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29 June 1965

Synaptic Connections of the Centrifugal Fibers in the Pigeon Retina

Abstract. *The centrifugal fibers in the pigeon retina end in the inner nuclear layer and form two kinds of terminals, convergent and divergent. In the inner nuclear layer the fibers synapse with amacrine and displaced ganglion cells. Because of their great number and their even distribution these fibers appear to constitute a system for the localized centrifugal control of the retinal functions.*

There is abundant and conclusive anatomic evidence demonstrating the existence in vertebrates of centrifugal fibers which come from the brain to end in the retina (*1*, *2*, *3*, *4*). However, their mode of connection and their functional role are not as yet well known. According to Cajal (*1*) and Dogiel (*2*), who first studied them, the centrifugal fibers end in the retina in the inner aspect of the layer of bipolar cells (sublayer of the amacrine cells, according to Cajal), forming synapses only with the association amacrines and ordinary amacrines. Physiological studies (*5*) in various vertebrates confirm the existence of centrifugal fibers.

We have made a careful examination of pigeon retinas vitally stained with methylene blue (*3*). Our observations indicate that the centrifugal fibers enter the retina at the optic papilla, follow a path amidst the optic axons, then cross obliquely the inner plexiform layer, and bend a few microns before reaching the inner nuclear layer, where they follow, parallel to it, a course of variable length and direction, and end in its innermost aspect. Although the course of each centrifugal fiber seems erratic, the fiber endings are evenly distributed over the entire expanse of the retina; the centrifugal fiber which enters a particular retinal segment ends in it. This relation is in agreement with the findings of McGill (*6*) with respect to the projection on the retina of the nuclei from which the centrifugal fibers originated.

Centrifugal fibers can be divided into two types according to their mode of terminal branching.

1) In the convergent type, the centrifugal fiber ends in several branches of different lengths and thicknesses. These, after following separate paths that make loops sometimes hundreds of microns wide, converge to form a synaptic nest on a single cell body (Figs. 1 and 2). The synaptic nests may give off small branches which make additional loops that end either on the same nest or on some neighboring cell. Sometimes these collateral loops, or others given off earlier, contribute to synaptic nests formed by other converging terminals. Convergent fibers make synapses with very few cells, usually not more than three or four, and frequently with only one. They penetrate the inner nuclear layer and form synaptic nests as deep as the second or third cell row, but some of their slender branches may penetrate deeper. However, most of their synapses are confined to the innermost quarter or fifth of this layer, where the amacrines lie.

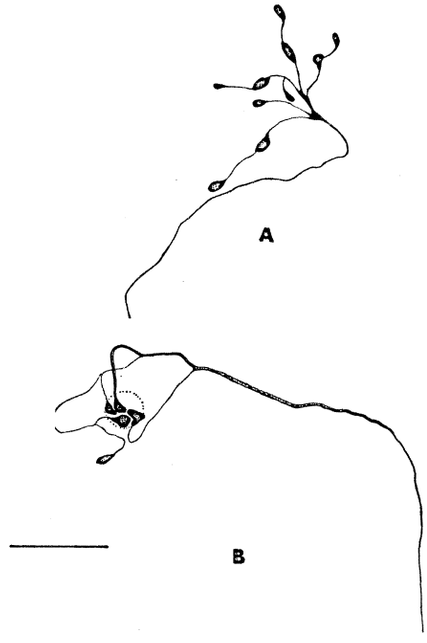

Fig. 1. Camera lucida drawings of retinas mounted flat, vitreal side uppermost. Oil immersion objective of the microscope was used. (*A*) Divergent terminal; (*B*) convergent terminal; the dotted circle represents the outline of a cell body, 7 μ in diameter, on which the terminal impinges. Scale, 20 μ.

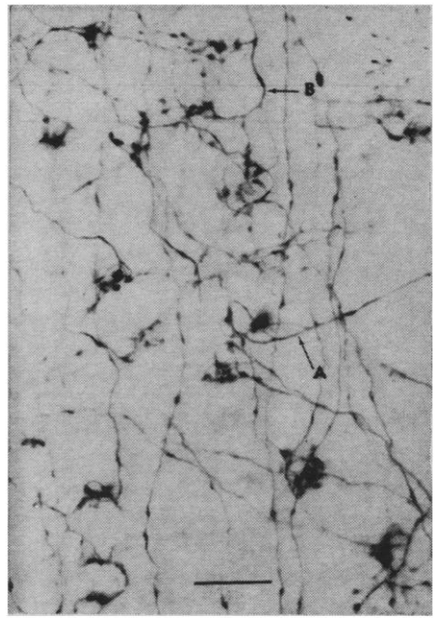

Fig. 2. Centrifugal terminals in a retina stained with methylene blue as seen from the vitreal side: Arrows: (*A*) convergent terminal, (*B*) divergent terminal. Scale, 40 μ.

