INTRODUCTION

At the first synapse of the vertebrate retina, the visual response is encoded in parallel by two classes of bipolar cells. One class, the hyperpolarizing bipolar cells (Bh), hyperpolarizes to light and thus conserve the sign of the input signal of the photoreceptor cells. The second class, the depolarizing bipolar cells (Bd), depolarizes to light, thereby inverting the photoreceptor input (Dowling 1987; Miller 1994; Werblin 1991; Wu 1994). It thus appears that the bipolar cells encode the visual world as two complementary images of opposite sign, and it is widely held that this scheme is somehow fundamental for the representation of visual contrast. Indeed, in primates, there is electrophysiological and behavioral evidence that signals arising from the Bd and Bh bipolar cells may underlie, respectively, the perception of positive and negative luminance contrast, i.e., the perception of objects brighter and dimmer than their backgrounds (Schiller 1992).

At the cellular level, there are several fundamental differences between the two classes of bipolar cells. The photoreceptors activate Bh cells via kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, whereas Bd cells are activated via amino-phosphonobutyric acid (APB) receptors and the subsequent activation of cyclic guanosine monophosphate (cGMP) second-messenger cascade (Miller 1994; Nawy and Jahr 1991). Bh cells may have higher synaptic gain (Capovilla et al. 1987), larger receptive field centers (Hare and Owen 1990), and faster kinetics than Bd cells (Copenhagen et al. 1983; Frumkes and Miller 1978; Nelson 1973; Wu 1994). Anatomically, some Bh and Bd cells may make different types of specialized synaptic contacts with photoreceptor cells (Kolb 1994; Lasansky 1978; Saito 1987), and proximally the axon terminals of Bh and Bd cells tend to synapse in different sublamina of the inner plexiform layer (Hare et al. 1986; Kolb 1994) to preferentially contact different classes of ganglion cells. In some vertebrate retinas, a number of morphological subtypes of bipolar cells have been described (Ammermuller and Kolb 1995; Boycott and Wassle 1991; Hare et al. 1986; Wassle 1996), suggesting that there may be considerable parallel processing of the visual image in addition to the underlying Bd/Bh dichotomy.

Despite the generally held view that the bipolar cells critically shape the visual response to luminance contrast, there is surprisingly little direct evidence on the issue. This seems largely due to the technical difficulties in recording from bipolar cells in most vertebrate retinas and because most previous electrophysiological work on bipolar cells has been restricted to responses evoked by light flashes in the dark. In this paper, we analyze the responses of bipolar cells to contrast steps by intracellular recording in the light-adapted retina of the tiger salamander (Ambystoma tigrinum). By quantitative measurements of contrast gain, contrast dominance, and response latency, we attempt to determine how the bipolar cell population encodes negative versus positive contrast and whether there are functional differences in contrast processing between the two bipolar classes. We also measure the contrast responses of horizontal cells and cone photoreceptors and find them to be much different from those of bipolar cells. We show that the contrast gain is similar and very high for both Bd and Bh cells as a consequence of an amplification of some 10 times across the cone → bipolar cell synapse, that Bh cells tend to have shorter
METHODS

Preparation and intracellular recording

Intracellular recordings were made from superfused eyecup preparations of the tiger salamander (A. tigrinum). The animal was decapitated rapidly and pithed. The cornea and iris cut were cut away so that the lens could be removed with a fine suction tube. Most of the vitreous was removed by small pieces of thin, absorbent paper. A razor blade tissue chopper then was used to slice away the remaining iris, leaving a rectangular strip of the back of the eye of ~2 × 4 mm. Mylar strips then were gently applied at the edges to flatten and secure the tissue in a small chamber. The retina was maintained at room temperature (20–23°C) and superfused at ~1 ml/min with a Ringer solution composed of the following (in mM): 111 NaCl, 22 NaHCO3, 2.5 KCl, 1.5 MgCl2, 1.5 CaCl2, and 9 dextrose. The pH was regulated at ~7.5 by bubbling the superfusate with 98% O2–2% CO2. The general condition of the retina was monitored by recording the electroretinogram. As a rule, the b wave showed high sensitivity and good stability for ≥8 h. Intracellular recordings were made with glass micropipettes (0.5 mm ID, 1 mm OD) pulled on a Brown-Flaming puller. They were filled with 2.0 or 0.25 M K-acetate and had resistances of 200–700 MΩ. Cells were penetrated by the common procedure of causing the microelectrode amplifier to oscillate via brief applications of excessive negative capacitance. Electrodes filled with 0.25 M K-acetate, perhaps due to their higher resistance, seemed more likely to penetrate cells and thus were generally preferred, despite their somewhat higher noise level.

Light-evoked responses were recorded permanently on video tape and later digitized (0- to 5,000-Hz bandwidth) for analysis with the aid of commercial software (Superscope, GWI Instruments). Response amplitude was measured from the baseline to peak of the response. Latency was taken as the time at which the rising phase of the response first deviated from the baseline. For the latter measurements, two best-fitting straight lines were drawn by eye to determine the intersection between baseline and the initial rising phase of the response. For the responses displayed in Figs. 1, 2, and 7, digital filtering was used to achieve the optimal reduction of electrode noise without producing detectable distortion of response amplitude and waveform.

