Intracellular Staining Reveals Different Levels of Stratification for On- and Off-Center Ganglion Cells in Cat Retina

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SUMMARY AND CONCLUSIONS

1. Ganglion cells in the retina of the cat were stained by intracellular dye injection after recording their responses to photic stimulation.

2. All cells encountered were divided into those giving on-responses and those producing off-responses, and the level of dendritic branching of these two groups was compared. Cells giving off-responses were found to branch high in the inner plexiform layer (IPL), near the amacrine cell bodies (sublamina a); those giving on-responses were found to branch lower in the inner plexiform layer (sublamina b).

3. Dye-injected cells varied widely in morphology and size, having cell bodies ranging in diameter from 8 to 32 μm and dendritic fields ranging from 25 to 490 μm in diameter; yet the sign of the response of each unit correlated only with the level of dendritic branching. Thus, no other morphological feature except stratification appears to be important in determining the sign of the response of these cells.

4. The stratification of ganglion cells into on- and off-layers parallels the distribution of the axon terminals of the flat and invaginating cone bipolars. Flat cone bipolars are in a position to contact off-center ganglion cells (in sublamina a) and invaginating cone bipolars are in a position to contact on-center ganglion cells (in sublamina b).

5. The rod and cone inputs to some cells were characterized by comparing their responses to deep red and blue rod matched stimuli over a 2-log unit range starting at dark-adapted threshold. About half the cells appeared to be rod dominated under these conditions, whereas the others appeared to have mixed rod and cone signals.

6. The nature of the rod and cone pathways to ganglion cells is discussed.

INTRODUCTION

In 1953 Kuffler (20) found that ganglion cells of the cat retina gave either on- or off-responses to spots flashed in their receptive-field centers and that these central responses were antagonized by stimulation of their receptive-field surrounds. Later it was shown that in many of the higher visual centers on- and off-center elements played an equal role within different physiological classes of cells (13, 14). Thus the organization of visual information into parallel on- and off-channels appears to be basic to the entire visual system.

The earliest stage at which the on/off dichotomy occurs is probably the bipolar cell level of the retina, for on-center and off-center varieties of bipolar have been found by intracellular-recording techniques in many nonmammalian species (15, 24, 34). These varieties of bipolar have been implicated as direct precursors of on-center and off-center ganglion cells in the particular species studied (22, 23).

Cone bipolar cells in mammals are known to come in flat and invaginating varieties, based on their different synapses with cones (3, 4, 16). It has been natural to speculate that these two different types of cone bipolar might be the morphological counterparts of on-center and off-center physiological varieties (10, 29, 32). Morphological counterparts for on-center and off-center ganglion cells, on the other hand, had hitherto been suspected.

Ganglion cells of the cat retina come in a
FIG. 1. Organization of cone bipolar cells and ganglion cells in the IPL of the cat retina. Flat cone bipolar cells (f) have axon terminals ending in sublamina a, contacting the dendrites of a-type ganglion cells (Ga). Invaginating cone bipolar cells (i) have axon terminals which ramify lower in the IPL, in sublamina b where they contact b-type ganglion cell dendrites (Gb). Ganglion cells of various morphologies branch either in sublamina a or sublamina b; these prove to be off-center and on-center, respectively. c, cones.

variety of morphological classes (5, 9, 21, 31) and examples of most of these can be found branching either in sublamina a (a-type, Ga, Fig. 1) or sublamina b (b-type, Gb, Fig. 1) of the IPL (9). Elsewhere (9, 18) we have shown by Golgi procedures and electron microscopy that flat cone bipolars (f, Fig. 1) in cat retina contact the dendrites of a-type ganglion cells; by contrast, invaginating cone bipolars (i, Fig. 1) provide synaptic input to the dendrites of b-type ganglion cells. The specificity of cone bipolar/ganglion cell connections in the IPL suggested to us a structural basis for on- and off-center responses in retinal ganglion cells (9).

In the present work we provide electrophysiological evidence from intracellular recording and marking with fluorescent dyes that ganglion cells with dendrites stratifying in sublamina a are off-center and ganglion cells stratifying in sublamina b are on-center.

