Contrast Rectification and Distributed Encoding By ON-OFF Amacrine Cells in the Retina

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Burkhardt, Dwight A. and Patrick K. Fahey. Contrast rectification and distributed encoding by ON-OFF amacrine cells in the retina. J. Neurophysiol. 261: 1676–1688, 1999. The encoding of luminance contrast by ON-OFF amacrine cells was investigated by intracellular recording in the retina of the tiger salamander (Ambystoma tigrinum). Contrast flashes of positive and negative polarity were applied at the center of the receptive field while the entire retina was light adapted to a background field of 20 cd/m\(^2\). Many amacrine cells showed remarkably high contrast gain: Up to 20–35% of the maximum response was evoked by a contrast step of only 1%. In the larger signal domain, C50, the contrast required to evoke a response 50% of the maximum, was often remarkably low: 24 of 25 cells had a C50 value of \(\leq 10\%\) for at least one contrast polarity. Across cells and contrast polarity, the dynamic ranges varied from extremely narrow to broad, thereby blanketing the range of reflectances associated with objects in natural environments. Although some cells resembled “contrast rectifiers,” by showing similar responses to contrasts of opposite polarity, many did not. Thus for contrast gain and C50, individual cells could show a strong preference for either negative or positive contrast. In the time domain, the preference was strong and unidirectional: for equal contrast steps, the latency of the response to negative contrast was 20–45 ms shorter than that for positive contrast. The present results, when compared with those for bipolar cells, suggest that, on average, amacrine cells add some amplification, particularly for negative contrast, to the high contrast gain already established by bipolar cells. In the time domain, our data reveal a striking transformation from bipolar to amacrine cells in favor of negative contrast. These and further observations have implications for the input and output of amacrine cell circuits. The present finding of substantial differences between cells reveals a potential substrate for distributed encoding of luminance contrast within the ON-OFF amacrine cell population.

INTRODUCTION

Contrast in luminance provides the primary dimension for the definition of most objects in natural environments. Some objects are brighter than their backgrounds (positive contrast), whereas others are darker (negative contrast), appearing as backlit objects, shadows, or the myriad of objects that reflect less light than their backgrounds. The retina transforms the resulting images into complex patterns of neural activity distributed in parallel across multiple cell types. Many of these cells have the capacity to preserve the sign of the contrast in the retinal image. These include the classical types known as the ON cells and the OFF cells (Dowling 1987; Miller 1994; Rodieck 1998; Schiller 1992; Werblin 1991). On the other hand, cells of the ON-OFF type seem indifferent to the contrast sign because they give similar responses to both decreases and increases of illumination. ON-OFF cells first were observed in recordings from the axons of retinal ganglion cells (Hartline 1938), and in pioneering intracellular recordings three decades later, Werblin and Dowling (1968) showed that mechanisms for the generation of ON-OFF responses first were elaborated earlier in the retina at the level of the ON-OFF amacrine cells. It now is recognized widely that the amacrine population of vertebrate retinas is diverse, encompassing a number of morphological and functional classes (Ammermuller and Kolb 1995; Bloomefield 1992; Kolb 1994; Kolb et al. 1992; Miller 1994; Morgan 1990; Rodieck 1998; Yang et al. 1991). Amacrine cells of the ON-OFF class, responding with transient, graded depolarizing potentials at both the onset and offset of a light flash, now have been found in a wide range of vertebrates (Ammermuller and Kolb 1995; Burkhardt 1975; Dowling 1987; Miller 1994; Morgan 1990; Sakuranaga and Naka 1985b; Stafford and Dacey 1995).

With respect to both retinal design and visual contrast, ON-OFF amacrine cells are particularly intriguing because they apparently act as agents for “contrast rectification” of the retinal image by encoding the contrast magnitude while discounting the contrast sign. However, little is known in detail about such contrast rectification or other aspects of the contrast response of ON-OFF amacrine cells. To date, most studies of ON-OFF amacrine cells have used light flashes in the dark or stimuli of unspecified contrast, whereas white-noise studies in the contrast domain (Naka et al. 1975; Sakai et al. 1995) do not make direct distinctions between the response to negative versus positive contrast.

