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Neuronal Architecture of On and Off Pathways to Ganglion Cells in Carp Retina

Abstract. *Bipolar, amacrine, and ganglion cells of carp retina, stained intracellularly with Procion yellow, can be divided into types a and b, according to the destination of terminals and dendritic trees in the inner plexiform layer (sublamina a and b, respectively). Type a cells showed hyperpolarizing, or off, responses and type b cells depolarizing, or on, responses. Carp thus resembles cat in the basic organization of on and off pathways in the retina.*

The inner plexiform layer (IPL) of cat retina can be divided into two sublaminae, one of which contains the terminals of "flat" cone bipolar cells (sublamina "a") and the other of which contains the terminals of "invaginating" cone bipolars (sublamina "b") (*1*). Moreover, the majority of retinal ganglion cells can be divided into types "a" and "b", according to the sublamina in which their dendritic trees are confined (*1*). It was proposed that sublamina a, nearest the amacrine cell bodies, contains the synaptic connections for off-center ganglion cells and sublamina b, nearest the ganglion cells, the connections for on-center ganglion cells (*1*). This hypothesis has been confirmed in the retina of the cat by iontophoresis of fluorescent dyes into ganglion cells, after intracellular recording of their responses (*2*).

We now report the results of a similar analysis applied to the retina of the carp, in which two advantages are obtained for examination of this hypothesis: (i) the distinctive stratification of the IPL (*3*) is much easier to appreciate in carp retina than in most mammalian retinas and is especially prominent under fluorescent light, and (ii) it is relatively easy to record from and to stain cyprinid bipolar and amacrine cells (*4*). Despite the very distant phylogenetic relationship of carp and cat, we find that all carp neurons contributing processes to the IPL (bipolar, amacrine, and ganglion cells) also follow the rule that cells branching in sublamina a [Cajal's strata (S) 1, 2, and 3 in carp] give hyperpolarizing (off) responses, and those branching in sublamina b (S4 and S5 in carp), give depolarizing (on) responses (*5*).

Retinas of light-adapted *Cyprinus carpio* were isolated in dim light with the receptor side up and transferred to a superfusion chamber, where they could be maintained in good condition for about 2 hours (*6*). Retinas of fish that were dark-adapted for more than 40 minutes were prepared in dim red light. Microelectrodes containing a 4 percent solution of Procion yellow MX-4R had resistances of 200 to 300 megohms; neurons were penetrated by electrical oscillation of the preamplifier applied to the electrode tip through the capacitance compensation feedback circuit. Each cell was identified by its response to 400-msec flashes of white or monochromatic lights of equal quanta, projected onto the retina as small spots (60 to 240 μm) or annuli (inside diameter, 0.5 mm; outside diameter, 3.5 mm) in a dual beam system in the presence of a background light (*7*). Dark-adpated retinas were studied without background light, with stimuli attenuated by 4 log units more than in photopic studies. Dye was passed through the electrode tip with 2 to 3 na of direct current for 200 to 600 seconds. Retinas were fixed in buffered aldehydes (*p*H 7.2), embedded in Spurr mixture E, sectioned at 15 μm, and examined with dark-field flu-