Light stimulation

Focused light stimuli, arising from a 100-W tungsten-halogen source, were applied to the retina. An optical system of conventional design was used for standard screening tests to determine the basic response properties of cells. To investigate responses to contrast steps, an active-matrix Liquid Crystal Display (Magnabyte m2x, Telex Communications, Minneapolis, MN) was inserted at an object plane in the optical system. The image on the retina was restricted to a field of 100 × 100 pixels. Each pixel illuminated a 30 × 30 μm square area on the retina. Custom software made it possible to stimulate the retina with spots and annuli of variable contrast and size. Light calibrations were made at the plane of the retina with a photodiode photometer. Contrast steps ranging from ±0.03 to ±2.0 log units were generated with the liquid crystal display (LCD) system on a steady background illumination of 20 cd/m2 on the retina. A centered spot of the Brown-Flaming puller. They were filled with 2.0 or 0.25 M K- optimal diameter and an annulus (typically 750 μm OD) at the center of the receptive field of the cell. For this report, contrast usually is specified as the logarithm of the flash/background intensity ratio: Contrast = log10 (F/B), where B is the steady background intensity and F is the light intensity prevailing during the flash. For example, contrasts of +0.30 and −0.30 refer to flashes that are two times and half of the background intensity.

Michelson contrast is a well-known metric that also can be used for simple contrast steps and defined: Contrast = (F − B)/(F + B), where F and B are as defined above (Burkhardt and Gottesman 1987) For contrast steps, log contrast and Michelson contrast are numerically equivalent over the range of ±0.70. In describing our results, we usually will use the logarithmic specification of contrast and refer to this as “contrast,” without further qualification. However, when reporting measurements of contrast gain, we use percent Michelson contrast and will refer to this simply as percent contrast. For low to moderate contrasts (<0.70), percent Michelson contrast is numerically equivalent to log contrast × 100.

Protocol

After a cell was penetrated, the following protocol was typically used to identify cell types and obtain contrast/response measurements: 1) the center of the receptive field was found by flashing a 100 × 2,000 μm slit at various positions on the retina. 2) The LCD stimulator was used to present low contrast stimuli in steps of variable diameter (from 100 to 2,000 μm) at the center of the receptive field to determine the optimum diameter, i.e., the stimulus diameter giving the largest response. 3) A centered spot of the optimal diameter and an annulus (typically 750 μm ID and 2,000 μm OD) were flashed at several contrast levels to screen for center/surround antagonism (see further). 4) The relation between contrast and response was investigated by presenting contrast flashes of the optimal stimulus diameter for 500 ms at the center of the receptive field. Flashes were presented every 10 s at each of 14 contrast levels covering a range from about −2.0 to +2.0. Because recordings from bipolar cells and cones were relatively noisy (see further), the series was repeated when recording time allowed, and average responses were computed. The retina was always light-adapted to steady background illumination of 20 cd/m2 covering the entire retina. Preliminary work showed that the interstimulus interval of 10 s was sufficiently long to eliminate effects of previous flashes, thus keeping the retina in a steady state of light adaptation. And 5) when recording time permitted, interference filters (~10 nm half-band) were used to present flashes of variable intensity at 630 and 530 nm on the background field. The resulting measurements were used to determine the cell’s 630/530 nm sensitivity ratio and thereby classify the cell for spectral type.

In the text, the term maximum response refers to the maximum change in voltage evoked by a contrast flash of positive or negative contrast, whichever is the more effective for the cell in question. The term total response refers to the voltage range measured from the largest response evoked by negative contrast to the largest response evoked by positive contrast. The maximum response, as defined above, was used as the reference for computing the normalized response amplitude. All statistical probabilities given in the text are based on Student’s t-test.

Identification of intracellular recordings

The origin of intracellular recordings was determined from functional criteria established in past work in the tiger salamander (Hare and Owen 1990; Hare et al. 1986; Wu 1991; Yang and Wu 1991). In brief, recordings assigned to bipolar cells had small receptive field centers, typically giving their largest response to stimuli of ~240 μm (range: 100–500 μm). Smaller responses always were evoked by stimuli of large diameter. In all cases, an
The response of bipolar cells to contrast varied greatly both within as well as across the Bd and Bh classes. Examples of this finding are given in Fig. 1, which shows selected responses to positive and negative contrast steps applied in the center of the receptive field (see METHODS). Because the stimuli are of very high contrast (±1.5), the responses shown for each cell pair are the largest (saturated) responses evoked by both contrast polarities. The Bd and Bh cells paired in Fig. 1, A and B, are similar in giving considerably larger depolarizing than hyperpolarizing responses but differ inversely in their response to contrast polarity. Thus the Bd cell responds maximally to positive contrast, whereas the Bh cell responds maximally to negative contrast. Figure 1, C and D, shows a pair of Bd and Bh cells the responses of which are relatively similar in amplitude as a function of contrast polarity. The third pair of cells (Fig. 1, E and F) gives considerably larger hyperpolarizing than depolarizing responses but differs inversely in the cells’ response to contrast polarity. The Bh cell responds maximally to positive contrast, whereas the Bd cell responds maximally to negative contrast. Taken together, Fig. 1 highlights a central finding, to be documented in more detail further on, that the optimal contrast polarity for driving bipolar cells may depend as much on the specific bipolar cell as the generic class to which it belongs.