MATERIALS AND METHODS

Preparation and recording system

Intracellular studies were carried out in a perfused eye cup preparation of the cat (25, 27). This preparation represents a simple modification of the in vitro perfused eye preparation (11, 28). An eye was removed from a heparinized cat, deeply anesthetized with 44 mg/kg of ketamine hydrochloride followed by 15 mg/kg of sodium pentobarbital, and the attached ophthalmic artery was cannulated within 10 min. The anterior portion of the eye and vitreous body were removed to make the retina more accessible to fine (150-1,000 MO) pipettes. These were filled with either Procion yellow (M4RS or M4RAN) or "lucifer" dyes, the latter (32a) generously supplied by W. Stewart.

Responses were led through a unity-gain amplifier to an oscilloscope and stored on tape. Since mammalian action potentials are rapid, it is wise to bear in mind the high-frequency limitations of the recording system. Rectangular pulses approximating the shape of action potentials (4 mV, 300-μs width) were found to be reduced to 64% of their original amplitude by the tape and to 58% of their amplitude by the unity-gain amplifier when led through a 1,000-MΩ input resistor. A 100-MΩ resistor did not cause any attenuation, however. Thus the impulses in the illustrations of this paper may be between 1.6 and 2.7 times larger than they appear.

The monochromatic stimuli used in these experiments have been measured in terms of (quanta/μm²)/(s) incident on the surface of the retina using a calibrated photodiode (United Detector Technology). A physiological calibration of these stimuli can be provided by rod
saturation in the responses of horizontal cell bodies of the cat retina (25). This occurred at about 3.5–4.0 log (quanta/μm²)/(s) with 441-nm stimuli and at about 5.5–6.0 log (quanta/μm²)/(s) with the 647-nm stimuli.

**Intracellular recording from ganglion cells**

While searching for cells, a large red stimulus (generally 5 mm² on the retina and 647 nm) was presented repetitively at 3- to 4-s intervals as the electrode was advanced through the ganglion cell layer and the negative capacitance amplifier was intermittently thrown into oscillation. Penetration of ganglion cell bodies was signaled by a negative shift in potential of about 30–50 mV, the appearance of light-modulated excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs), and the presence of impulse activity, ranging in amplitude from about 5–15 mV, driven by these postsynaptic potentials. Since spikes recorded inside ganglion cell bodies were hardly large enough to overshoot the resting potential, it seems likely that the cell body itself did not fire and that impulses invaded this region passively from the ganglion cell axon. Larger spikes, 20–35 mV in amplitude, similar to those reported by Wiesel (35), have also been recorded (see Fig. 8); however it has not been possible to recover a stained cell from such units. In one case such a unit was identified as an axon since the receptive field was a centimeter away from the recording site. Intra-axonal recordings have been previously reported (24, 35) so this is a likely recording site for responses with large-amplitude spikes.

Postsynaptic potentials recorded in ganglion cells could be rather small compared to the responses of other retinal neurons, and yet were remarkably effective in modulating impulse activity. Depolarizations of only 2–3 mV produced vigorous bursts of impulses while hyperpolarizations of less than 0.5 mV could abolish spontaneous activity.

In order to identify on- and off-center ganglion cells morphologically, a cell’s responses were characterized as either on or off by just a few presentations of a broad-field stimulus before injection with stain by the passage of 5–10 nA of hyperpolarizing current for 1 min. In this way cells which might be only briefly recorded intracellularly could be stained and their essential on- or off-center nature established.

In six instances, four of them being large-bodied cells (about 30 μm), it was possible to maintain stable intracellular recordings after staining, generally with somewhat reduced spike amplitudes. In these cases attempts were made to measure the receptive fields of ganglion cells and to measure the contributions of rod and cone signals to their responses. In no case has a more precise receptive-field map caused us to reverse our interpretation of the sign of the center response based on broad-field stimulation. Receptive-field maps were obtained by recording intracellular responses to a flashed slit stimulus positioned at regular intervals across a unit’s receptive field. The slit stimulus was 5.4 mm long and 50 or 100 μm wide. Intensities of stimulation were selected to be suprathreshold, but below saturation for a particular unit, and no background illumination was used. The diameter of the receptive-field center was judged to be the distance between the positions at which the initial EPSP or IPSP disappeared, less one slit width if this was large compared to the center size. The sizes of field centers measured by this technique ranged from 60 to 960 μm.