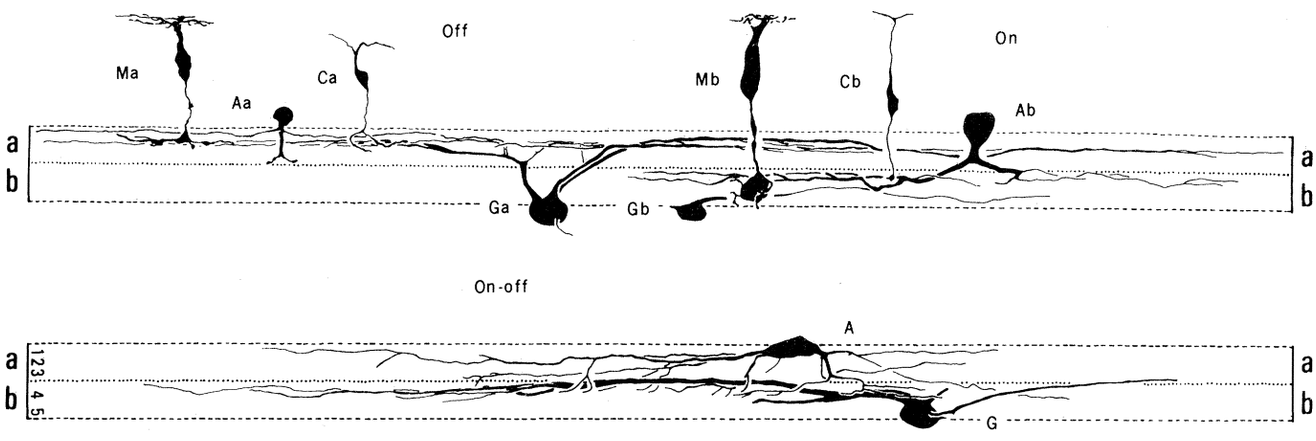

Fig. 1. Drawings of bipolar, amacrine, and ganglion cells in the retina of the carp, stained with Procion yellow after intracellular recording of their responses (Fig. 2). All cells send synaptic processes into the inner plexiform layer (IPL), which is divided into two sublaminae, a and b, about equally thick. Sublamina a is composed of strata 1, 2, and 3, while sublamina b includes only strata 4 and 5. Synaptic processes of type a cells are confined to sublamina a, and those of type b cells to sublamina b. The former are all hyperpolarizing (off) in their responses, while the latter are all depolarizing (on) (see Fig. 2). Bipolar cells are of two main varieties: large "M" bipolars and small "C" bipolars. Ma bipolars end in strata 1 and 2, and Mb bipolars in strata 4 and 5 with large globular terminals. Many varieties of amacrine cells were stained, three of which are shown here. "Ab" is the most commonly found sustained amacrine, and "A" is the typical on-off amacrine, the former confined to stratum 4 and the latter broadly stratified in S1 through S4. "Aa" is a bistratified amacrine, but is confined to sublamina a and is hyperpolarizing (Fig. 2). Though unusual in appearance, this cell appears to be almost completely stained. "Ga" is an example of Cajal's "giant" ganglion cell, branching at the border of S1 and S2. "Gb" represents one of the few type b (on) ganglion cells of which any dendrites were stained. "G" is typical of the large on-off ganglion cells. Dendritic branches enter both sublaminae, strata 3 and 4. Scale: thickness of the IPL is 40 μm.

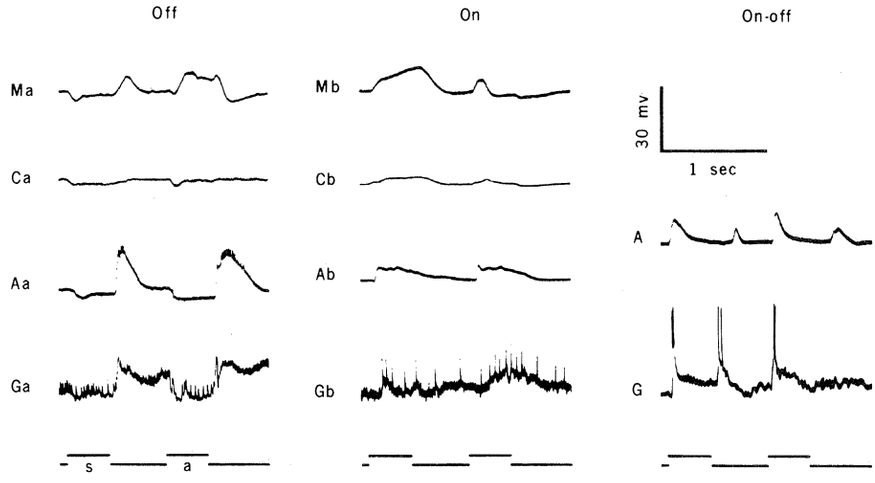

Fig. 2. Responses recorded from type a (off) and type b (on) bipolar, amacrine, and ganglion cells in the retina of the carp, prior to iontophoresis of Procion yellow dye. Each record was obtained from a cell labeled identically in Fig. 1. A spot (60 to 240 μm in diameter) and an annulus (inside diameter, 0.5 mm; outside diameter, 3.5 mm) of white light (400-msec flashes, 0.12 mw/cm^2) were projected successively on the light-adapted retina. Spike traces were retouched.

orescence microscopy. Drawings were made from photographs, or directly from the tissue slides, by a method of graphic interpolation (*2*). Altogether, 36 bipolar cells and 32 amacrine cells were stained sufficiently well to mark the destination of their processes in the IPL, but ganglion cells were much more difficult to stain.