2) In the divergent type, the centrifugal fiber ends in several fanlike branches which make successive synapses with different cells and sometimes at several separate places on the same cell (Figs. 1 and 2). In general, they penetrate less deeply into the inner nuclear layer than the convergent type does, and usually they are confined to the boundary area between this layer and the inner plexiform layer.

Centrifugal fibers synapse on at least three types of cells. Of these, (i) displaced ganglion cells have their perikaryon (9 to 15 μ in diameter) amidst the amacrines of the inner nuclear layer at the boundary with the inner plexiform layer. A single expansion which enters the inner plexiform layer, where it divides into several thick dendrites, sprouts from the cell body. The dendrites give off many secondary and tertiary branches which form a single dendritic stratum 300 to 600 μ wide and 8 to 10 μ below the inner nuclear layer. The axon issues from one of the main dendrites, crosses the internal plexiform layer, and enters the layer of optic fibers, leaving the retina with these fibers. At the origin of their main dendrites the displaced ganglion cells receive at least one synaptic bouton from one centrif-

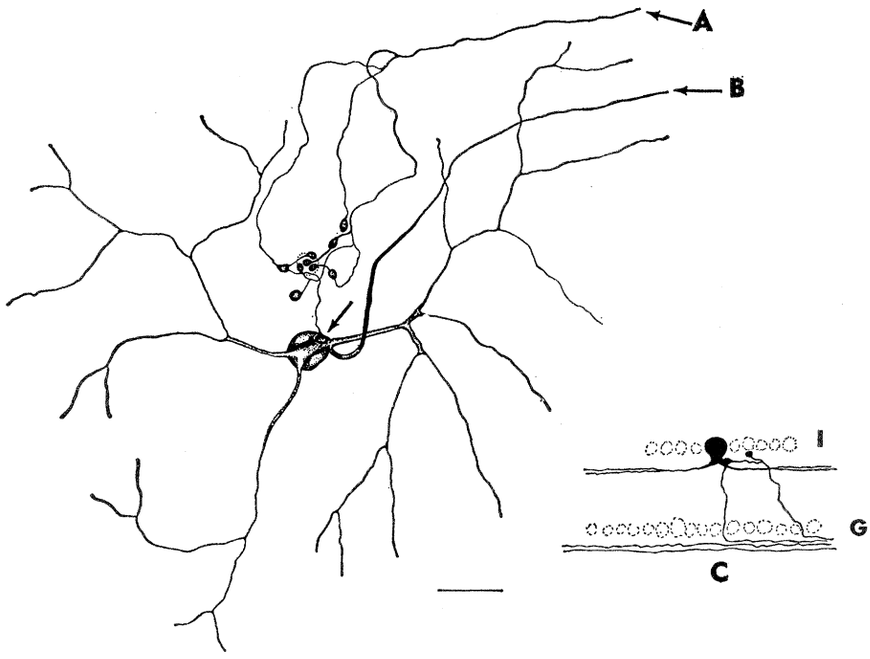

Fig. 3. Camera lucida drawing of displaced ganglion cell and convergent terminal, from the vitreal side. The relative position of the cell and the terminal in the retina are shown in (*C*); (*G*) ganglion cell layer; (*I*) inner nuclear layer. Unmarked arrow, synapse of the convergent terminal on the displaced ganglion cell near the axon hillock. Arrows: (*A*); centrifugal fiber; (*B*) axon from displaced ganglion cell. Scale, 20 μ.

ugal terminal (which can be of either type), but no synaptic nest is formed on them (Fig. 3). (ii) Flat amacrine cells lie at the boundary between the inner plexiform and the inner nuclear layer. From their flat bodies issue four or five dendrites that subdivide frequently, forming a single stratum which also lies at this boundary (Fig. 4). Of the two kinds of centrifugal fibers, only the diverging type synapses with these amacrines, reaching them from the inner plexiform side to form boutons at the base of the dendrites, and possibly also along them. (iii) Small amacrine cells of the parasol type lie slightly deeper in the inner nuclear layer. A single expansion arises from the perikaryon (6 to 7 μ in diameter) and ends in the inner plexiform layer in numerous branches which spread in a single stratum 100 to 200 μ wide. The converging type of centrifugal fibers form, on the cell body, a little synaptic nest which surrounds the origin of the expansion (Fig. 4).

