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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5. During a flash of light, off ganglion cells are hyperpolarized and their spike discharge decreases. When the light is turned off, there is a rebound of membrane potential, accompanied by a discharge of spikes. During a similar stimulus, on ganglion cells produce a depolarizing response, accompanied by an increase in spike discharge. Although bipolar cells and most amacrine cells do not produce "fast" spikes typical of ganglion cells, we shall call off cells those bipolar and amacrine cells which produce hyperpolarizing slow potentials in response to a flash of light, and we shall call on cells those which depolarize in response to such a stimulus.
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14. Naka's recent demonstration of direct excitatory connections between on bipolar cells and on ganglion cells and between off bipolar cells and off ganglion cells in catfish retina is in good accord with the present findings. See K.-I. Naka, *J. Neurophysiol.* **40**, 26 (1977).
15. We thank Dr. W. K. Stell for sharing his unpublished results with us and commenting on our work and on this manuscript. Dr. N. W. Daw also made helpful comments on the manuscript. Supported in part by a Bionics Research grant from the Japanese Ministry of International Trade and Industry, by a grant to A.K. from the Takada Science Foundation, and by a research promotion grant to M.T. from the Keio University School of Medicine. E.F. was supported by a grant from the Leopold Schepp Foundation.
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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

on and off responses in ganglion cells is not fully understood, recent observations indicate a crucial role for bipolar cells: (i) bipolar cell receptive fields comprise concentrically organized, opponent center and surround regions, and some bipolar cells are depolarized by centered light spots while others are hyperpolarized (*2*, *3*); (ii) depolarization of bipolar cells of either type by extrinsic current causes on responses in ganglion cells while hyperpolarization causes off responses (*4*); and (iii) the axons of depolarizing bipolars appear to terminate in the same sublamina of the inner plexiform layer as the dendrites of on-center ganglion cells, and the axons of hyperpolarizing bipolars in the sublamina of dendrites of off-center ganglion cells (*5*, *6*). Thus, the responses of bipolar cells appear to be the major determinants of ganglion cell responses, at least with respect to certain aspects of spatial organization, contrast sensitivity, and response sign.

Intracellular recording and dye injection in cyprinid (carplike) fishes by Kaneko (*3*) and Famiglietti *et al.* (*6*) have shown that hyperpolarizing (off)-center bipolar cell axons terminate in the outer half or sublamina a of the inner plexiform layer, while depolarizing (on)-center bipolars terminate in the inner half or sublamina b (*5*). Using Golgi methods, we have identified cells morphologically similar to these dye-injected cells and described structurally specialized dendritic contacts which may be instrumental in generating their responses.

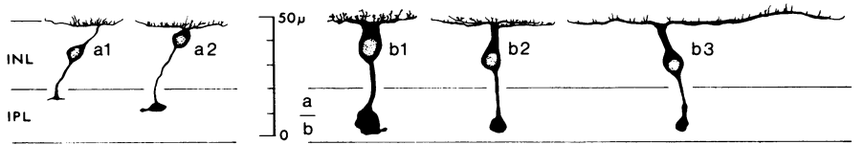

Fig. 1. Camera lucida drawings of Golgi-stained mixed rod-cone bipolar cells in goldfish retina. (a and b) Sublaminae of inner plexiform layer (*IPL*); *INL*, inner nuclear layer.

Table 1. Dendritic properties of mixed rod-cone bipolar cells in goldfish retina, derived from light microscopic reconstructions from serial semithin (0.5 or 1.0 μm) sections of cells impregnated with silver chromate.

Cell type	*N*	Average dendritic field dimensions (μm)	Average number of receptors contacted		
			R cones	G cones	Rods
a1	5	65.2 × 23.2	11.4	0	25
a2	6	43.7 × 36.6	7.3	4.8	20
b1	6	41.2 × 30.6	8.3	0	218
b2	5	55.6 × 48.0	9.7	8.0	223
b3	3	104.5 × 71.3	35.0	22.5	100

