

Structural Basis for ON- and OFF-Center Responses in Retinal Ganglion Cells

Abstract. *The inner plexiform layer of the mammalian retina has a bisublaminal organization determined by restricted branching of the terminals of cone bipolar cells and dendrites of class I (large) and class II (small) ganglion cells. Comparison of dendritic field diameters and receptive field center sizes of large ganglion cells suggests that neural circuitry in sublamina a conveys "OFF"-center properties and connections in sublamina b "ON"-center properties to retinal ganglion cells.*

The eye is often compared to a camera, yet its sensitive layer of retinal photoreceptors, rather than merely counting photons, builds contrast between the receptive field "center" and "surround" [for example (1)]. "Center-surround" receptive field organization, fully developed in certain retinal ganglion cells, is characterized by mutual antagonism between spatially concentric retinal areas (2). Center may be ON or OFF, according to the response elicited by a flashing light; antagonistic surrounds give the opposite response. A second aspect of center-surround organization, typical of mammals and of particular interest to us, is the presence of approximately equal numbers of ON- and OFF-center units in a single physiological class of ganglion cell (2). At present it is not known precisely how ON- and OFF-center properties are conveyed from photoreceptors to ganglion cells and separately maintained in two subsets of otherwise similar ganglion cells, except that bipolar cells must be involved.

Cone bipolar cells of the cat (3), the monkey (4), and other mammals contact photoreceptors only (i) at ribbons (invaginating cone bipolar cells) or (ii) at superficial junctions (flat cone bipolar cells). In addition, the synaptic terminals of flat cone bipolar cells end high in the inner plexiform layer (IPL) (near the inner nuclear layer), and invaginating cone bipolar cells end low (toward the ganglion cell layer) (5) (Fig. 1). The stratification of ganglion cell dendrites in the IPL also contributes to this dual organization. Distinctive morphological classes of ganglion cells in the retina of the cat may be subdivided into two subclasses, those that branch exclusively in the domain of flat bipolar cells (in sublamina *a* of the IPL), and those that branch only among the terminals of invaginating cone bipolar cells (in sublamina *b*) (Fig. 1). We shall demonstrate here that anatomically distinct pathways and a bisublaminal organization of the IPL form a likely structural basis for the symmetry of ON- and OFF-center responses in mammalian reti-

nal ganglion cells. Moreover, we propose that if a retinal ganglion cell has significant input to a bipolar cell, it will be ON-center or OFF-center depending upon the sublamina in which it branches.

Recently, Boycott and Wässle (6) measured the diameters of the dendritic fields and the cell bodies of retinal ganglion cells in flat-mounted Golgi preparations of the cat retina as these parameters varied with retinal eccentricity (distance from the center of the area centralis) and divided the cells into three groups. We have divided retinal ganglion cells of the cat into several groups, two of which we shall describe here. Our class II cells appear to be identical to Boycott and Wässle's beta cells, but our class I includes a broader range of dimensions (in a single retina) and is morphologically more diverse than their alpha type.

In all classes of ganglion cells, we have studied branching patterns in flat-mounted retinas and dendritic stratification in vertically sectioned rapid Golgi or Golgi-Kopsch-Colonnier preparations. In some cases, we have used computer reconstruction and rotation of dendritic trees (7) to demonstrate stratification (as well as the branching pattern) in obliquely sectioned material (Fig. 1). We confine our attention here to cells of classes I and II, because each of these classes is internally quite homogeneous and because each follows the bisublaminal organization of the IPL in the strati-