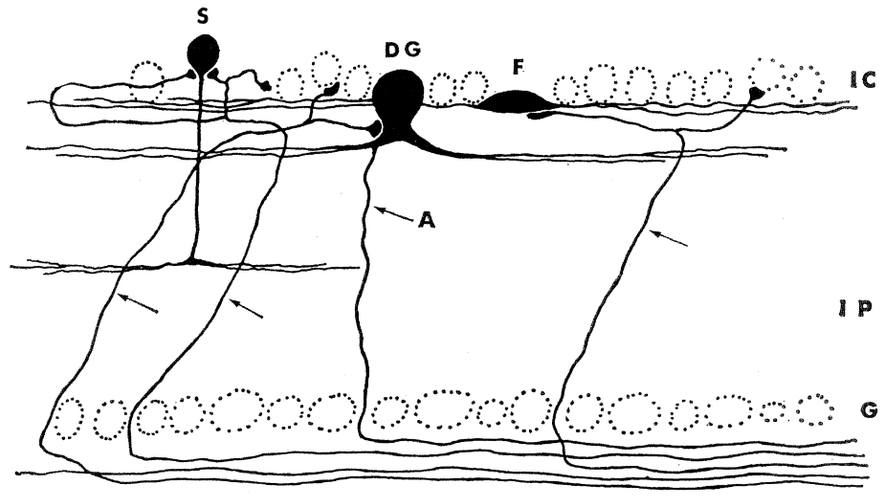

Fig. 4. Drawing of cells and terminals as seen in a transverse section of the retina at the inner plexiform layer. (*IC*) Inner nuclear layer; (*IP*) inner plexiform layer; (*G*) ganglion cell layer; (*DG*) displaced ganglion cell; (*F*) flat amacrine; (*S*) small parasol amacrine. Arrow (*A*) axon from the displaced ganglion cell; other arrows, centrifugal fibers.

Methylene blue cannot be used for quantitative studies because it does not stain every element. However, an assessment of a lower limit for the number of centrifugal fibers can be made by counting the terminals in a unit area of a well-stained preparation, if one assumes, as is apparently the case, that they are uniformly distributed over the retina. These calculations give a density no lower than 1 terminal per each 1500 μ^2 and a total number of no less than 10^5 terminals.

It is thus apparent that the brain should be able to exert some control over the visual information received from the eye—directly by means of the centrifugal fibers which synapse with the displaced ganglion cells, and indirectly through the centrifugal fibers which synapse with the amacrines. In this regard several considerations are pertinent.

The synapses of the centrifugal fibers on the displaced ganglion cells are close to the axon hillock, and, because of this closeness, the centrifugal fibers could be expected to control the overall output of these cells through either facilitation or inhibition, and thus enhance or diminish their output. As the displaced ganglion cells seem to belong to only one morphological type, only one kind of visual function should be expected to be directly affected in this manner.

The association amacrines have long axons and therefore can be expected to conduct propagated spikes. The ordinary amacrines lack a standard axon, and it is not at present possible to say how they transmit information. That they do so, however, is apparent because they receive direct synapses from the centrifugal fibers.

The output of the association amacrines, which receive nest synapses from the centrifugal fibers (*I*), should also be expected to be under strong centrifugal influence, or perhaps even complete control, if there is no other input.

We have not yet identified all the types of amacrines with which the centrifugal fibers synapse. However, given the great number of centrifugal

fibers and the fact that they end almost exclusively in the inner aspect of the inner nuclear layer, we can reasonably expect the majority of the amacrines to be under direct centrifugal control if centrifugal fibers are the only input. The rest could be under indirect centrifugal control through the association amacrines which, according to Cajal, end on these fibers.

To the extent that the centrifugal fibers make different kinds of synapses on different kinds of amacrines, it can be expected that these should be affected in different ways by the centrifugal processes. Similarly, the different kinds of terminals can be expected to function differently.

The abundance of centrifugal fibers and their ordered and complex synapses suggest that their role in the visual processes is to exert localized influences in the retina, affecting in different ways the displaced ganglion cells and the various kinds of amacrines. They would thus constitute a system which would serve to not only enhance or diminish some of the different retinal functions (*7*), but also to do so locally, and perhaps in a different manner, in different retinal areas at the same time.