Many bipolar cells showed remarkable sensitivity to small contrast steps. Figure 2 shows this finding for two very sensitive cells, one Bd and one Bh. Both cells generate vigorous responses to a small contrast step of 0.03 and generate nearly maximum responses at 0.15 contrast. To analyze the contrast response quantitatively, complete contrast/response plots were obtained for 20 Bd and 24 Bh cells. On average, the maximum total response (see METHODS) did not differ significantly between the two classes [Bd cells: mean = 10.0 ± 1.5 (SE) mV; Bh cells: mean = 13.1 ± 1.3 mV; P = 0.12]. On the other hand, the maximum response varied considerably from cell to cell regardless of type. The factors responsible for these differences are unknown, so most of the present report concentrates on the analysis of normalized contrast/response measurements. Figure 3 shows representative plots for a sample of eight Bd cells. The cells were ordered from top left to bottom right with respect to the relative strength of their response to positive contrast. Figure 4 shows representative contrast/response plots for eight Bh cells, ordered as in Fig. 3 with respect to the relative strength of their response to positive contrast. Three primary mea-
sures were used to analyze the contrast response plots: contrast gain, $C_{50}$, the contrast for half-maximal response, and the ratio of the maximal responses evoked by positive and negative contrast.

**CONTRAST GAIN.** The contrast gain was estimated for positive and negative contrasts by projecting the first points of the contrast/response curve (typically, the responses to $+0.03$ and $-0.03$ contrast) to the origin and computing the resulting slope in units of percent normalized amplitude/percent contrast. Because contrast gain most often has been expressed in percent Michelson contrast in past work, we use this contrast metric here. For low contrasts, percent Michelson contrast is equivalent to $\log$ contrast $\times 100$, as noted in METHODS. Figure 5 shows measurements for all Bd cells (○) and Bh cells (●). This shows that there is both considerable overlap and dispersion in contrast gain as a function of contrast polarity and cell type. Many cells are relatively symmetrical with respect to contrast gain because their values fall near the diagonal. Symmetry in contrast gain should be found for very small excursions about the background light level, where linear response behavior is expected. However, several cells in Fig. 5 are asymmetrical, showing considerably lower gain for one polarity than the other. This implies that the response to that contrast polarity is already outside the linear contrast/response range, showing reduced gain as it starts to approach saturation. Asymmetries around zero contrast are hard to appreciate in Figs. 3 and 4 due to the compressed nature of these plots but two clear examples may be seen in Fig. 2, by comparing each cell’s response to $+0.03$ and $-0.03$ contrast.

The average contrast gain for cones is shown by the cross (for details, see further). Thus as a rule, Fig. 5 shows that the contrast gain of bipolars is much greater than that of cones. A number of bipolar cells showed very high contrast gain in the range of 15–20% amplitude/percent contrast, and across the total sample of 44 cells, it was found that on
average, ~10% of the maximal bipolar response was evoked by a change in contrast of only 1%. The high sensitivities found in this report are probably close to the maximum contrast sensitivity because the stimulus diameter and position were optimized for each cell (see METHODS). The mean results for contrast gain measurements are given in Table 1. They show that, on average, contrast gain did not differ appreciably between Bd versus Bh cell types. For Bd cells, contrast gain was also clearly independent of contrast polarity. Bh cells, however, showed higher contrast gain for positive versus negative contrast, the only difference in Table 1 that reached statistical significance ($P = 0.008$). This difference is evident in Fig. 5 because the majority of the filled circles fall to the right of the diagonal. When expressed in millivolts rather than normalized amplitude, the average contrast gain of Bd cells was ~0.7 mV/% contrast for both contrast polarities, whereas Bh cells showed values of ~1.4 and ~0.9 mV/% for positive and negative contrast, respectively. Thus in either normalized or absolute units, the highest contrast gain was found for the response of Bh cells to positive contrast.

C50, THE CONTRAST NECESSARY TO EVOKE A HALF-MAXIMAL RESPONSE. From each cell’s contrast/response plot, $C_{50}$ was calculated by linear interpolation between the pair of data points spanning 50% of the maximum response. For cells with relatively balanced responses, a $C_{50p}$ value for positive contrast and a $C_{50n}$ value for negative contrast could be determined. On the other hand, a number of cells showed highly asymmetric responses as a function of contrast polarity so only $C_{50p}$ (e.g., Figs. 3A and 4A) or only a $C_{50n}$ could be determined (e.g., Fig. 3H). For such asymmetric cases where $C_{50p}$ or $C_{50n}$ were indeterminate, values were assigned as $>2$ or $<2$, respectively, so that results for all cells in the sample could be displayed graphically. Figure 6 shows the resulting plot for Bd (○) and Bh (●) cells for all 44 cells studied. Several points are notable: 1) few data points cluster around the 45° locus, thus showing that there is considerable diversity and asymmetry for both Bd and Bh cells in their suprathreshold response to contrast. 2) Many $C_{50}$ values are impressively low: 35 of the 44 cells in Fig. 6 have $C_{50}$ values of $<0.10$ contrast for at least one contrast polarity. 3) The $C_{50}$ values for bipolar cells are generally much lower than that of cones—the rectangle in Fig. 6 encloses the range of $C_{50}$ values found for cones, as will be discussed in more detail below. 4) A good number of Bh cells show small $C_{50p}$ values and thus seem particularly responsive to positive contrasts.