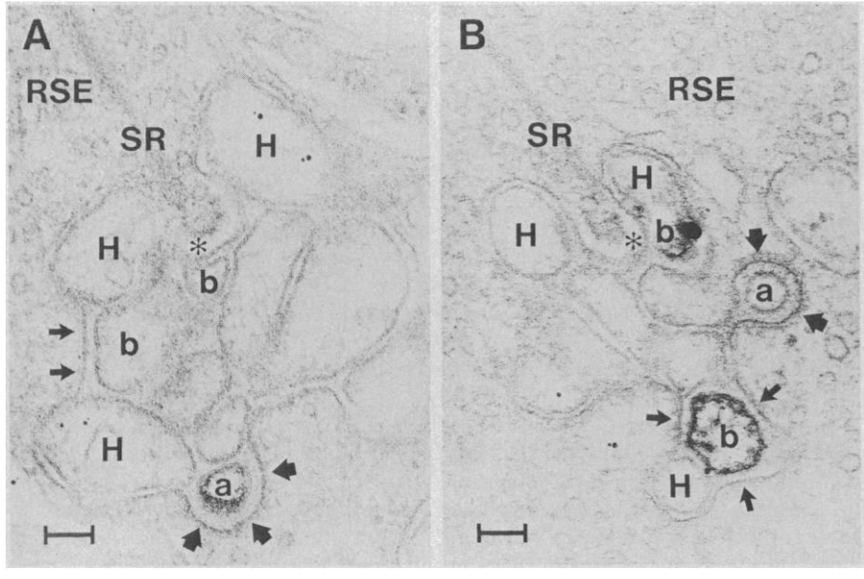

Fig. 2. Electron micrographs of rod-bipolar synapses in goldfish retina. *RSE*, rod synaptic ending; *SR*, synaptic ribbon; *H*, rod horizontal cell process; *a*, dendrite of class a bipolar; *b*, dendrite of class b bipolar; asterisk, ribbon contact; small arrow, narrow cleft junction; large arrow, wide cleft junction. (A) RSE contacts dendrite of Golgi-impregnated type a1 bipolar at wide cleft junction. (B) RSE contacts dendrite of Golgi-impregnated type b1 bipolar at ribbon and narrow cleft junction. Calibration, 0.1 μm.

Goldfish retinas were fixed for electron microscopy, impregnated with silver chromate by a Golgi method, embedded in epoxy resin, and sectioned transversely at 80 to 90 μm. These sections were surveyed under the light microscope to locate well-impregnated, isolated cells for further study; selected cells were remounted and sectioned serially at 0.5 to 1.0 μm for light microscopic reconstruction and identification of receptors contacted (*7*, *8*), or at 60 to 100 nm for electron microscopic reconstruction and identification of contacts between photoreceptor and bipolar cells (*9*, *10*). At present we have extensive data for the larger-bodied mixed bipolar cells, whose dendrites contact both rods and cones (*10–13*). These were selected for study because they seemed more likely to be penetrated by micropipette electrodes than small-bodied pure-cone bipolars and, therefore, to permit direct correlation of structure and function. We have not examined enough cone bipolars to draw conclusions about them.

We identified two major types of mixed rod and cone bipolar cells, which comprise five subtypes (Fig. 1) (*8*, *14*). Type a [hyperpolarizing or off-center (*6*)] mixed bipolars have dendrites, cell bodies, and axons of medium caliber and axonal terminations in the distal or outer half (sublamina a) of the inner plexiform layer. Type b [depolarizing or on-center (*6*)] mixed bipolars have dendrites, cell bodies, and axons of larger caliber and axonal terminations in the proximal or inner half (sublamina b) of the inner plexiform layer. Subtypes a1 and b1 contact rods and red-sensitive cones, while subtypes a2, b2, and b3 contact these as well as green-sensitive cones (Table 1).

Examination of the "invaginated" synaptic complexes of goldfish rods and cones reveals a variety of contact specializations (Fig. 2, A and B), including (i) ribbon contacts, in which a dendrite ends between horizontal cell processes close to the photoreceptor's synaptic ribbon; (ii) narrow cleft junctions, characterized by a thin, patchy electron-dense undercoating of the photoreceptor membrane and an irregular (11 to 13 nm wide) intercellular cleft which may contain pe-

riodic densities; and (iii) wide cleft junctions, characterized by a thick, continuous undercoating of the photoreceptor membrane and a regular (16 nm wide) cleft which usually contains periodic densities (*13*). Narrow and wide cleft junctions in goldfish are entirely different and readily distinguishable (*15, 16*).

We sectioned 17 Golgi-stained mixed bipolars (at least two of each subtype) serially through their contact with photoreceptor cells and examined them in the electron microscope. Both the type and extent of junctions are characteristic for each bipolar cell subtype, independent of photoreceptor type. Type a1 bipolars make extensive wide cleft junctions with rods (Fig. 2A) and cones. Type a2 bipolars make minimal wide cleft junctions with rods and cones. Type b1 bipolars make extensive narrow cleft junctions with both receptors and frequently ribbon contacts with rods (Fig. 2B). Type b2 bipolars make less extensive narrow cleft junctions and often terminate near the synaptic ribbon in cones as well as rods. Type b3 bipolars make minimal or no narrow cleft junctions and usually end near the ribbon in rods and cones.

These observations show that type a (hyperpolarizing, off-center) bipolars make only wide cleft junctions, unassociated with ribbon synapses, with both rods and cones, whereas type b (depolarizing, on-center) bipolars make only ribbon contacts or narrow cleft junctions (or both) with both rods and cones. It is reasonable to suppose that these specialized appositions represent the sites which mediate chemical synaptic transmission from photoreceptor to bipolar cell (*15*). Goldfish type a bipolars appear homologous to mammalian "flat" cone bipolars, and goldfish type b bipolars to mammalian "invaginating" cone bipolars (*5, 17, 18*). Our findings support the hypothesis, drawn indirectly from freeze-fracture studies of synaptic membranes in retinas and brains of different species (*18*), that the synapses upon "flat" bipolars are "excitatory" or sign-conserving whereas the synapses upon "invaginating" bipolars are "inhibitory" or sign-inverting (*13*). It remains for further studies to test the predictive value of the correlations between synaptic structure and function reported here (*19*).