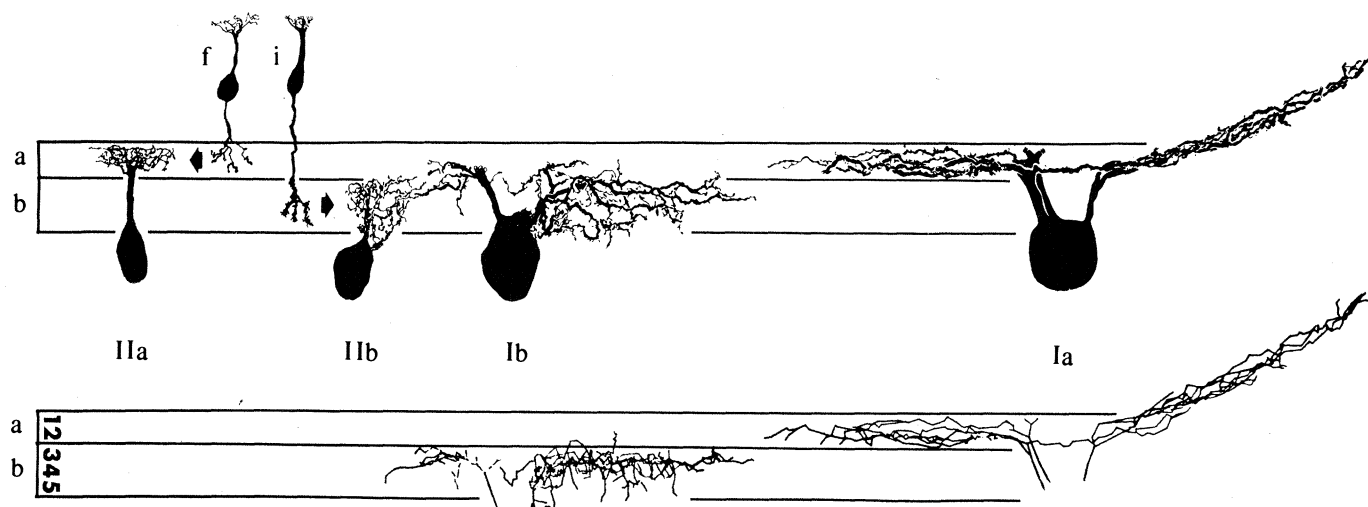


Fig. 1. Camera lucida drawings (top) and computer drawings (bottom) of Golgi impregnated cone bipolar cells and two classes of ganglion cell. Each contributes to the bisublaminal organization of the inner plexiform layer (IPL) in the area centralis of the cat's retina. An effort was made to orient the dendritic trees (and thus the strata of the IPL) perpendicular to the surface of the paper. (Top) Sublamina *a* contains the terminals of flat cone bipolar cells (*f*) and the dendrites of type Ia and IIa ganglion cells. Sublamina *b* contains terminals of invaginating cone bipolar cells (*i*), and dendrites of type Ib and type IIb ganglion cells. Class II ganglion cells of both subtypes have complex tufted branching, while the type Ia cell has a simpler and more regular branching pattern when viewed flat (8). The type Ib cell has more dendritic appendages, a greater frequency of branching per unit area, and a more tufted appearance than the type Ia cell. Its dendritic field diameter is about 30 percent smaller than the type Ia cell (Fig. 2). Arrows indicate the direction of transmission from bipolar cells to ganglion cells. (Bottom) Unretouched computer drawings of the dendritic trees of the same two class I cells (7). The type Ia cell [retinal eccentricity (r.e.) = 450 μ m] needs no axial rotation, and it branches in a stratum (Cajal's S2) narrower than sublamina *a*. The type Ib cell (r.e. = 400 μ m), when rotated is still broadly stratified, as are the class IIb cell (r.e. = 450 μ m) and the invaginating cone bipolar cell (r.e. = 250 μ m). Method: Golgi (rapid), sectioned vertically at 100 μ m. Scale: *a* + *b* = 40 μ m.

fication of dendritic branches. A third, morphologically heterogeneous class of ganglion cell (not described here) also includes paired, paramorphic types contributing to bisublamina organization.