In recent work, retinal ganglion cells were classified on the basis of $C_{50p}$ and $C_{50n}$ values (Burkhardt et al. 1998). In short, cells the $C_{50}$ values of which differed by two or more times were classified as positive or negative contrast dominant, and the remainder were classified as “balanced.” Applying these criteria to the present sample of Bh cells yielded 12% as negative contrast dominant, 73% as positive contrast dominant, and 15% as balanced. The sample of Bd cells yielded values of 35% negative dominant, 35% positive dominant, and 30% balanced.

### TABLE 1. Contrast gain for bipolar cells

<table>
<thead>
<tr>
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<th>Bd</th>
<th>Bh</th>
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<tbody>
<tr>
<td>$CG_{pos}$</td>
<td>$+11.6 \pm 1.4$</td>
<td>$-14.4 \pm 1.7$</td>
</tr>
<tr>
<td>$CG_{neg}$</td>
<td>$-11.6 \pm 1.5$</td>
<td>$+9.4 \pm 1.5$</td>
</tr>
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Values are means ± SE. Mean contrast gain (% amplitude/% contrast) for contrast steps of positive (CG pos) and negative (CG neg) polarity for depolarizing (Bd) and hyperpolarizing (Bh) bipolar cells.
a similar mean value (1.96) was obtained for a sample of eight cones (see further), the variation from cell to cell was much larger for the bipolar cells. Hence, the $P:N$ ratio and thus the contrast asymmetry found in an individual bipolar cell can be of much different magnitude or even of opposite direction from that of the cone input.

**Small Signal Linearity.** When the light step was specified in linear light units of $\Delta L$, rather than contrast, the overall form of the resulting stimulus/response plots (not shown) was even more nonlinear than is apparent for the contrast/response plots of Figs. 3 and 4. For cells like those in Fig. 2, the departure from linearity was extreme. This may be appreciated by noting that as the contrast increases from 0.03 to 0.15, the light step, $\Delta L$, increases by about five times. However, the corresponding increase in response is only about twice for responses at the Fig. 2, top left, and 1.3–1.4 times for the other three cases. An analysis of all cells in our sample showed that although, on average, the degree of nonlinearity decreased as the stimulus range was gradually reduced, even when the range of the light steps was reduced to only about ±15% of the background (equivalent to contrasts of ±0.07), the plots of response versus $\Delta L$ were still noticeably sigmoidal in shape. In the limit, linearity should be found for very small light steps of either polarity, and it will be of interest to pursue this issue in the future. The present results are sufficient to support the conclusion that the linear response range of most bipolar cells in our light-adapted conditions is very small, corresponding to contrasts of ≤0.07.

**Contrast/response of cones**

Contrast/response measurements were obtained for cones using identical stimulus conditions as those typically used for bipolar cells, i.e., stimuli of 240 $\mu$m in diameter (see METHODS). Responses of a cone are shown in Fig. 7. Responses to low contrasts are very small and the largest response is evoked by very high positive contrast. Unlike bipolar cells, the contrast/response plots were very similar from cone to cone, so it was possible to compute a representative mean curve. This is shown in Fig. 8. The contrast/response plot is very shallow for small contrasts and is positive-contrast dominant overall, so that very large positive contrasts (+1.5 to +2.0) are required to evoke the maximum response. Values for the average cone contrast/response curve were: $C_{50}^p = +0.51$; $C_{50}^n = -1.75$; $P:N = 1.96$.

For relatively weak stimuli, it was found that the cone response was linearly related to the size of the light step, $\Delta L$. For responses spanning ±20% of maximum, the linear best fit, constrained to pass through the origin, yielded an $r^2$ value of 0.98 for the relation: $R = -0.36 \Delta L$, where $R$ is the normalized response amplitude and $\Delta L$ is the size of the light step scaled relative to a background intensity of 1.0. This relation is plotted as the dotted line in Fig. 8 and can be seen to approximate the data satisfactorily over a range of contrasts from about −0.30 to +0.30 and thus a considerably larger range than found for bipolar cells, as discussed earlier. From the linear best-fit equation given earlier, the mean contrast
gain of the cone can be calculated as 0.9%/% contrast for both contrast polarities. This value is \(\sim 10\) times less than that typically found for bipolar cells (see preceding text).