Our goal was to investigate contrast encoding in quantitative detail, giving special attention to the limits of contrast rectification and how encoding might be distributed across the ON-OFF amacrine population. We took the simple approach of applying contrast steps of variable magnitude and opposite sign so we could compare responses to negative and positive contrast. The experiments were carried out in the retina of the tiger salamander (Ambystoma tigrinum). Because it offers several technical advantages, including relatively large cells that facilitate intracellular recording and other analytic procedures (Dong and Werblin 1998; Jacobs and Werblin 1998; Werblin 1991; Yang et al. 1991; Yu and Miller 1996), it is currently one of the most intensively studied preparations for the analysis of retinal function. In this report, we present quantitative data on contrast gain, half-maximal contrasts, contrast dominance, dynamic range, and contrast/latency relations for 25 ON-OFF amacrine cells along with some related intracellular recordings from other inner retinal neurons and ON-OFF ganglion cells.

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CONTRAST ENCODING BY AMACRINE CELLS

Our results show that the on-off amacrine cells are remarkably sensitive to negative and positive contrast steps but show substantial variation from cell to cell and a considerable degree of independence with respect to contrast polarity. The present results, when compared with recent measurements on bipolar cells obtained under identical conditions (Burkhardt and Fahey 1998), suggest that, on average, amacrine cells add some amplification, particularly for negative contrast, to the high-contrast sensitivity already established by the bipolar cells. In the time domain, we find a striking transformation from bipolar to amacrine cells in favor of negative contrast, such that amacrine cells respond with shorter latencies to negative than to positive contrasts of comparable magnitude. These and other results have implications for the nature of the input and output of amacrine cell circuits. The considerable diversity of amacrine cell types in vertebrate retinas (Dowling 1987; Miller 1994; Morgan 1990; Rodieck 1998; Yang et al. 1991) would seem to provide a clear basis for distributed encoding of multiple dimensions of the visual stimulus across the amacrine population as a whole. The substantial cell-to-cell variations in the contrast response reported here suggest that distributed encoding may be extended to on-off amacrine cells for a single stimulus dimension—achromatic contrast.

METHODS

Preparation and intracellular recording

Intracellular recordings were made from flat-mounted sections (∼2 × 4 mm) of the eyecup of the tiger salamander (A. tigrinum), as described in detail previously (Burkhardt and Fahey 1998). The retina was maintained at room temperature (20–23°C) and superfused at ∼1 ml/min with a Ringer solution composed of (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. 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 Kacetate 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 Kacetate, perhaps due to their higher resistance, seemed more likely to penetrate cells and thus generally were preferred despite their somewhat higher noise level.

Light-evoked responses were recorded permanently on video tape and later digitized (0–5,000 Hz bandwidth) for analysis with the aid of commercial software (Superscope, GWI Instruments). For the responses displayed in this paper, low-pass filtering (3 dB down at ≤250 Hz) was used to achieve the optimal reduction of noise without producing detectable distortion of response amplitude and waveform. Low-pass filtering also was used for all quantitative measurements of response amplitude in this report. Response amplitude was measured from the baseline to the peak of the response. All latency measurements were made on unfiltered records. Latency was taken as the time at which the rising phase of the response first deviated from the baseline. To make 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.

Light stimulation and contrast metrics

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 120 × 120 pixels. Each pixel illuminated a 17 × 17 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 United Detector Technology Model 350 photodiode photometer. Contrast steps ranging from −0.02 to ±2.0 log units were generated with the LCD system on a steady background illumination of 20 cd/m², a light level ∼4–5 log units above ganglion cell threshold measured in the dark-adapted retina. Thus a moderately high level of light adaptation was achieved. Further details of the LCD stimulator are given elsewhere (Burkhardt et al. 1998).