Class II cells have relatively small cell bodies and the smallest dendritic field diameters in the central retina; their dendritic trees, studded with appendages, have a "tufted" (8) appearance (Fig. 1). The primary dendrite or dendrites of type IIa cells branch in the outer third of the IPL (sublamina *a*). They generate a spray of dendritic branches that arise in Cajal's stratum 2 (S2) and spread into S1, next to the inner nuclear layer. Type IIb cells branch in the inner two-thirds of the IPL (sublamina *b*). They usually have one major and several minor primary dendrites, which branch in S3 and S4. In the area centralis some branches ramify in S5 (Fig. 1).

In the branching and stratification of their dendrites, class II cells follow most closely the distribution of the terminals of the cone bipolar cells. Throughout the retina, the terminals of flat cone bipolar

cells are narrowly confined to sublamina *a* (Cajal's S1 and S2), thus overlapping the dendrites of type IIa ganglion cells (Fig. 1). The overlapping stratification of the terminals of invaginating cone bipolar cells and type IIb cell dendrites is even more strikingly confirmed, however, for in the central retina both are broadly stratified, extending into S3, S4, and S5 (Fig. 1), while in the peripheral retina both bipolar and ganglion cells are narrowly stratified. From the study of serial thin sections, we know that both types of class II cells receive a large proportion of their synapses from cone bipolar cells (5). Thus, (i) class II cells of type *a* are dominated by a bipolar input only from flat cone bipolar cells and (ii) class II cells of type *b* are dominated by a bipolar input exclusively from invaginating cone bipolar cells. Members of other ganglion cell classes, whose dendritic trees do not straddle the *a/b* sublamina border, will be governed by the same restrictions in their bipolar input.

Class I ganglion cells have large cell bodies, large dendritic fields, and, in the

peripheral retina, highly stratified dendrites branching in a single stratum, either in S2 (occasionally in S1) or in S3. These may be designated types Ia and Ib, respectively. Throughout the retina the dendrites of type Ia cells maintain a simple "radiate" pattern of dichotomous branching (8) with few dendritic appendages and narrow stratification (Fig. 1). On the other hand, the dendrites of type Ib cells, although narrowly stratified in the peripheral retina and generally radiate in their branching pattern, are always more complex and have more appendages than type Ia cells at a given retinal location. The differences between types Ia and Ib are most striking in the central retina, where most type Ib cells exhibit a tufted appearance and broad stratification similar to that of type IIb cells (Fig. 1). It is likely that both the complex, tufted branching and the broad stratification of central type Ib cells increase their potential surface of contact with the comparatively simple and vertically elongated, invaginating cone bipolar terminals.