**Contrast transfer across the cone → bipolar synapse**

The majority of cones in the tiger salamander retina are red-sensitive, containing a photopigment with maximal absorption at \(\sim 610\) nm (Perry and McNaughton 1991). According to the nomogram for cone photopigments (Ebrey and Honig 1974), the action spectra of these cones should show a 630/530 nm sensitivity ratio (see METHODS) of \(\sim 0.22\). In good agreement, the mean log sensitivity ratio for the sample of cones of Fig. 8 was \(+0.25 \pm 0.04\) (SE), thus clearly identifying these recordings as arising from red-sensitive cones. When recordings could be held for sufficiently long periods (see METHODS), sensitivity measurements also were made for bipolar cells. The mean log 630/530 ratios were: Bd: \(+0.25 \pm 0.03\) \((n = 13)\); Bh: \(+0.22 \pm 0.04\) \((n = 16)\). No cells had sensitivity ratios remotely close to that expected for rod input (log 630/530 = \(-1.20\)). Thus although both predominantly rod-driven and cone-driven bipolar cells are found in the dark-adapted retina (Hensley et al. 1993), it is clear that under the light-adapted conditions of our experiments, all tested bipolar cells were driven by the red-sensitive cones. It is therefore possible to examine the transfer of information between red-sensitive cones and these bipolar cells by plotting the cone response, obtained from the mean curve of Fig. 8, against the response of the individual cone-driven bipolar cells.

Figure 9 shows a sample of cone/bipolar plots for four Bd and four Bh cells. The measurements in the left and right quadrants correspond, respectively, to responses to positive and negative contrast. For all cells, the slope for very small contrasts is very steep, implying high gain across the cone → bipolar synapse, and in most cases, the response quickly saturates at somewhat higher contrasts. The normalized amplitude data from all cells were analyzed to determine the relative bipolar/cone slope gain. This was estimated by projecting the first point of the cone versus bipolar plot to the origin and computing the resulting slope in dimensionless units. As shown in the two top rows of Table 2, mean values were about \(-9\) and \(-10\) for Bd cells and \(+12\) and \(+7\) for Bh cells. Of the four possible comparisons in the top two rows of Table 2, only the difference between the gain for positive versus negative contrast for Bh cells reached statistical significance (\(P = 0.006\)).

Unlike the relative gain calculations above, the determination of the voltage gain between cones and bipolar is not straightforward because the experimentally measured voltage may be attenuated spuriously due to an imperfect electrode seal or other factors. Attenuation seems particularly likely for cones because our recordings were of relatively small amplitude [mean total response: \(6.8 \pm 1.0\) (SE) mV] compared with responses of \(\sim 20\) mV for several of the best cases previously reported for flashes in the dark (Attwell et al. 1982; Lasansky 1984; Wu 1991). To calculate the voltage gain between cones and bipolar, we have therefore assumed that the cone response (Fig. 8) covers a total response range of \(20\) mV, namely, about \(-13\) and \(+7\) mV for positive and negative contrast, respectively. The resulting calculations of...
voltage gain should be conservative because they use the experimentally measured responses of bipolar cells, some of which were relatively small and thus, probably considerably less than the true amplitude. When so computed, the mean values for voltage gain, as shown in the lower part of Table 2, were about –4 and –5 for Bd cells and +5 and +9 for Bh cells. The largest voltage gains were about 1.7 times greater than the mean values in Table 2 and thus ranged up to a maximum of about +15 for the response of Bh cells to positive contrast.

Over the full response range, the relation between the cone and bipolar response was typically quite nonlinear. If the bipolar cell response were strictly a linearly scaled version of the cone response only accounted for about 65% of the variance of the bipolar response (Bd: $R^2 = 0.65 \pm 0.03$; Bh: $R^2 = 0.61 \pm 0.06$). To achieve a $r^2$ value of about 0.90, it was necessary to restrict the cone response range even further to about 5% of the maximum, equivalent to a contrast range of about 0.07. Thus under the conditions of our experiments, there is only a very narrow range of small signals within which the cone → bipolar transfer is approximately linear. To at least a rough approximation, the sigmoidal form of the contrast/response curves might be accounted for by the operation of a quasilinear transfer at very low contrasts followed by a saturating nonlinearity at higher contrasts.

C → $B_{50}$, the cone amplitude required to evoke a half-maximal response in bipolar cells, was evaluated to provide a suprathreshold index of the transfer of the contrast response. In agreement with Fig. 9, very small cone responses, in the 2–15% range, were sufficient to evoke a half-maximal response in many bipolar cells. However, much larger values were found in some cells, and in 6 of 16 Bh cells with highly asymmetric responses, the C → $B_{50}$ value for the depolarizing response was indeterminate because a 50% response was not achieved (e.g., see Fig. 9A). Because of this limitation, median values are used in Table 3 to summarize the results. These data show that in half of our bipolar cells, half-maximal responses in the hyperpolarizing direction were evoked by cone responses of ≤8% while responses in the depolarizing direction were evoked by cone responses of ≤17%. If it is assumed (as discussed earlier), that the typical cone response (Fig. 8) covers a total response range of 20 mV, then the median cone responses to evoke a half-maximal bipolar cell response would range from about –1 to +2 mV.