Contrast may be quantified in several ways: as the scaled difference between spot and background (Shapley and Enroth-Cugell 1984), as the Michelson contrast (e.g., Burkhardt et al. 1984; Westheimer et al. 1999), or as the contrast ratio. Each specification has merits and limitations. We use each when appropriate but place primary emphasis on the contrast ratio because it avoids problems of scale compression, allows direct comparison with our recent work on bipolar cells (Burkhardt and Fahey 1998), and there is much evidence that contrast ratios are fundamental for suprathreshold vision (Goldstein 1998). Hence in this report, contrast usually is specified as the logarithm of the contrast ratio: contrast = log₁₀(F/B), where B is the steady background intensity and F is the light intensity prevailing during the flash. In this metric, contrast of steps that increase or decrease the prevailing light by the same factor differ only in sign: e.g., 2× increases or 2× decreases from the background are specified as contrasts of +0.30 and −0.30, respectively; 10× increases or decreases, amount to contrasts of +1.0 and −1.0, and so forth. In describing our results, we will usually use the logarithmic specification of contrast and refer to this as contrast without further qualification. Michelson contrast is a well-known metric for sinusoidal stimuli that also can be used for contrast steps (e.g., Burkhardt and Gottesman 1987; Burkhardt et al. 1984; Westheimer et al. 1999). In the terms used here, it is defined: Michelson contrast = (F − B)/(F + B), where F and B are as defined earlier in the paragraph. For contrast steps, log contrast and Michelson contrast differ insignificantly over the range of ±0.70, but Michelson contrast compresses all higher contrasts to the narrow range of 0.7–1.0. Much past work on contrast gain of retinal and cortical neurons has been reported in units of percentage Michelson contrast. Hence, when reporting measurements of contrast gain, we use percentage Michelson contrast and will refer to this simply as “% contrast.” For low to moderate contrasts (<0.70), percentage Michelson contrast is numerically equivalent to log contrast × 100.

Protocol

After a cell was penetrated, the following protocol typically was 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 optimum diameter and an annulus (typically 600 µm ID and 2000 µm OD) were flashed at several contrast levels to screen for center/surround antagonism (see following text). 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 amacrine and ganglion cells were relatively noisy (see following text), the series was repeated, at least once when possible, and average responses were computed. The retina was always light-adapted to a steady back-
ground field 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. 3) 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 of 20 cd/m². The resulting measurements were used to determine the cell’s 630/530 nm sensitivity ratio and thereby classify the cell for spectral type.

Identification of intracellular recordings

The origin of intracellular recordings was determined from functional criteria based on past work in amphibian retinas (Burkhardt and Fahey 1998; Frumkes et al. 1981; Hare and Owen 1990; Hare et al. 1986; Vallerga 1981; Werblin 1977; Werblin and Dowling 1968). Cells penetrated within 0–30 μm of the retinal surface that exhibited graded depolarizing responses to contrast steps with superimposed bursts of ~ 5–20 impulses, were identified as ganglion cells. The present analysis is restricted to ON-OFF ganglion cells because very few ON cells were detected, and recordings from OFF cells, although more common, were rarely sufficiently stable to permit quantitative studies. Recordings assigned to ON-OFF amacrine cells were identified according to the following functional criteria: 1) recordings were obtained after the electrode was advanced through an inactive region some 30–40 μm distal to the level of the ganglion cells. 2) The response waveform consisted of a transient, graded depolarization at the onset and at offset of light flashes. Only graded responses were seen in most recordings but a few cells showed a brief, stereotypical burst of one to five impulses, were identified as bipolar cells. 3) Flashed annuli (600 μm OD/2000 μm OD), unlike the case for bipolar cells (Burkhardt and Fahey 1998; Hare et al. 1986), never inverted the response polarity. 4) Cells typically gave their largest response to stimuli of 250–500 μm. Larger spots attenuated the response but never reversed the polarity of the response.

On the criteria outlined in the preceding paragraph, ~60% of recordings obtained in the inner region of the inner plexiform were classified as depolarizing ON-OFF amacrine cells. Of the remaining 40%, some clearly resembled responses of ON-OFF hyperpolarizing or sustained, depolarizing amacrine cell types, as will be discussed in more detail in RESULTS. Other recordings in the inner retina were not readily classified as a simple or known functional type and will not be considered further. When the electrode was advanced more distally in the inner nuclear layer, recordings were observed that clearly satisfied the functional criteria for bipolar cells (Burkhardt and Fahey 1998; Hare et al. 1986). Resting membrane potentials of ON-OFF amacrine cells were in the 25- to 40-mV range but were not studied in detail. All statistical probabilities cited in the text are based on Student’s t-test unless noted otherwise.

RESULTS

ON-OFF amacrine cells

RECEPTIVE FIELD ORGANIZATION: A SUPPRESSIVE SURROUND. The receptive field of the majority (84%) of ON-OFF amacrine cells showed evidence for surround antagonism. When the diameter of a centered flash of fixed contrast was varied in steps from 100 to 2,000 μm, the response typically increased to reach a maximum response at some optimal diameter and then decreased for larger fields. As a rule, the optimal diameter was either 240 or 500 μm, and the response to a 2,000-μm field was attenuated by ~35 ± 5% (mean ± SE, n = 25) relative to the response evoked by the optimum diameter. The response to large fields as well as to annuli were attenuated but never reversed in polarity, the surround mechanism, as in turtle ON-OFF amacrine cells (Marchiafava and Torre 1978), is suppressive and not overtly opponent in nature. By contrast, the surround mechanism of bipolar cells is polarity reversing, i.e., overtly opponent (see METHODS).