Rod and cone contributions to the responses of ganglion cells were assessed using the principal of univariance. Intensity response series were obtained at two wavelengths, 441 and 647 nm, in the dark-adapted state. These wavelengths were selected to maximize the difference in the ratio of absorption by the 502-nm rods and the 556-nm cones. (If the ratio of cone to rod absorption is taken as unity at 441 nm, it is over 100 to 1 at 647 nm.) If cells produced indistinguishable intensity response series at these two wavelengths, they were said to be univariant, implying input from just one photoreceptor mechanism over the range tested. Univariant cells always followed the rod match, identifying the photoreceptor input with the rods. Cells which had distinguishable intensity response series at the two wavelengths were termed nonunivariant, implying input from more than one photoreceptor mechanism, principally rods and 556-nm cones.

**Determination of ganglion cell stratification**

After an experiment, the approximate positions of stained cells were marked extracellularly on the retinal surface by microelectrode iontophoresis of fast green dye (33). Retinas were fixed in situ with 4% paraformaldehyde either in acetate buffer (pH 4) or phosphate buffer (pH 7). Maps were drawn of the fast green marks noting the positions of the optic disc and the area centralis. The appropriate piece of the eye cup, generally containing from four to eight injected units, was cut out and the retina was peeled away from the pigment epithelium, dehydrated through acetone, embedded in soft Epon, and sectioned vertically at 20–40 μm with a steel knife on a sliding microtome. Sections were scanned with dark-field fluorescence microscopy.
Fig. 2. Photomicrographs of cat retinas showing ganglion cells injected with fluorescent stains after intracellular recording. A: on-center ganglion cell. Double arrows indicate the IPL capillary bed at the a/b sublaminae border. Dendrites of the ganglion cell branch below the capillary bed in sublamin a b. B: small-field on-center ganglion cell, seen in flat section (above) and vertical section (below) to branch throughout sublamin a b. C: off-center ganglion cell, narrowly stratified in sublamin a under the amacrine cells. D: off-center ganglion cell branching in sublamin a at the amacrine cell/IPL border. E: off-center ganglion cell with a profusely branched dendritic tree restricted to sublamin a. F: ganglion cell giving off-center responses branching in sublamin a. The photoreceptor side of the retina is up in all micrographs. Scale bar, 100 μm. A and F: Procion yellow stain. B–E: lucifer stain.
After recovery of a stained ganglion cell in serially sectioned material, the layering of the cell’s dendrites with respect to sublamina a and b was determined. In most cases this was apparent from inspection of single vertical sections. However, the branching pattern of many ganglion cells is more easily appreciated in drawings reconstructed from serial sections. These drawings were made on graph paper with the aid of an eyepiece grid. Because of the compressive distortion of these drawings, the central plane of dendritic branching is apparent and it will be seen that cells branching in sublamina a always send terminal branches to the very top of the inner plexiform layer, abutting the amacrine cell bodies, whereas those branching in sublamina b send their deviant processes downward, leaving the upper third of the inner plexiform layer (sublamina a) vacant. These drawings thus clarify any ambiguity created by dendritic branches running close to the a/b border.

After making drawings we discovered that the inner plexiform layer contains a single stratum of capillaries which distinctly separates sublamina a from sublamina b. In the fluorescence microscope this capillary bed has the appearance of a dark line or a series of small dark holes against the olive green background of the inner plexiform layer (see Fig. 2A double arrow). It lies about one-third of the distance from the top of the inner plexiform layer, marked by the dark layer of large amacrine cell bodies, to the bottom of the inner plexiform layer, marked by the bodies of the ganglion cells themselves. Branches can be followed downward into large arteries lying on the surface of the retina; however, this

![Diagram of a Concentrically Organized On-Center Ganglion Cell](http://jn.physiology.org/)