### Contrast responses of horizontal cells

Contrast/responses were measured for 18 horizontal cells, using large diameter fields (1.8 mm) to fully illuminate the receptive field. The form of the contrast/response plot of the majority of these cells was very similar to that found for cones. Thus the contrast/response was very shallow for small contrasts and quite positive-contrast dominant, so that very large positive contrasts (+1.5 to +2.0) were required to evoke the maximum response. These points are illustrated by the contrast/response plots for two typical horizontal cells shown in Fig. 10, A and B. On the other hand, a minority of horizontal cells were more nearly balanced in their response to negative versus positive contrast steps, as shown in Fig. 10C. However, such cells still showed relatively shallow slopes for small contrasts along with relatively high $C_{50}$ values and thus clearly differed from Bh cells (Fig. 4). No horizontal cells showed strong negative contrast dominance, and there was no indication for discrete subtypes of horizontal cells based on their contrast response or other response properties. Mean values for the complete horizontal cell sample were: relative contrast gain for negative and positive contrast = 1.0% and –1.1%/% contrast, respectively; $C_{50}p = +0.62$; $C_{50}h = < –2.0$; $P:N = 2.19$. These values are markedly different from those found for bipolar cells but are similar to those found for cones (see preceding text).

On average, horizontal cells showed slightly lower 630/530 sensitivity ratios (mean = +0.09 ± 0.03) than bipolar cells, suggesting that some horizontal cells may have received a weak input from rods or blue-sensitive cones. In support of the former possibility, a few intracellular recordings were made from rods. Although the rod response was attenuated markedly by our steady background illumination, small responses were still present and might have been capable of affecting some horizontal cells.

### Contrast/latency relations and response waveforms of bipolar cells and cones

Figure 11A summarizes measurements of the latency of the response of Bd and Bh cells to the onset of contrast steps of both polarities. The data support two main conclusions: (1) for stimuli of equal contrast, the latency of Bd cells is

| Table 3. Cone response for half-maximal bipolar response |
|-----------------|-----------------|
|                | Bd              | Bh              |
| C hyperpol → $B_{50}$ | –8 (–0.05, –16) | –8 (–0.04, –23) |
| C depol → $B_{50}$     | +12 (+0.08, +22) | +17 (+0.10, +53) |

Median amplitude (in %) of the cone response required to evoke a half-maximal response in depolarizing (Bd) and hyperpolarizing (Bh) bipolar cells for the case of depolarizing responses in cones (C depol → $B_{50}$) and hyperpolarizing responses in cones (C hyperpol → $B_{50}$). Numbers in parentheses give the interquartile range.
longer than that of Bh cells. This is shown in more detail in Fig. 11B. The mean latency difference was 21.6 ± 1.29 (SE) ms. There was no obvious dependence of the latency difference on contrast magnitude or polarity. Hence, these data indicate that there is a relatively constant latency difference of ~20 ms between the two classes of bipolars, with the Bd lagging the Bh bipolar cells. And 2) for both Bd and Bh cells, the latency to high positive contrasts tends to be shorter than that to high negative contrasts, and the minimum latency always is evoked by the highest positive contrast (+2.0). These results would be expected if the latency of response were primarily dependent on the absolute size of the light step. In Fig. 12, the circles show the mean latency of the Bh cells plotted against the logarithm of the absolute size of the light step, $\Delta L$. The data for positive and negative contrast (open and filled symbols, respectively) are reasonably well described by a single smooth curve, showing that the latency is largely dependent on the absolute size of the light step. Figure 12 shows that this generalization also seems to hold for Bd cells (squares). Although the data are more noisy, all differences between positive and negative contrast (i.e., open and filled squares) are within the variability of the measurements.

Cone latency measurements are shown by the triangles in Fig. 12. Although the cone latencies span a smaller time range and could not be reliably measured for very small light steps due to signal-to-noise limitations, Fig. 12 shows results that are similar in form to those of the hyperpolarizing bipolars. It also shows that the minimum latency of the cones is, as expected, shorter than that of Bh cells (~22 vs. 35 ms). Overall, the results of Fig. 12 are consistent with the suggestion that the form of the bipolar cell’s contrast/latency relation depends largely on the size of the light step, $\Delta L$, and that this relation is largely determined earlier in the cones and only altered by addition of a fixed delay. A similar dependence of contrast/latency has been found in cones of the walleye pike (Burkhardt and Gottesman 1987) and turtle (Burkhardt, unpublished data), as well as for horizontal cells (not shown) in the present work.

In addition to the differences in kinetics revealed by the latency measurements in Figs. 11 and 12, it was observed that the response waveform varied considerably across the bipolar cell population both within and between classes. Some of the variations consisted of differences in the prominence of peaks and overshoots, as suggested by the range of waveforms shown in Fig. 1. Rather striking differences also were observed in peak times of on and off responses evoked by steps of opposite contrast polarity.

**DISCUSSION**

**Contrast responses in bipolar cells and cones**

Our measurements on contrast gain for small signals argue against the simple hypothesis that, on average, the Bd and Bh cells might be strongly biased for the detection of positive and negative contrast, respectively. To the contrary, the average contrast gains are similar across cell types (Table 1), and there is considerable dispersion within and between the two classes (Fig. 5). A clear and striking feature of Fig. 5 is that many bipolar cells of both types show impressive contrast gain. Thus in the more sensitive cells, up to 15–20% of the bipolar response was evoked by a contrast step of 1%.