RESPONSES TO CONTRAST STEPS IN THE CENTRAL RECEPTIVE FIELD. To analyze the response of the central receptive field mechanism, centered spots of optimal diameter were used in all the following experiments. The retina was light adapted to a steady background field of 20 cd/m² that covered the entire retina. Preliminary observations indicate that this background is sufficiently intense to bring ON-OFF amacrine cells close to their maximum achievable contrast sensitivity. At this level of light adaptation, it has been shown previously that input from rods is negligible and the 610 nm cones provide the overwhelming input to bipolar cells (Burkhardt and Fahey 1998), so it would be expected that the responses of ON-OFF amacrine cells also would be cone-driven. This was confirmed by measurements of the 630/530 sensitivity ratio in a subset of cells (see METHODS). They yielded a mean value of +0.23 ± 0.03 log units (n = 11), in very close agreement with the value of 0.22 expected for exclusive input from 610-nm cones (Burkhardt and Fahey 1998).

Figure 1 shows responses to contrast steps of opposite polarity and variable magnitude evoked by a 240-μm spot in the center of the receptive field. Each row shows response pairs evoked by contrasts of equal absolute magnitude and opposite polarity. The smallest contrast steps (~0.03) generate remarkably large responses, and thus the contrast sensitivity is very high for both contrast polarities. For all paired contrasts, this cell generates somewhat larger responses to the onset of negative contrast. The response evoked by +0.15 contrast (left) is shown (right) as a superimposed with the response to −0.15 contrast (—). Although the time scale is compressed, it is still apparent that the latency of response to the onset of negative contrast is shorter than that for positive contrast. As will be shown in quantitative detail later, this finding was very robust, holding clearly across contrast levels and cells. On the other hand, at the offset of the contrast step, the reverse was found, as may be seen in the superimposed records in Fig. 1. Here, the onset and peak of the depolarization is delayed for negative relative to positive contrast. This delay may be due to the opposing influence of an initial, hyperpolarizing deflection that was only seen for the offset of negative contrast. This small hyperpolarizing deflection (†) is evident in all the responses to negative contrast in Fig. 1.

In addition to the small hyperpolarizing deflection, Fig. 1 shows a clear difference in waveform due to contrast polarity. After the transient peak, the responses to negative contrast show much less decay to baseline and thus appear to contain a relatively large sustained component. Such waveform differences were not uncommon, amounting to about one-third of the cells in our sample, although in about half of these, the sustained component was more evident for the response to positive rather than negative contrast. Thus for this subset of cells, it is clear that the waveforms of responses to negative and positive contrast are not equivalent and thus clearly fail to
satisfy a stringent criterion for "contrast rectification." The other two-thirds of the cells in our sample showed relatively similar waveforms to both negative and positive contrast, but there was some variation between cells in the time constant of decay after the initial peak response.

CONTRAST/RESPONSE FUNCTIONS: SYMMETRY AND CONTRAST DOMINANCE. Quantitative contrast/response curves were analyzed by plotting peak response to contrast onset versus contrast magnitude. The maximum response varied from cell to cell [mean = 11.6 ± 6.6 (SD) mV]. The factors responsible for the variation are unknown, although differences in electrode seal are one likely possibility. However, a regression analysis failed to reveal any significant correlations between the magnitude of the maximum response and other aspects of the contrast response, so the present report concentrates exclusively on the analysis of normalized contrast/response measurements. The features of the response that will be analyzed are illustrated in the hypothetical contrast/response curve of Fig. 2. These features are defined in the legend and will be discussed in detail later.