**FIG. 3.** Concentrically organized on-center ganglion cell. The cell (drawn above) branches profusely in sublamina b of the IPL (I9 of Table 1). Its on-center responses (inner solid circle) occurred for slits positioned between 70 and -210 μm (left). The antagonistic surround (shaded annulus) can be seen on flanks of the center mechanism. The dotted circle indicates the dendritic field of the stained cell drawn to the same scale as the receptive field. The response to broad-field stimulation is shown below. The 100-μm wide stimuli are drawn slightly narrower than scale, but greatly shortened. Their wavelength was 441 nm at a retinal illumination of 3.1 log (quanta/μm²)/(s). Broad-field stimulus: 647 nm at a retinal illumination of 4.6 log (quanta/μm²)/(s). Stimulus duration: 560 ms.
STAINS OF ON AND OFF GANGLION CELLS

10mV

FIG. 4. Drawings of two on-center ganglion cells with their intracellularly recorded responses. Both cells branch in sublamina b although they have very different morphologies. These are drawings of cells A and B of Fig. 2 (cells 1 and 9 of Table 1, respectively). Stimuli: broad field, about 0.5 s duration. A: 400 nm, 2 log units above threshold. B: 647 nm, 4.9 log (quanta/μm²)/(s).

RESULTS

On-center ganglion cells

The physiology and morphology of an on-center ganglion cell in the cat retina is shown in Fig. 3. The drawing of this Procion-stained cell (above) shows that it is clearly a ganglion cell because it has a large soma lying in the ganglion cell layer and a distinct axon. The dendritic field diameter is about 150 μm and the dendrites branch close to the cell body in the lower portion of the IPL, sublamina b. Below (right) in Fig. 3 is the response of this cell to a broad-field stimulus (before staining), while on the left are the responses to slits positioned at intervals across its receptive field (made after staining).

The receptive-field center was characterized by depolarizing responses with sustained impulse activity. It was about 280 μm in diameter, not much larger than the actual dendritic field size of the cell, taking into account histological shrinkage and the size of the 100-μm-wide stimulus. On the flanks of the center mechanism a surround mechanism (shaded area), which inhibited impulse activity, can be seen.

This ganglion cell, thus, has response characteristics typical of a concentrically organized on-center cell of the cat retina and has a dendritic arborization restricted to sublamina b of the IPL.

Two other on-center ganglion cells are illustrated in Fig. 4, while A and B of Fig. 2 are micrographs of the original stained cells. Cell A gave a simple on-response to photic stimulation. The drawing of the cell indicates that it has a large cell body (30 x 32 μm) and a well-stained axon. The large dendritic tree (260 μm) arborized exclusively below the capillary bed (double arrows, Fig. 2A) in sublamina b of the IPL.

Cell B (Fig. 4) was first sectioned flat (Fig. 2B, above) and then resectioned vertically to determine its stratification (Plate 1B below). This ganglion cell’s small compact dendritic tree (80 μm) is confined to sublamina b close to its cell body. Note that its axon is stained (Fig. 4B).

The light responses of this cell consisted of an initial transient depolarization accompanied by a high-frequency burst of spikes. Its spontaneous activity was high, about 80 impulses/s, similar to average pulse densities reported by Enroth-Cugell and Robson (7) for X cells.

Off-center ganglion cells

The ganglion cell of Fig. 5C and Fig. 2C is an example of a large cell with an axon of large dimensions and with thick dendrites branching in a regular radiate pattern over a diameter of about 500 μm. In fact this stained cell could be followed through 13 sections (40 μm thick), 4 of which are represented in the drawing (Fig. 5C). The dendrites of this cell lie exclusively in sublamina a of the IPL just under the amacrine cell bodies (Fig. 2C). Its response to broad-field photic stimulation is illustrated in Fig. 5, bottom. This unit gave a maintained off-center response. On the left are the responses to a 50-μm-wide slit for different locations in the receptive field. The shaded circle gives our estimate of the physiological limits of the receptive-field center of this unit for the initial IPSP. This
FIG. 5. Receptive-field properties and morphology of an intracellularly stained off-center ganglion cell. The cell (above) has a large dendritic field branching exclusively in sublamina a (cell C, 8 of Table 1). Intracellular responses (left) to slits at different positions indicate that the central IPSP lies between 210 and -70 µm (shaded circle). The dotted circle gives the cells dendritic field (490 µm) drawn to the same scale. The response to a broad-field stimulus is shown below. Slit stimuli: 50 µm wide. Wavelength: 441 nm at a retinal illumination of 2.7 log (quanta/µm²)/(s). Stimulus duration: 542 ms.

