terminal branches are intraretinal. From its structure it appears that this cell might play an associational role at the level of the ganglion cells.*

A new technique, the Gros silver impregnation of the entire retina of mammals (*1*), allows us to map the distribution of fibers in the optic nerve, to study the ganglion cells and, particularly, to measure their "dendritic fields." A clear picture of the centrifugal fibers can be obtained (*2, 3*). The transverse association system in the retina can be analyzed by this technique. In this way one of us has described (*4*) a clearly defined layer of horizontal cells at the level of the outer plexiform layer. These cells have no axis cylinder and are organized into a plexus that extends across the whole retina. The amacrine cells can also be studied and can be interpreted as elements of transverse association at the level of the inner plexiform layer (*2*).

In the dog's retina there are cells (Fig. 1) that are identical with the giant ganglion cells described by Cajal (*5*) in location and in structure of their bodies and dendrites. The axis cylinder of these cells does not follow the course of the optic nerve fibers but crosses it in several directions. After covering a varying distance—in some cases more than 5 mm—it divides abruptly into several branches, usually five or six. These branches follow a rectilinear course through a wide field of the retina.

We are thus confronted by a new type of cell located in the layer of ganglion cells, but different from other ganglion cells in that it is purely intraretinal; the axon and its terminal branches do not leave the retina. The body of this cell is usually located in the first row of ganglion cells, in contact with the inner limiting membrane. Sometimes it is close to the inner plexiform layer. The axon originates in the cell body or in the base of a large dendrite, and in some cases produces one to four collaterals (recurrent collaterals?) (Fig. 1*c*) after proceeding a short distance. The length of the axons in the cells varies between 0.5 and 6 mm. After running undivided for these distances, each axon suddenly divides into five or six terminal branches (Fig. 2) in a characteristic way. The terminal branches follow a course at the level of the outer portion of the ganglion cell layer, passing through the origin of the dendrites of the ganglion cells and extending over a large field. We have not been able to show clearly the type of synapse established by these terminal branches. In their course they look as though they make contact with the bodies or main dendrites of the ganglion cells. Usually they end in a small knob (possibly a synaptic ending) in the vicinity of the body of a ganglion cell, but we cannot exclude the possibility of a synapse at the level of the amacrine cells. The "dendritic field"—that is, the area covered by the dendrites—averages 200,000 square microns in the cells we have been able to study.

Discovery of this new type of cell in the ganglion cell layer may help to clarify the question of whether or not collaterals exist in the fibers of the optic nerve. This question was raised (*6*) as a consequence of Marenghi's old description (*7*) of the layer of ganglion cells in the mammalian retina. Marenghi mentioned the existence of ganglion cells each of whose axons produced numerous collaterals at right angles before and during the incorporation of these axons into the bundles of optic nerve fibers, and stated that such fibers and cells "must represent another channel of connection between the retina and the centers independently of the classical fibers." Marenghi cells have not been found since. Cajal (*5*) and Polyak (*8*), who worked with the Golgi method, were not able to see collaterals in the optic nerve fibers other than the centrifugal fibers. In hundreds of retinas (cat, dog, rabbit, guinea pig, rat, monkey, and man) studied by our technique, we did not find a single collateral on the axons of true ganglion cells, that is, the ones forming the optic nerve fibers.

We have also been able to stain this type of cell in the human retina (Fig. 3); here its axon is shorter than it is in the dog's retina, but its terminal branches cover areas of similarly large size.