It was found that maximum responses varied considerably from cell to cell as a function of the contrast polarity. This is shown in Fig. 3 where normalized contrast/response curves for eight cells have been ordered from top left to bottom right with respect to the relative amplitude of the response maxima evoked by positive contrast. For the cell in A, positive contrast gives about twice as large a response as does negative contrast, whereas negative contrast is about twice as potent as positive contrast for the cell in H. Other cells fall between these extremes, with the cells in D and E showing nearly symmetrical or balanced maximum responses to contrast polarity. Taken together, the results of Fig. 3 show that for response maxima, the contrast dominance can vary from cell to cell by as much as 2 to 1 in either direction. The distribution was relatively symmetrical when evaluated by the ratio, $P_{\text{max}} / (P_{\text{max}} + N_{\text{max}})$, where $P_{\text{max}}$ and $N_{\text{max}}$ are the maximum amplitudes evoked by positive and negative contrast, respectively. The mean ratio was 0.46 ± 0.09 (mean ± SD, n = 25), close to the value of 0.50 required for a symmetrical distribution.

To emphasize differences due to contrast polarity, most of our analysis focuses on measurements of responses normalized with respect to the maximum response evoked by the most effective contrast polarity. However, as may be seen in Fig. 3, the opposite limbs of the contrast/response curves were often fairly similar in shape. Thus the response curve for the less effective contrast polarity approximated a scaled version of the curve for the more effective contrast polarity.

CONTRAST GAIN. The plots in Fig. 3 are compressed to show the entire contrast/response curve, but it is still obvious that most cells are exceedingly sensitive to small contrast steps. This characteristic was analyzed quantitatively by calculating the contrast gain: for each contrast polarity, the lowest point of

![Fig. 1. Responses of an ON-OFF amacrine cell to contrast steps of positive and negative polarity as a function of the contrast magnitude (numbers at left). Stimulus diameter is 240 μm. - - -, copy of the trace evoked by +0.15 contrast shown in the left column. ↑, initial hyperpolarization seen at the offset of negative but not positive steps (see text).](image-url)
The contrast/response curve was used to estimate the gain in units of percentage normalized amplitude/percentage contrast. Two values were obtained: \( C_{\text{Gp}} \) and \( C_{\text{Gn}} \), the contrast gain for positive and negative contrast steps, respectively, calculated from the initial slope of the contrast/response curve. \( C_{\text{Gp}} \) and \( C_{\text{Gn}} \): contrast gain for positive and negative contrast steps, respectively. \( C_{50P} \) and \( C_{50N} \): the positive and negative contrasts, respectively, required to evoke a response of 50% of the maximum response. Dynamic range: 10–90% of the maximum response.

The measurements for each cell in our sample are plotted in Fig. 4. The + near the origin shows the contrast gain, of \( \sim 0.9\% \), previously measured for cones (Burkhardt and Fahey 1998). Thus 84% of the amacrine cells in Fig. 4 have contrast gains that are 10–30 times higher than that of cones. Some of the data points fall very near the 45° locus, providing clear examples of cells with nearly equivalent (balanced) negative and positive contrast gains, whereas other cells have very different contrast gains for negative versus positive steps. Although the present measurements are not adequate for a rigorous analysis of the issue of linearity, it seems quite likely that the balanced cells in Fig. 4 may generate quasi-linear responses for small contrasts (\( \# 0.02 \)), whereas those cells showing significant departures from contrast equivalence, of which there are many in Fig. 4, must already be responding in a nonlinear fashion. Overall, Fig. 4 suggests a fair degree of independence for negative versus positive contrast because a regression analysis showed that \( C_{\text{Gp}} \) could only account for \( \sim 43\% \) of the variance of \( C_{\text{Gn}} \).
C50. For each cell, the positive and negative contrasts required to evoke a half-maximal response, C50p and C50n, were determined by interpolation from the contrast/response curve. The results, plotted in logarithmic coordinates in Fig. 5, show cases of clear symmetry as well as cases of marked disparity between negative and positive contrast. Many of the values are remarkably low. Thus 12 of 25 cells have values of ≤0.10 for both contrast polarities and 24 of the 25 cells have a C50 value of ≤0.10 for at least one polarity. The means for C50n and C50p, were 0.12 and 0.22, respectively. However, the standard deviations were large and the difference in the means was not significant (P = 0.37).