amounts to a 280-µm-diameter off-center response. Notice that the receptive field for the discharges at off may be somewhat larger than this (about 420 µm). Thus, the physiological receptive field was considerably smaller than the cell's dendritic field (dotted lines); corrections for retinal shrinkage, which can amount to 20%, would make the disparity between receptive field and dendritic field even larger. In contrast, other off-center ganglion cells have been found with receptive-field centers from 3 to 6 times larger than their dendritic fields. No surround responses could be elicited from the unit of Fig. 5 under these (dark adapted) conditions, even using very large surround stimuli.

An additional three ganglion cells which gave off-center responses are illustrated in Fig. 6. All the cells have the common feature that their dendrites branch high in the IPL, in sublamina a. Cells D and F (illustrated in Fig. 2D, F) have small cell bodies and very fine axons. The example of Fig. 6D exhibited a slow sustained hyperpolarization with an apparent reduction of noise during the light flash, and a discharge at off. The receptive field of this unit was between 200 and 400 µm. In the cell of Fig. 6F, a high rate of unmodulated spontaneous discharge was observed initially, probably the result of injury. Subsequently, spiking activity ceased but a small hyperpolarizing response to the light flash remained (F in Fig. 6). It has generally been difficult to obtain normal-appearing impulse activity from small ganglion cells such as D and F, possibly because their impulse-generating mechanism is particularly susceptible to injury.

Cell E (Fig. 6E and Fig. 2E) has an extremely dense, narrow-field branching pattern to its dendritic tree, which contrasts with the wider ranging, fine dendrites of the other two ganglion cells (D and F). However, like the latter two cells, its dendrites branch only in sublamina a. E has a small cell body but a rather sturdy axon. Like the unit of Fig. 3B, this cell exhibited high spontaneous activity, but in this case the activity was reduced during the maintained hyperpolarizing off-response.

Physiological and morphological features of all the ganglion cells studied

A total of 21 ganglion cells have been recovered after intracellular recording and dye injection. Table 1 summarizes the essential morphological features and physiological classifica-
FIG. 6. Intracellular recordings and morphology of off-center ganglion cells. D, E, and F are drawings of cells D, E, and F of Fig. 2 (6, 16, and 7 of Table 1). All have in common that they branch in sublamina a of the IPL. E and F: broad-field stimulation. D: 200-μm-wide centered slit. Wavelength: all 647 nm. Duration: 0.5 s. Retinal illumination: 6.1, 4.7, and 6.1 log (quanta/μm²)/s in D, E, and F, respectively.

TABLE 1. Morphological properties of on- and off-center ganglion cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Sublamina</th>
<th>On/Off</th>
<th>C.B., μm</th>
<th>D.F., μm</th>
<th>Ecc/Quad</th>
<th>Rod/Cone</th>
<th>Axon/Spikes</th>
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<td>1</td>
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<td>r</td>
<td>++/++</td>
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<td>14</td>
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<td>r</td>
<td>++/−</td>
</tr>
<tr>
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<td>150</td>
<td>6.0/i*</td>
<td>r/c</td>
<td>−/−</td>
</tr>
<tr>
<td>4</td>
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<td>20</td>
<td>150</td>
<td>4.5/n</td>
<td>r</td>
<td>−/−</td>
</tr>
<tr>
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<td>215</td>
<td>1.5/n*</td>
<td>r/c</td>
<td>++/−</td>
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<tr>
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Explanation of headings: cell, cell number in chronological order (2/73 12/76); sublamina, sublamina of dendritic branching, either a or b (see text); on/off, response sign of the center mechanism; C.B., μm, diameter of the cell body (the mean of the major and minor axes was used in the case of irregularly shaped cell bodies); D. F., μm, dendritic field diameter (also a mean diameter); ecc/quad, eccentricity from the central area in millimeters per quadrant or direction in which the cell was located (t, temporal; n, nasal; i, inferior; s, superior); all were left eyes except those marked with an asterisk: rod/cone, r indicates a purely rod driven cell while r/c indicates a strongly mixed (e.g., 50/50) rod-cone unit (see text); axon/spikes, + indicates the presence of an axon morphologically/or spikes physiologically. Morphological measurements have not been corrected for shrinkage.
rod-dominated ganglion cell to rod-matched stimuli. The effect of stimulus intensity on the response pattern is compared at two wavelengths, one blue (441 nm) and the other deep red (647 nm). The two intensity response series are indistinguishable at these two wavelengths (univariant) and are separated in the intensity domain by the rod match. Stimuli, broad field (5.4 mm²); duration, 542 ms; retinal illumination, given to the left of each trace in log (quanta/μm²)/s). This is the same unit as in Fig. 4 (cell 8 of Table 1).