Measurements of $C_{50}$ show that many bipolar cells have
very good contrast sensitivity in the larger signal domain: In 35 of 44 cells, a half-maximal response for at least one contrast polarity was evoked by a contrast of <0.10, and many Bh cells showed particularly small C<sub>50</sub> values. However, both Bh and Bd cells showed considerable cell-to-cell variation in C<sub>50</sub>, including some cells with markedly asymmetrical responses to contrast polarity (Figs. 1–4 and 6). As result, one can often find a Bd cell that responds more strongly to negative than positive contrast or a Bh cell that responds more strongly to positive than negative contrast. Thus the optimal contrast polarity for driving bipolar cells may depend as much on the specific bipolar cell as on the generic class to which it belongs.

The range of C<sub>50</sub> values for bipolar cells is comparable with that recently reported for tiger salamander ganglion cells (0.02–1.0) measured under identical experimental conditions (Burkhardt et al. 1998). When C<sub>50</sub> measurements were used to classify cells for contrast dominance, 12% of Bh and 35% of Bd cells were classified as negative dominant. Using the same criteria based on C<sub>50</sub>, no cones were even close to qualifying as negative dominant (Fig. 8). On the other hand, >50% of the ganglion cell population was found to be negative dominant under the same conditions (Burkhardt et al. 1998). Taken together, these observations suggest that the response to negative contrast is amplified in a subset of bipolar cells and then amplified further in ganglion cells and/or amacrine cells.

The second index of large signal behavior, the P/N ratio, did not show significant differences between the means of the Bh and Bd classes but varied rather widely within and between both classes, further underscoring the heterogeneity of the bipolar population. The origin(s) of these and other differences between bipolar cells is largely unknown but because there seems to be little variability across the cones (Fig. 8), the differences presumably arise in the bipolar cells, as also suggested by recent results in the dark-adapted retina (Yang and Wu 1997). Differences in rod versus cone input, recently described in the dark-adapted retina (Hensel et al. 1993), apparently can be ruled out because all bipolar cells tested were cone-driven under our light-adapted conditions. Differences in the resting membrane potential impressed by the background illumination might contribute to differences in contrast dominance between bipolar cells, but we cannot resolve this issue from the data at hand. The high contrast gain of bipolar cells may be due, at least in part, to convergence from many cones over the receptive field center. However, convergence cannot account for the radically different shape and C<sub>50</sub> values of the contrast/response of bipolar cells and cones. Some of the differences in waveform and the contrast response might arise from cell-to-cell differences in the strength of several voltage-sensitive conductances previously found in bipolar cells (Kaneko and Tachibana 1985).

The contrast/response of cones was quite nonlinear over the full contrast range but showed approximate linearity for small-to-moderate contrasts of about ±0.30 (Fig. 8). The overall response of bipolar cells was even more nonlinear than that of the cones, and the linear range was very narrow, corresponding to contrasts of less than ±0.07. This result appears at odds with the generalization reached from white-noise analysis in the light-adapted catfish retina that the bipolar cell response is predominantly linear (Sakai and Nakajima 1987; Sakai et al. 1995). At least part of the apparent disagreement might be due to differences in species, the stimulus (steps vs. white noise), criteria for linearity, or level of light adaptation. For light steps in the dark, the responses of rod-driven bipolar cells in the isolated retina of the tiger salamander are nonlinear overall but show small-signal linearity (Capovilla et al. 1987), analogous to the present results.

The contrast/responses of horizontal cells were typically similar to cones. They showed relatively shallow slopes for small contrasts and were never strongly negative-contrast dominant. Thus even though the two cell types are immediately postsynaptic to the photoreceptors, both hyperpolarize to light and may employ similar synaptic receptors (Miller 1994), horizontal and Bh cells typically differ in their contrast/response, and usually can be distinguished on that basis alone.

**Contrast enhancement across the cone → bipolar synapse**

By quantitatively comparing the response of cones and bipolar cells, we have found clear evidence for contrast enhancement of small signals across the cone → bipolar synapse in the light-adapted retina, such that typically, ~10% of the bipolar response is evoked by a cone response of ~1% of maximum for either response polarity. These relative gain factors ranged from ~7 to 12 times (Table 2, top) whereas a range of ~5–9 times was estimated for voltage gain (Table 2, bottom). The latter estimates are probably conservative (see results). They are compatible in magnitude with past findings of voltage gains between 2.5 and 10 for small signals transmitted across the rod → bipolar synapse in the isolated retina of the tiger salamander (Capovilla et al. 1987; Wu 1994; Yang and Wu 1993). However, contrary to these past findings for rod-driven bipolar cells, we did not find a significant difference between the mean gains for Bh versus Bd cells for the cone-driven cells studied here. Moreover, although Bh cells showed a tendency for generating a somewhat larger total responses than Bd cells, the difference in the means (13.1 vs. 10 mV) was not statistically significant. As was the case for contrast gain (see preceding text and Table 1), the results in Table 2 show that cone-driven Bh cells tended to have slightly higher slope gains for positive versus negative contrast steps and thus for hyperpolarizing rather than for depolarizing input. Similar differences were not apparent in Bd cells (Table 2).