Although our observations are, in principle, relevant to the visual systems of all vertebrates, we are aware that the role of the centrifugal control of the retinal function may be different in different groups of animals. After all, sense organs are instruments of perception, and as such their functional organization can be expected to vary according to their purpose.

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12 July 1965

Macroglobulin-Producing Plasma-Cell Tumor in Mice: Identification of a New Light Chain

Abstract. *A transplantable plasma-cell tumor in mice produces both a γM-macroglobulin serum protein with a sedimentation coefficient of 17S and a kappa-type urinary protein. The reduced and alkylated macroglobulin, when examined by electrophoresis in acid-urea polyacrylamide gel, had a fast component which migrated in the same position as the urinary protein and also a slow component. These two components, as shown by exclusion chromatography, represent the light and heavy polypeptide chains of the γM-macroglobulin. The aforementioned macroglobulin was antigenically related to that in normal mouse serum.*

The high-molecular-weight γ*M* (*1*) antibodies (also called γ1M-, β2M-, 19*S*γ-, or γ-macroglobulin) have only recently been demonstrated in mice (*2*, *3*). Plasma-cell tumors in mice secrete proteins which are antigenically related to normal immunoglobulin counterparts (*4*), and because they each synthesize a single class of protein these tumors serve as convenient sources of material for study of the immunoglobulins. The mouse immunoglobulin classes thus far studied (γA, γG — Be1, γG — Be2, and γF) are composed of two types of polypeptide chain subunits, light and heavy (*5*, *6*); thus they have a similar pattern of chemical structure to that established for human immunoglobulins and immunoglobulins of other species (*7*). Clausen *et al.* described a transplantable plasma-cell leukemia in mice which was accompanied by high concentrations of macroglobulin in the serum (*8*). This macroglobulin migrates in two bands on acid-urea starch-gel electrophoresis after reduction and alkylation (*9*).

Investigation of the Bence Jones proteins produced in mice with transplantable plasma-cell tumors led to the identification (*10*) of three types based on antigenicity and structure: the light-chain type, the γA type, and the RPC-20 type. Proteins of the first type were related to the light chain most commonly found in γA-, γG-, and γF- myeloma proteins of the BALB/c mouse and are now designated as urinary λ (lambda)- globulins. Proteins of the second group represented combinations of one λ-chain and one γA- or heavy α-chain (*5*). The third (RPC-20) type was not immunologically or structurally related to the λ-chain or to any known heavy chain (*10*). The molecular weight of the RPC-20 urinary protein was 24,000 (*11*), which is in the size range of light-chain proteins. Three urinary proteins similar to the RPC-20 protein in antigenic and tryptic peptide structure have since been found, and this group is now designated κ (kappa). We now report a γM-macroglobulin, produced by a transplantable plasma-cell tumor in the BALB/c mouse, which has a light chain related to the κ-type of urinary globulins.

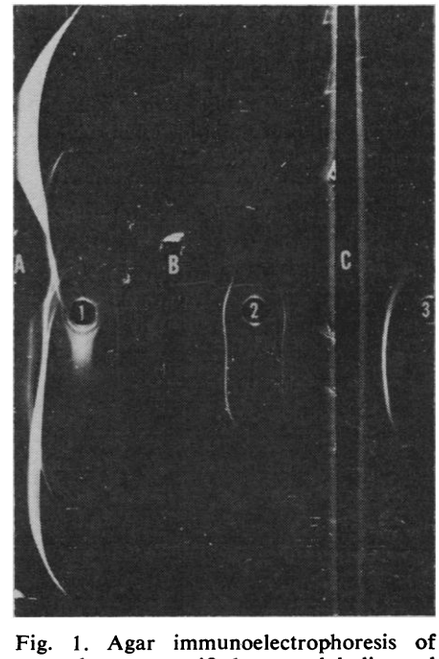

Fig. 1. Agar immunoelectrophoresis of normal serum, purified macroglobulin, and urinary κ-protein. Trench A contains rabbit antiserum against whole mouse serum, B contains rabbit antiserum against the MOPC-104 macroglobulin, and C contains rabbit antiserum against the κ-type protein (no cross reactivity with λ-type protein). Well 1 contains pooled serum from C57BL mice immunized with ovalbumin, well 2 contains purified MOPC-104 macroglobulin, and well 3 contains the urinary protein produced by MOPC-104.