The three measures of the contrast response analyzed in the preceding text, i.e., \( P_{\text{max}}/(P_{\text{max}} + N_{\text{max}}) \), contrast gain, and C50, reflect somewhat different facets of contrast processing because they were found to be only weakly correlated when evaluated by regression analysis. In the best case, it was found that C50 could account for a modest 45% of the variance of \( P_{\text{max}}/(P_{\text{max}} + N_{\text{max}}) \). Thus some but by no means all of the asymmetry in Fig. 5 is a reflection of differences in the respective response maxima for positive and negative contrast. Hence, the data would fall closer to the 45° locus if C50n and C50p were scaled separately to the respective negative and positive response maxima. Contrast gain accounted for only 12% of the variance of \( P_{\text{max}}/(P_{\text{max}} + N_{\text{max}}) \), suggesting substantial differences in the generation of small and large signals. These differences also interact with contrast polarity because Fig. 4 shows that for small signals, a majority of cells had highest gain for negative contrast, whereas for the largest signals, the distribution for \( P_{\text{max}}/(P_{\text{max}} + N_{\text{max}}) \) was relatively symmetrical, as noted earlier.

**Dynamic Range.** The dynamic range of the contrast/response curve was assessed for both contrast polarities by measuring C10 and C90, the contrasts required to evoke responses of 10 and 90% of the cell’s maximum response, respectively (see Fig. 2). Figure 6 shows C10–C90 measurements (—) for all cells in our sample. For cells with highly asymmetric contrast/response curves (e.g., Fig. 3, A and H), a C90 value could not be determined for the less effective contrast polarity. These instances are shown by Δ. The cells in Fig. 6 are sorted in ascending order with respect to the magnitude of C90n. Several main points are evident: a number of cells show remarkably narrow ranges, particularly for negative contrast; other cells show larger dynamic ranges, particularly for positive contrast, although C90p is indeterminate for nearly half of the cells; if C90 is recalculated relative to the maximum response of the less effective contrast polarity, then these recalculated C90 values are typically small, as shown by ● in Fig. 6. This is expected from the finding that, in many cells, the slower limb of the contrast response curve rises quite rapidly (see Fig. 3). Also evident is that the ordering of the dynamic range for positive contrast is erratic with respect to that for negative contrast, suggesting that dynamic range varies somewhat independently with the contrast polarity in any given cell, i.e., cells that have small dynamic ranges for negative contrast do not necessarily have small dynamic ranges for positive contrast.

**Effect of Contrast Polarity on Response Latency.** In discussing Fig. 1, it was noted that the response latency to contrast onset was shorter for negative than for positive contrast steps. Quantitative evidence for this generalization is summarized in Fig. 7. For the cell of Fig. 7A, it can be seen that the minimum (asymptotic) latency is ~70 ms for negative contrast and 65 ms for positive contrast. Over all 25 cells, the minimum latency averaged 68 ± 3.6 and 79.3 ± 4.3 (SE) ms for negative and positive contrast, respectively, a highly significant difference (P = <0.001). Figure 7B shows results for another cell with the measurements now plotted against the absolute value of the contrast. This illustrates the general finding that the difference in latency holds for all comparisons between responses to steps of equal absolute contrasts. This effect was quantified by measuring the latency difference, \( L_p - L_n \), for all negative/positive contrast pairs, where \( L_p \) and \( L_n \) are the latencies evoked by the onset of positive and negative contrasts.
contrast steps of equal absolute magnitude. Thus a positive value indicates that the latency to negative contrast is shorter than that to positive contrast. Figure 7C summarizes data for all cells in our sample. The difference decreases in quasieponential manner from ~45 ms at very low contrast to ~20 ms at maximum contrast. The differences found in Fig. 7, A–C, are impressive when it is realized that the absolute magnitude of the light step (ΔL) is considerably smaller for negative than for positive contrast steps. To highlight this point, all the data for the cell of Fig. 7B are replotted in terms of ΔL in Fig. 7D. This clearly illustrates that for light steps of equal absolute magnitude (ΔL), the latency of the on-off amacrine cell is invariably shorter for the negative step.

Other inner retinal neurons

In addition to the preceding recordings from depolarizing ON-OFF amacrine cells, several other response types were seen in the inner retina (see METHODS). As displayed in Fig. 8, one type showed hyperpolarizing transients at stimulus onset and offset regardless of the spatial configuration or contrast polarity and thus appeared similar to ON-OFF hyperpolarizing amacrine cells described previously by others (Ammermuller and Kolb 1995; Werblin 1970). Such recordings were typically very noisy, but eight were studied quantitatively. Their contrast/response curves were within the range of variation described in the preceding text for depolarizing ON-OFF amacrine cells and included several cells with exceedingly steep curves. Mean contrast gain for the eight cells were: $C_{Gp} = 17.3 \pm 2.9\%$, $C_{Gn} = 20.0 \pm 6\%$.