Two morphological varieties of bipolar cell were stained, a large cell and a small cell. While these correspond to the classical "rod" and "cone" bipolars, respectively, of fish retina (*3*), recent light- and electron-microscope studies of goldfish and rudd retinas show that the former bipolar cells have mixed rod and cone input and that the latter are connected only to cones (*8*). We shall therefore call the large cells "M" (mixed) bipolar cells and the small cells "C" (cone) bipolar cells (Fig. 1).

Physiologically, bipolar cells are classified according to the polarity of their responses, whether off or on, to light falling on the receptive field center (*4, 9*) (Fig. 2). Intracellular staining of recorded bipolar cells shows (i) that the primary morphological distinction between off and on bipolar cells is in the level at which their synaptic terminals are found in the IPL (either in sublamina a or in sublamina b) and (ii) that this distinction cuts across the between "M" and "C" bipolar cells, yielding Ma (mixed, off), Ca (cone, off), Mb (mixed, on), and Cb (cone, on) bipolar cells (Figs. 1 and 2).

Ma bipolar cells, hyperpolarizing to light in the receptive field center, support a profusely branching dendritic tree and a synaptic terminal in sublamina a of the IPL which is confined to S1 and S2, as defined by Cajal (*3*) (Fig. 1). Ma bipolars resemble the "a" bipolars impregnated by the Golgi method in goldfish retina (*10*). Their cell bodies, bipolar processes, and synaptic terminals are large, when compared to those of the other variety of type a (off) bipolar cell, the Ca bipolar. The example in Fig. 1 has a slender process extending into the IPL and terminating in two irregular elongated processes "broadly stratified" in S1 and S2. Unistratified Ca bipolar cells have also been stained, and they may exist in bistratified form, as well, both strata within sublamina a.

Mb bipolar cells, depolarizing to light in the receptive field center, are the largest and most frequently stained bipolar cells in carp retina (Fig. 1). They appear to be the morphological and physiological complement of the Ma bipolar cell (Figs. 1 and 2). All 23 stained Mb bipolars were characterized by large cell bodies, dendritic field diameters of 35 to 50 μm, and enormous globular terminal expansions. These terminals lie in S4 and S5 and give rise to many small appendages (Fig. 1). Mb bipolar cells are similar to the Golgi-impregnated "b" bipolar cells of goldfish retina (*10*). The second variety of type b (on) bipolar cell (Cb in Figs. 1 and 2) is the complement of the Ca bipolar described above and, like it, has a small cell body and slender bipolar processes. The descending process of the Cb bipolar in Fig. 1 terminates in a small irregular expansion which lies in the upper part of S4.

In light-adapted retinas we observed only two spectral types of bipolar cell, which have been described (*7*). Both gave a maximal response to 620-nm light shone in the receptive field center. In some cells the surround response, opposite in polarity to the center response, was again maximal to 620-nm light, but for a second group there was a shift toward green-dominance in the surround and hence red-green color opponency.

In dark-adapted retinas all 15 bipolar cells studied were most sensitive to monochromatic light of 520 nm near the absorption maximum for cyprinid porphyropsin (*11*), and all showed a Purkinje shift from 520 nm to 620 nm on the application of background illumination, with response polarity unchanged. Only five of these cells were stained, however, and all were Ma (off) or Mb (on) bipolar cells.

Amacrine cells of carp retina have been classified in two physiological groups: those with sustained responses and those with transient (on-off) responses (*7*). It has been shown that some of the former are unistratified, while the latter are regarded as "bistratified" or (multi) "stratified" (*12*). In our study attention was directed to sustained amacrine cells, for it was our working hypothesis that amacrine cells with "sustained" hyperpolarizing or depolarizing slow potentials are the amacrines most likely to receive direct input from bipolar cells and also most likely to be confined to one sublamina, if not to a single stratum.

About half of the sustained amacrine cells successfully filled with dye were of uniform morphology and physiology (Ab in Figs. 1 and 2). Like type b bipolar cells, the synaptic terminals of which overlap their dendrites in S4, these wide-field type b amacrine cells give maximal depolarizing responses to red light. On the other hand, their spatial summation is considerable, and spatially distinct surrounds are rarely found. Off amacrine cells, like off bipolar cells, were infrequently encountered. Among these are unistratified, broadly stratified, or even bistratified (Aa in Fig. 1) type a amacrine cells, all with dendrites confined to sublamina a (S1, S2, and S3). A few wide-field, broadly stratified amacrine cells branching in S1 and S2 appear to be the morphological and physiological counterparts of the wide-field type b amacrine cells. Other type a and type b amacrine cells, all predominantely sustained, and some with more complex chromatic responses, were also stained.