As can be seen from Table 1, the sample of cells studied here consisted of stained cell types that could, in most cases, be positively identified as ganglion cells by their location in the ganglion cell layer and by the presence of an axon. Among these ganglion cells a wide range of morphological cell types were encountered: some had small cell bodies and others large, dendritic field sizes varied from very small (25 μm, 20) to very large (490 μm, 8), and there was considerable variety in dendritic branching pattern among these cells (see Figs. 2–6). In addition to dendritic field and cell body dimensions, the eccentricity of each ganglion cell from the area centralis is listed since this is relevant to several classification schemes (5, 9). The only consistent feature of all cells studied, however, is that the sign of their respective receptive-field center responses is related to stratification of their dendrites in the IPL.

Rod pathways through the cat retina follow quite different neural circuitry than cone pathways (18, 26) and it, therefore, seemed of considerable interest to determine whether the ganglion cell responses studied originated with the rods or with the cones. Table 1 indicates rod and cone contributions to the responses of many of the cells studied. About half the cells appeared to be rod dominated, whereas the rest seemed to have a mixture of rod and cone signals. There appeared to be no segregation to sublaminae on the basis of rod or cone input. Details of the intensity response characteristics for a rod-dominated ganglion cell (r) and a mixed rod-cone unit (r/c) are described below.

**Rod and cone input**

We have used rod-matched red (647 nm) and blue (441 nm) stimuli in order to analyze rod and cone contributions to the responses of ganglion cells. This match was determined from the dark-adapted thresholds for the electroretinographic b-wave and has also been used to distinguish the responses of horizontal cell bodies from those of the terminal arborizations of horizontal cell axons (27). The intensity
response characteristics of an off-center ganglion cell (same unit as \( \delta \) of Table 1, Figs. 2C and SC) are shown in Fig. 7; the responses were nearly identical (univariant) for these two wavelengths and followed the rod match very closely. Retinal illuminations have been given to the left of each response in log (quanta/\( \mu m^2 \))/s). The rod match is thus identified as a 2.7–3.0 log unit shift at these two wavelengths when computed on an equal quantum basis. Under these (dark adapted) conditions, then, this ganglion cell appeared to be driven nearly purely by rod input.

A ganglion cell with quite different intensity response characteristics at these two wavelengths is illustrated in Fig. 8. The responses were matched at threshold giving a displacement of 2.0–2.2 log units on an equal quantum basis. This is somewhat less than the match used in Fig. 7 and in the direction of the match for 556-nm cones which, on a quanta basis, appear to be equally sensitive at these two wavelengths (25). Furthermore, the 441-nm threshold is somewhat higher than for the cell of Fig. 7, about 2.3 versus 0.5 log (quanta/\( \mu m^2 \))/s). But the most striking difference between the cell of Fig. 8 and that of Fig. 7 is that the responses increase in amplitude much more rapidly with increments in the 647-nm stimulus than they do with similar increments in the 441-nm stimulus. Thus the intensity response characteristics of this cell were definitely not univariant, which indicates more than one photoreceptor input. Since, under these approximately rod-matched conditions, the unit responds much more strongly to the red (647 nm) stimulus than the blue (441 nm), a likely candidate for one of these inputs is signals from the 556-nm cones, whereas the other input probably originates in rods, since the threshold responses were close to the rod match. Although no stain could be recovered from this unit, its small receptive field (60–80 \( \mu m \)) suggests that this may have been a type with a small dense dendritic field.