In our opinion the Marenghi ganglion cells are the same type as those we have described. Because of the technique Marenghi used, he missed the important fact that the axon ends in the retina itself. From his description and drawings it appears that he never saw the end of the axon, not even

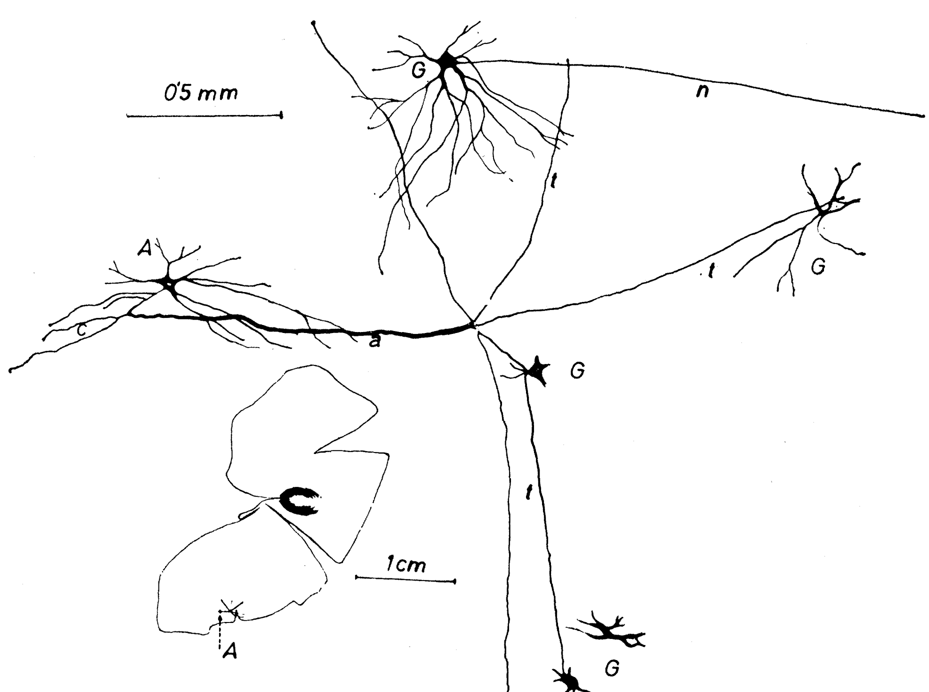

Fig. 1. Associational cell (*A*) of the ganglion cell layer of the dog's retina: *a*, axon; *t*, terminal branches; *c*, recurrent collaterals; *G*, ganglion cell; *n*, optic nerve fiber.

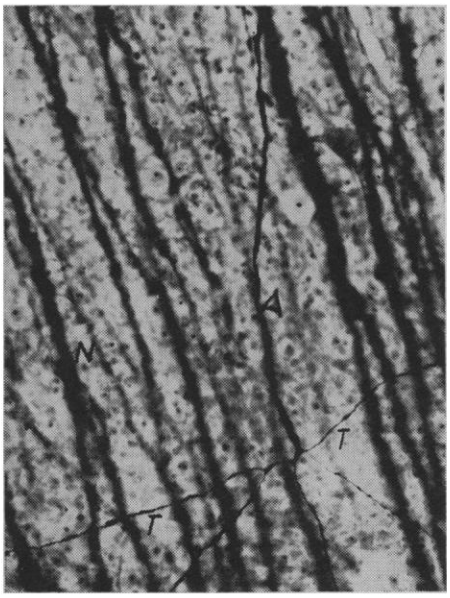

Fig. 2. Dog's retina. *A*, axon of one associational cell of the ganglion cell layer; *t*, terminal branches; *n*, optic nerve fibers. Unretouched photomicrograph.

its division into terminal branches; and he supposed that the axon was joined to the optic nerve fibers leading to the centers. His description does not exactly match that of the cells we have described. He states that the axon puts out branches over its entire length. In our stainings the salient characteristic of the type of cells described is the lack of collaterals in the segment between the "recurrent" ones and the sudden division into several branches. The