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14. We have adopted cell names consistent with the nomenclature of (*6*). Types a1, a2, b1, b2, and b3 correspond, respectively, to types 5, 4, 1, 2, and 3 of (*13*), and types B1, B2, A1, A2, and A3 of (*11*).
15. A. Lasansky [*Philos. Trans. R. Soc. London Ser. B* **262**, 365 (1971)] described two forms of "basal junction" (BJ) (nonribbon contact) between cones and unidentified bipolars in turtle retina. Truly basal (superficial) BJ's in turtle appear identical to goldfish wide cleft junctions, whereas invaginated BJ's present "narrow-gap segments" which appear identical to goldfish narrow cleft junctions. The intermingling of wide and narrow features described in invaginated turtle BJ's is not observed in goldfish. We have avoided the term "basal junction," (i) because many junctions to which the term might be applied are not basal in location and (ii) because we wish to avoid any implication that wide and narrow nonribbon junctions might be identical [see also (*16*)].
16. A. R. Nagy and W. K. Stell (in preparation) have shown by freeze-fracture and replication of membrane interiors that characteristic aggregates of particles are present in goldfish bipolar cell membranes at wide cleft junctions but not at narrow cleft junctions or ribbon contacts.
17. A. Lasansky [*Invest. Ophthalmol.* **11**, 265 (1972)], observed apparently narrow-type junctions between receptors and invaginating bipolars en route to ribbon contacts in monkey retina, and Raviola and Gilula (*18*) observed no intramembrane particle aggregates in invaginating bipolars.
18. E. Raviola and N. B. Gilula, *J. Cell Biol.* **65**, 192 (1975).
19. A. Kaneko [*J. Physiol. (London)* **235**, 133 (1973)], has described "color-opponent" (R-center, R+G surround) and "noncolor-opponent" (R center, R surround) on- and off-center bipolar cells in goldfish. Assuming some of these to be mixed rod-cone bipolars, it is reasonable to suppose that type a1 and b1 cells are noncolor-opponent, since they have no apparent connection with either G cones or green-controlled horizontal cells (*7*); a2, b2, and b3 cells might then be color-opponent. Analyses of rod connectivity indicate that the absolute and relative contributions of rod activity to bipolars vary markedly with subtype [Table 1 and (*8, 12*)], as do the probable contributions of rod horizontal cells to their receptive fields (*10, 13*). Studies of the physiology and morphology of dye-injected cells, however elegant, have not yet provided sufficient data to test these hypotheses.
20. We thank E. V. Famiglietti, Jr., A. Kaneko, and M. Tachibana for sharing and discussing the results of their experiments with us, and for offering comments on this report. D. Bougn and A. Williams prepared the manuscript. Supported by grants EY 00331 and EY 01190 from National Institutes of Health.

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Body Weight: Reduction by Long-Term Glycerol Treatment

Abstract. *Elevation of body glycerol concentration by multiple daily injections of glycerol was shown to lead to hypophagia and body weight loss followed by normal food intake and normal rate of body weight increase in rats. Termination of injections was followed by hyperphagia and an accelerated rate of growth. These findings suggest that the blood glycerol concentration plays an important role in the control of body weight and may be one signal by which the central nervous system monitors body lipid content.*

It is generally believed that body weight is a regulated variable, and it has often been suggested that the regulation is achieved by the control of body lipid content (*1*). If this is the case, then the system which controls body weight must be able to sense the size of the adipose tissue stores, or some correlate of it, in order to correct deviations from "normal." There is accumulating evidence that a humoral signal might be utilized for this purpose. Studies involving the cross circulation of blood between pairs of rats, either by means of the parabiotic preparation (*2*) or by blood mixing in nonjoined animals (*3*), have shown that the blood of normal nonfasted or of obese rats contains a factor capable of suppressing food intake in a recipient animal.

The nature of a humoral signal which could be involved in the regulation of body weight through the control of food intake is unknown, but evidence exists which suggests that glycerol may be involved. A number of investigators have reported a positive relation between adipose cell size and blood glycerol concentrations (*4*). Since under some circumstances variations in body lipid content can be accounted for by variations in adipose cell size (*5*), blood glycerol concentrations in nonfasted animals should be directly related to the total lipid content of the body. If glycerol actually functions as an indicator of body lipid content, then raising the blood glycerol concentration should lead to a reduction of body weight to a new, lower settling point (*6*), at which it should be regulated. This follows from the assumption that an increase in blood glycerol would be interpreted by a control system as an increase in fat cell size and thus an increase in body weight. A body weight control system would then be expected