This second unit, then (Fig. 8), confirms the widely reported observation that there is no change in the polarity of the center responses of ganglion cells depending on whether they are driven by rod or cone signals (2). Daw and Pearlman (6), and Rodieck and Rushton (30) have claimed that all cat ganglion cells can be driven by both rods and cones. Purely rod-driven ganglion cells have not been so extensively documented, although Andrews and Hammond (1) reported a few such cells in the dark-adapted cat retina; moreover Granit (12) said that only 36% of the cat ganglion cells in his studies could be shifted from the scotopic to the photopic spectral sensitivity function by light adaptation, implying a large population of rod-driven cells.

**Displaced amacrine cells?**

We are mindful of the possible existence of displaced amacrine cells in the cat's ganglion cell layer and the possibility that some of the cells listed in Table 1 might, in fact, have been amacrine cells. Although the proof of the existence of displaced amacrines would rest with a demonstration of presynaptic dendrites, we have taken notice of whether or not all units studied had either an axon or impulse activity. As can be seen by reference to Table 1,
three of the units were not observed to have either an axon or spikes. Unit 14 with its small 8-μm cell body is particularly suspect. These three units without axon or impulses bore some similarity to each other both physiologically and anatomically. All gave sustained depolarizing responses with hyperpolarizations at off. The cell bodies of these units, although located in the ganglion cell layer, were all flattened against the IPL and tended to be half-moon in shape. Their dendrites arborized much lower than other cells, tending toward the very lowest strata of the IPL. In any event, if some displaced amacrine cells with regard to branching, since these cells, tending toward the very lowest strata of the IPL. The likelihood that on- and off-center ganglion cells are stratified randomly with respect to sublaminae a and b is evidently 1 in 2^{20}, or a probability of about 1 in a million. Thus, the idea that the polarity of the center responses of retinal ganglion cells is related only to their level of dendritic branching, irrespective of major differences in cell body size or branching pattern of the dendritic tree, appears to be true.

Combining our previous anatomical findings (9, 18, 19) with the results of this study, we are now in a position to state that in cat retina off-center ganglion cells are a type and are contacted by flat cone bipolars, whereas on-center ganglion cells are b-type and receive input from invaginating cone bipolars (summarized in Fig. 1). If bipolar-to-ganglion cell synapses are excitatory, as suggested by experiments in nonmammalian species (22, 23) it follows that invaginating cone bipolars ought to be on-center and flat cone bipolars ought to be off-center in the cat retina. Thus on- and off-pathways may originate with the first synapse in the visual system.

A natural extrapolation from these arguments might be that all neurons stratifying in sublamina a would be off-center and all neurons stratifying in sublamina b would be on-center, and that this organization might be a general feature of all vertebrate retinas. In fact this idea has since been confirmed in carp retina (8). As far as the cat retina is concerned, though, sublamina a can only be regarded as off-center, and sublamina b can only be regarded as on-center, in respect to synaptic output to ganglion cell receptive field centers, for it appears that, particularly in the rod system, on-center elements, such as the A II amacrine (19, 26), feed sublamina a, and off-center elements, such as the rod bipolar, have input to sublamina b (26).

In this study we found about half the ganglion cells to be rod dominated, whereas the remainder received equally mixed rod and cone input (see Table 1, Figs. 7 and 8). This distribution of rod and cone input is strongly reminiscent of the distinction found between the rod and cone systems of the cat at the level of the outer plexiform layer. The terminal arborizations of horizontal cells, which contact rods exclusively, have rod-dominated responses, whereas the horizontal cell bodies, which are known to contact only cones, have equally mixed rod and cone signals (17, 25–27). This organization of the rod and cone channels may be carried through to the ganglion cell level relatively unaltered.

Some varieties of ganglion cells in cat retina have been found to be richly innervated by cone bipolars (18, 19). Such cells might thus be expected to reflect the 50% mixture of the cone system. On the other hand, other ganglion cell types, innervated by the rod amacrine pathways of the IPL (18, 19), could reflect rod-dominated responses typical of the rod system (26). Thus, although the bisublaminar organization of the IPL of cat retina appears to rest solely with specific connections established between cone bipolars and ganglion cells (Fig. 1), probably the rod system utilizes these cone pathways, in addition to its own independent circuitry, to maintain on- and off-channels through sublaminae b and a, respectively, to the ganglion cells.

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REFERENCES