Plots of the bipolar versus the cone response generally show very steep slopes for only a modest contrast range followed by a relatively abrupt saturation (Fig. 9). Thus the overall signal transfer across the synapse is quite nonlinear. In many cells, the segment with steep slope continues into moderately high suprathreshold levels. As a result, in many bipolar cells, cone responses of ~10% of maximum evoked half-maximal bipolar responses (Fig. 9 and Table 3). There appear to be no striking differences between Bh and Bd cells with respect to median C → B<sub>50</sub> values.

**Contrast/latency relations for cones, bipolar, and ganglion cells**

The results of Fig. 12 suggest that the form of the bipolar cell’s contrast/latency relation depends largely on the size of
the light step, $\Delta L$, and that this relation is largely determined earlier, in the cones. Hence in horizontal cells and both classes of bipolar cells, the latency for high positive contrast is shorter than that for negative contrast. On the other hand, precisely the opposite result is found in most ON/OFF ganglion cells (Burkhardt et al. 1998). Thus the present results suggest that the contrast/latency relation in the ON/OFF pathway is transformed markedly beyond the bipolar cells. This transformation might be partially explained by the data in Fig. 11. They show that, for any given contrast, the average latency of Bh cells is shorter than that of Bd cells. Therefore, if it is assumed that the ON/OFF ganglion cell’s responses to negative and positive contrasts are driven primarily by Bh and Bd cells, respectively, the latency for negative contrast should be shorter than that for positive contrast for equal steps of opposite polarity, as is found (Burkhardt et al. 1998). However, additional factors also seem involved because the latency difference between the Bh and Bd cells is approximately 20 ms (Fig. 11B), whereas 40–80 ms differences typically are found for ON/OFF ganglion cells (Burkhardt et al. 1998).

Shorter latencies for Bh over Bd cells have been reported previously for light flashes in the dark in mudpuppy and turtle retinas (Frumkes and Miller 1978; Kim and Miller 1993; Marchiafava and Torre 1978; Nelson 1973). It was suggested that the longer Bd latency might be due to the involvement of a second messenger (Frumkes and Miller 1978), a possibility that now seems quite likely in view of recent evidence for cGMP as a second messenger in Bd cells (Nawy and Jahr 1991). The present report provides the first evidence that differences between the latency of Bh and Bd cells also are found in the light-adapted retina and that the difference holds for contrasts of both polarities (Fig. 11).

**Distributed encoding by bipolar cells**

Contrast gain, $C_{50}$, the $P:N$ ratio, $C \rightarrow B_{50}$, and response waveforms all varied considerably from cell to cell, thus raising the likelihood of distributed encoding at the level of the bipolar cells. Selective connections from bipolar to ganglion cells could thereby provide a basis for the very substantial range in contrast gain and negative/positive contrast dominance that recently has been documented within the ganglion cell population under identical experimental conditions as used in the present report (Burkhardt et al. 1998). Although Fig. 9 and a body of past work (Frumkes and Miller 1978; Marchiafava and Torre 1978; Sakai et al. 1995; Sakuranaga and Naka 1985; Thibos and Werblin 1978; Wu 1994) provides some evidence for the generalization that the intensity/response curves of retinal neurons may progressively steepen as the signal progresses from receptors to ganglion cells, our results in the contrast domain also highlight many exceptions to this generalization with respect to bipolar and ganglion cells. In particular, some bipolar cells have considerably steeper contrast/response curves and smaller $C_{50}$ values than that found in a goodly number of ganglion cells but overall the range of distributed encoding of contrast seems similar and extensive in both the bipolar (this report) and ganglion cell populations (Burkhardt et al. 1998).

Even in the latency domain, there would appear to be enough variance across the bipolar population (Fig. 11A) to allow selective bipolar-ganglion connections to explain recent findings that a small minority of ON/OFF ganglion cells show shorter latencies for positive than for negative contrast. Selective connections within the Bh and ganglion cell population also could explain the findings that OFF ganglion cells tend to have lower contrast gains and longer latencies to negative contrast than do ON/OFF cells (Burkhardt et al. 1998).

The postsynaptic consequences of distributed encoding in bipolar cells need to be investigated in more detail. In addition to possible selective connections and temporal differences in the input to amacrine and ganglion cells, differential scaling or nonlinear transmission at the output synapses of bipolar cells might amplify or attenuate some of the effects monitored at the cell body in the present report. It is also pertinent to recall that our results apply specifically to contrast steps applied at the receptive field center at a single level of background illumination (20 cd/m$^2$). Whether the contrast response of bipolar cells described in this paper will show substantial modification as a function of the level of light adaptation is an important question for future research, particularly because background-dependent changes in synaptic transfer from rods to bipolars (Wu and Yang 1992) and from cones to horizontal cells have been reported (Wu and Yang 1996). Although several important issues remain for future study, the present results clearly suggest that the bipolar cell population in the light-adapted retina is functionally diverse and has the potential for providing a rich substrate for distributed encoding of the visual image.

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**REFERENCES**


CONTRAST RESPONSES OF RETINAL BIPOLAR CELLS