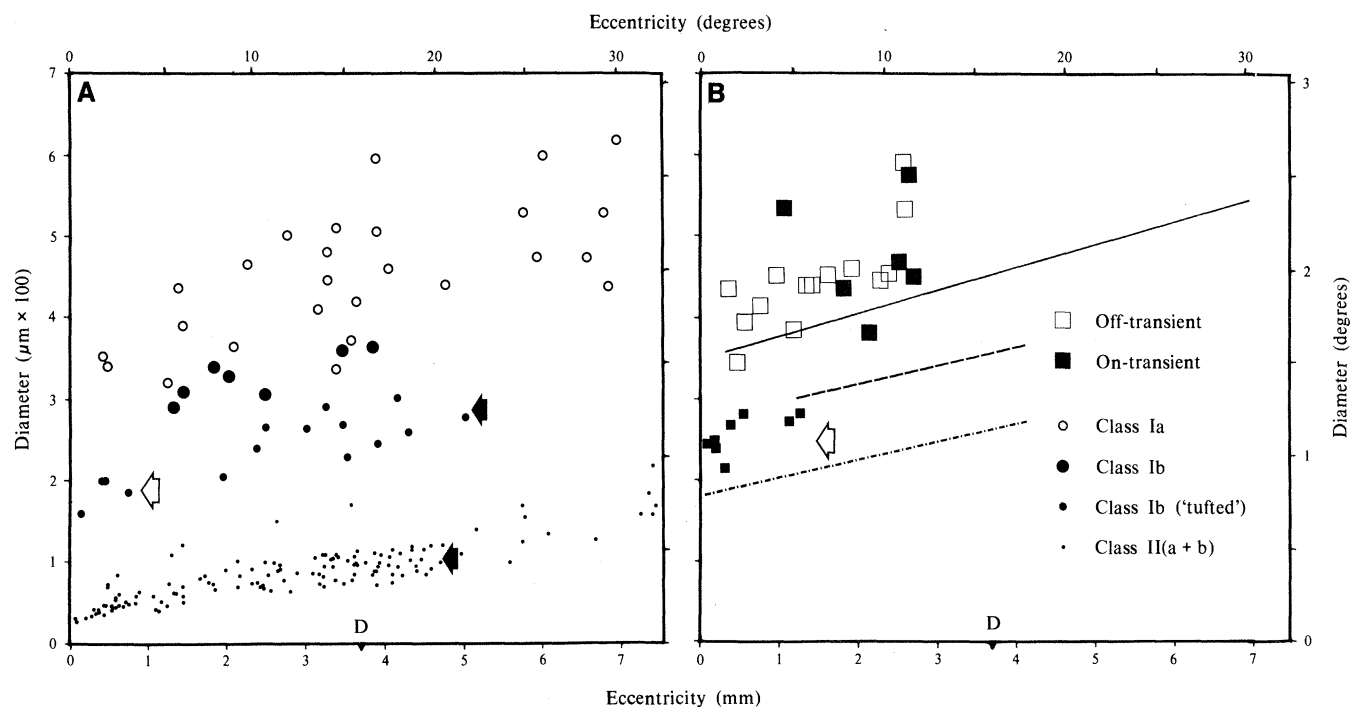


Fig. 2. Dendritic field diameter (A) and receptive field "center" diameter (B) of ganglion cells in the cat retina as a function of distance from the center of the area centralis (eccentricity). Data in (A) are from a single, vertically sectioned rapid Golgi preparation. Data in (B) are from figure 6 of Cleland and Levick's physiological study (9). Regression lines (B) are fitted to the data of the three groups of class I cells (A). The distance of the optic disk from the center of the area centralis is 3.7 mm in this retina (A) [regression lines (B)]; its position on the abscissa is indicated by D. The data in both (A) and (B) can be read from both sets of axes. Open arrows point to a group of "tufted" class Ib cells (A) with dendritic field diameters comparable to the receptive field center diameters of a group of ON-center "brisk-transient" cells (B). (A) Class I type *a* cells (open circles) are consistently larger than those class I type *b* cells (large closed circles) with similar (though more frequent) dendritic branching of the simple radiate type (8). These are larger in turn than those class Ib cells (medium closed circles) with tufted branching (8) and broader stratification. At any given eccentricity there is no overlap between class II cells (small closed circles) and class I cells. By far the largest number of cells impregnated in this retina lay along the projection of the horizontal meridian onto the retina between the area centralis and the optic disk. Class Ib and especially class II cells lying along this radius form clusters (closed arrows) with a lower mean rate of increase in dendritic field diameter than the average rate for the whole retina. (B) OFF-center, "brisk transient" cells (open squares) tend to be larger than ON-center, brisk-transient cells (closed squares) here, as in Hammond's study (9). Apparently, OFF-center cells constitute a homogeneous group like the class Ia cells (A), and the ON-center cells may fall into two groups (large and small closed squares) like the class Ib cells. In each comparison the disparity in receptive field center and dendritic field diameters can be minimized by adding to the latter the dimensions of the dendritic field and terminal field of a single cone bipolar cell, plus a small factor determined by the receptive field size of a single cone photoreceptor (12).

Type Ia and type Ib ganglion cells also differ in the diameters of their dendritic fields at a given retinal eccentricity. This disparity exists throughout the retina but is marked in the central retina (Fig. 2). Dendritic field diameter of both types increases with increasing retinal eccentricity, but type Ia cells are consistently larger than type Ib cells (Fig. 2A).