Most on-off amacrine cells were broadly stratified in both sublaminae (A in Figs. 1 and 2). Similarly, on-off ganglion cells usually branched in both sub-

laminae (G in Figs. 1 and 2), where they are in a position to receive direct bipolar input from both type a (off) and from type b (on) bipolar cells.

Off ganglion cells included neurons with relatively large cell bodies (Ga in Fig. 1), as we anticipated from inspection of Cajal's Golgi studies (*3*). Their responses were red-dominant, when, as in this example, branching occurred in the region of Ma bipolar cell synaptic terminals (S1 and S2). On ganglion cells were rarely encountered and difficult to stain, probably because of the small size of the cell bodies. Three type b ganglion cells branching in S4 and S5 gave on responses maximal to red light, but we could demonstrate a receptive field surround in only one of these. Some type a ganglion cells had receptive field surrounds, including one color-coded unit: red-off, green-on in the receptive field center and red-on, green-off in the surround.

Bipolar cells of carp retina studied with microelectrodes, whether off or on, have been shown in all cases to give maximal receptive field responses to red light (*7*), despite the fact that cyprinids have retinal ganglion cells with blue, as well as green, center responses (*13*). Even "pure cone" (C) bipolar cells may be red-dominant in the receptive field center. Since we too have found, however, that some amacrine and ganglion cells have prominent short wavelength responses, we suspect that at least some "C" bipolar cells must be blue, or blue-green-dominant in their receptive field centers (*8*). On the other hand, the green centers of green-red color-opponent ganglion cells may be formed by the surrounds of bipolar cells (*7*).

More than half of a randomly selected sample of bipolar cells in carp retina are color-coded, that is, they have red-dominant centers and green-dominant surrounds (*7*). Of the first 18 bipolar cells stained at random in our study, 16 were "M" bipolar and 14 were Mb bipolar cells. Thus statistics, as well as incomplete spectral data on a few stained cells, reveals that "M" bipolar cells include color-coded units. It is possible that a more detailed morphological analysis (for example, an examination of the bipolar cell dendritic trees in flat view) would permit a morphological subclassification of our 23 Mb bipolar cells (*10*) which would separate colar-coded from noncolor-coded cells, but at present distinctions are not obvious. Physiological studies indicate that color-coded Mb bipolar cells ought to contact "H II" (blue-green hyperpolarizing) horizontal cells, and it will be interesting to see how such synaptic connections might be made in the outer plexiform layer of cyprinid retina (*8, 10*).

It seems clear that the morphological and physiological distinctions between type a and type b bipolar cells provide an explicit model of the means by which off and on signals are conducted separately from photoreceptors to the two sublaminae of the inner plexiform layer. Moreover, dendritic stratification plays a crucial role in the formation of off and on responses of amacrine and ganglion cells, including color-coded cells (*14*). There is evidently a general similarity between carp and cat in the principles of retinal architecture which maintain on and off pathways through the vertebrate retina.

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Structural Basis for On- and Off-Center Responses in Retinal Bipolar Cells

Abstract. *Electron microscopy of Golgi preparations of goldfish retina shows that dendrites of type a (hyperpolarizing, off-center) bipolar cells make wide cleft junctions unassociated with synaptic ribbons, while those of type b (depolarizing, on-center) bipolar cells make narrow cleft junctions and synaptic ribbon contacts, with rods and cones. This suggests that wide cleft junctions are the site of sign-conserving, and narrow cleft junctions or ribbon contacts (or both) are the site of sign-inverting synaptic transmission from photoreceptors to bipolars.*

Visual information is sent from the eye to the brain exclusively by the nerve fibers of retinal ganglion cells. Many of these cells respond to illumination of the center of their receptive field with an increase in rate of nerve impulse generation (on-center response) and to illumination of the surrounding area with a decrease in rate of firing followed by an increase when illumination ceases (off-surround response); many others, while also having concentric antagonistic receptive field regions, show off-center and on-surround responses (*1*). The on-center and off-center responses appear to encode, respectively, light-on-dark and dark-on-light contrast for visual images of restricted size and location.

The ganglion cells are excited in large part by bipolar cells, which relay to them the responses evoked by light in rod and cone photoreceptors. While the basis of