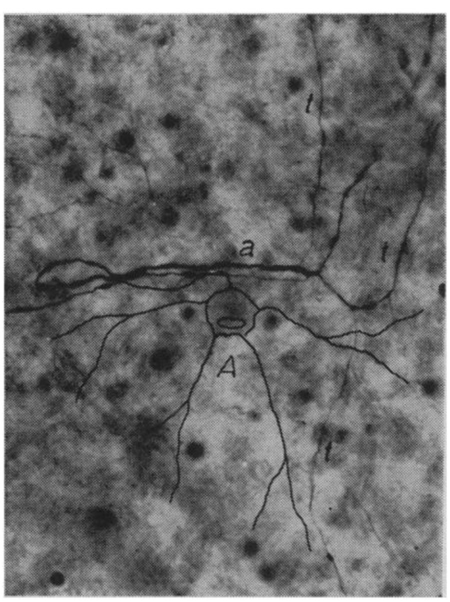

Fig. 3. Human retina. *A*, Associational cell of the ganglion cell layer; *a*, axon; *t*, terminal branches. The body and dendrites, being out of focus, have been drawn.

reason for this interpretation probably is that he had seen only the segment of the axon close to the cell body where it puts out the "recurrent" collaterals. The sections made from retinas stained by means of the Golgi method—even by Cajal's "enroulement" technique—do not permit us to follow the axon and its terminal branches over the whole extent of their distribution in the retina.

The area of the retina covered by the axon and terminal branches of these cells is very large (Fig. 1). We observed the cells in the peripheral part of the retina. At the moment it is practically impossible to ascertain how numerous they are. As far as we can judge from the stainings we have studied, we can only guess that they are present in a proportion of less than 1 percent of the giant ganglion cells.

When studying a retina we can see in practically all of it, by means of our technique. A great number of fibers of different thicknesses cross the bundles of the optic nerve fibers in several directions. There is no doubt that some of the fibers are the terminal branches of these new cells, but there also exist others, besides the centrifugal fibers, whose origin has not yet been identified. Hence a more complex system of intraretinal association than had been hitherto supposed may exist.

In the retina several types of cells have been regarded (*9*) as elements of transverse intraretinal association. Such cells are found (i) at the level of the outer plexiform layer, and (ii) at the level of the inner plexiform layer. Cells in the outer layer are of two main types: the outer, small, horizontal cells, without a cylinder axis, forming a plexus that spreads all over the retina, and the inner, large, horizontal cells whose axon terminals end at some distance from the body but still within the outer plexiform layer. Cells in the inner layer are the several types of amacrine cells.

From a morphological point of view the cells we are describing look as if they were playing an associative role at the level of the second neuron: the ganglion cells. Through their dendrites they might be activated by excitation from the visual cells which connect to the bipolars in their "dendritic field," and by the impulses from their axons they might modify the responses of the ganglion cells located at some distance. It is not going to be simple to record the electrical responses of these cells because of the smaller number found in the retina and their similarity to the regular ganglion cells, but their presence must be borne in mind when the electrical responses obtained at the level of the ganglion cells or the optic nerve fibers are being interpreted.

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References and Notes

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Anopheles balabacensis balabacensis Identified as Vector of Simian Malaria in Malaysia

Abstract. *The mosquito* Anopheles balabacensis balabacensis *has been identified as a natural vector of at least two species of simian malaria in the monsoon forests of the northern Malay States. This mosquito is also a serious vector of human malaria from Viet Nam to northern Malaya. This is the first report of a mosquito which transmits both human and simian malaria in nature.*

Natural vectors of simian malaria are known only from Malaysia, and all are members of the *Anopheles leucosphyrus* group of mosquitoes. Wharton and Eyles (*1*) identified *A. hackeri* as a vector of *Plasmodium knowlesi*, and this mosquito has since been found naturally infected with *P. cynomolgi*, *P. coatneyi*, and *P. fieldi*. In 1962 Wharton *et al.* (*2*) reported that *A. leucosphyrus* is a vector of *P. inui*, and Eyles *et al.* (*3*) found *A. balabacensis introlatus* naturally infected with *P. cynomolgi*. We now record the presence of both *P. cynomolgi* and *P. inui* in *A. b. balabacensis* in Malaysia.

The *A. leucosphyrus* group of mosquitoes is broadly distributed through