The morphological differences (and differences in connections) between class I and class II ganglion cells probably underlie the physiological distinction between "transient" and "sustained" cells and perhaps also between "Y" (non-linearly summing) and "X" (linearly summing) cells, respectively (6, 9, 10). If we assume a relationship between dendritic field diameter and receptive field center size (11), we can compare the dimensions of class I cells in Golgi preparations with Cleland and Levick's data (9) on field center size of "brisk" transient cells (Fig. 2B). Both the dendritic fields of class I cells and the field centers of transient cells increase in diameter with retinal eccentricity (6, 9). OFF-center transient cells are larger than ON-center transient cells, just as type Ia cells are larger than type Ib cells at a given retinal eccentricity. Hammond's more extensive physiological data (9), from units with eccentricities up to 35°, also support this finding. Finally, some central, tufted, broadly stratified, type Ib cells have particularly small dendritic field diameters (Fig. 2A) as do some ON-center transient cells (Fig. 2, A and B). This comparison supports the hypothesis that ganglion cells branching in sublamina *a* will be OFF-center and those branching in sublamina *b*, ON-center.

Our hypothesis pertains to the responses of ganglion cells with direct cone bipolar input but implies nothing concerning the response polarity of either of the two types of cone bipolar cells. ON- or OFF-center responses of ganglion cells could be determined by any of several disynaptic sequences of excitatory and inhibitory synapses. Nevertheless, there is ultrastructural evidence that flat cone bipolar cells receive excitatory synapses from cone photoreceptors [which hyperpolarize to photic stimulation (12)], but invaginating cone bipolar cells do not (13). The simplest interpretation of these findings is that the flat cone bipolar cell is hyperpolarizing to photic stimulation, or OFF-center, when the flow of excitatory transmitter from the photoreceptor is diminished, and the invaginating cone bipolar cell is depolarizing, or ON-center, under the same conditions of illumination. The presence of ON/OFF dualism in cone bipo-

lar cells of the cat is at least plausible, for such ON/OFF symmetry has been found among bipolar cells in nonmammalian vertebrates (14). If both flat and invaginating cone bipolar types make excitatory synapses on ganglion cells, as Naka has found in catfish for both ON- and OFF-center bipolar cells (15), then inferences made from the synaptic ultrastructure (13) are consistent with our hypothesis that sublamina *a* contains the processes of OFF-center cells and sublamina *b* the processes of ON-center cells.

It has been supposed that stratification of the IPL [so prominent in typical nonmammalian vertebrates (16, 17)] is of little functional significance in mammals (18). This opinion stands in contrast to Cajal's view that the IPL is similarly organized in all vertebrates (16). More study is needed to determine the precise meanings of multiple stratification in the IPL of the vertebrate retina. It is now evident, however, that a bisublaminal organization of the IPL is characteristic of the mammalian retina, that it may obscure but never obliterates Cajal's many-tiered stratification, and that it is designed to serve ON/OFF symmetry in retinal ganglion cells.

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25 March 1976; revised 18 June 1976

Cytochrome P-450 and Drug Metabolism in *Trypanosoma cruzi*: Effects of Phenobarbital

Abstract. *The epimastigotes of Trypanosoma cruzi hydroxylate drugs at substantial rates. The activity, which is of the mixed-function oxidase type, is increased by phenobarbital and is inhibited by CO, SKF 525-A, and metyrapone. The hydroxylation is paralleled by increases in free and membrane-bound ribosomes.*

Although Chagas' disease, which is caused by *Trypanosoma cruzi*, is a major health problem, no chemotherapeutic agents have been found that will cure the disease in animals or man. The failure of drugs to affect the parasite may be due

to a permeability barrier in the flagellate (1) or to rapid conversion of the drugs to nontoxic compounds. We now present data indicating that the epimastigote forms of *T. cruzi* possess an active detoxifying system whose character-