![Figure 7](image-url)  
**FIG. 7.** Contrast/latency relations for ON-OFF amacrine cells. A: latency vs. contrast for 1 cell. ○ and ●, latencies to positive and negative contrast, respectively. B: latency vs. contrast for another cell with latency plotted against the absolute value of the contrast. Convention for symbols as in A. C: average latency difference between positive and negative contrast for 25 ON-OFF amacrine cells. –, SE. D: data shown in B replotted with the stimulus scaled as the logarithm of the absolute value of the magnitude of the light step, $\Delta L$ (see text).

![Figure 8](image-url)  
**FIG. 8.** Responses of a hyperpolarizing cell in the inner retina to contrast steps of ±0.30. - - - in the response to the +0.30 step corresponds to the latency of the response to the −0.30 contrast step. Inset: latency/contrast plot for another hyperpolarizing cell. ○ and ●, positive and negative contrast, respectively.

3.1. Like their depolarizing counterparts, they consistently showed shorter latencies to negative than to positive contrast steps. Despite the compressed time scale, this effect can be seen in Fig. 8. The - - - in the +0.30 trace corresponds to the latency of the onset of the −0.30 trace and falls ~20 ms before the onset of the response to the +0.30 contrast step. Figure 8, inset, shows a full set of contrast/latency measurements. Over all contrasts and cells in our sample, the mean difference in latency for positive and negative contrast was 22 ms, a robust difference ($P < 0.002$).

Several recordings were obtained in the inner retina that resembled those described for sustained, depolarizing amacrine cells (Ammermuller and Kolb 1995; Chan and Naka 1976; Dixon and Copenhagen 1992; Frumkes et al. 1981; Maguire et al. 1989; Vallerga 1981). In response to positive steps, these cells showed a peak depolarization followed by a sustained phase. An example is shown in Fig. 9. Usually, oscillations of ~5–20 Hz were superimposed on the depolarization, as found for sustained amacrine cells (type Na) in catfish retina (Sakai and Naka 1992; Sakuranaga and Naka 1985a). Negative contrast steps evoked an ON-OFF response. Seven of these cells were studied quantitatively. Without exception, they were strongly positive-contrast dominant, showing steep contrast/response curves for positive contrast and considerably weaker responses for negative contrast. An example is shown by Fig. 9, inset. Mean results for the seven cells sampled were: $C_{Gp} = 19 \pm 5.3\%$; $C_{Gn} = 3 \pm 1.5\%$; $C_{50p} = 0.06 \pm 0.02$; $C_{50n} = -1.64 \pm 0.28$. When responses to negative contrast could be adequately measured, they usually showed shorter latencies than those for positive contrasts for most contrasts of ≤1.0 (mean difference = 15.6 ms, $P < 0.01$, $n = 27$), whereas in some cells, the difference was reduced or reversed at very high contrasts.
ON-OFF ganglion cells

Figure 10 shows intracellular responses of an ON-OFF ganglion cell to positive and negative contrast steps (light and dark traces, respectively). At both contrast onset and offset, the response consists of a burst of nerve impulses riding on a graded, excitatory postsynaptic potential (EPSP). For both EPSP and spikes, the cell generates vigorous responses to very small contrast steps and shows clear latency differences in favor of negative contrast at onset and positive contrast at offset, respectively, as previously found for amacrine cells (Figs. 1 and 7). These properties were characteristic of the 15 ON-OFF ganglion cells studied. As shown in Fig. 10, the latency of the first spike of the impulse discharge was triggered on the rising phase of the EPSP. Thus depending on the cell and contrast magnitude, the first spike occurred; 5–15 ms after the onset of the EPSP and showed the same dependence on contrast as the EPSP, i.e., for ON responses, the first spike latency was typically shorter for negative than for positive contrast of comparable magnitude, whereas the reverse was typically found at contrast offset. As with amacrine cells, a small hyperpolarizing deflection (Fig. 10, 3) is apparent at the offset of negative contrast and may be responsible for the increased latency of the subsequent EPSP and spike discharge.

Although the waveform of the EPSPs of Fig. 10 differ from those of Fig. 1, on the whole, no consistent differences were apparent in the range of the EPSP waveforms observed across our amacrine and ganglion cell samples. The EPSPs of ganglion cells in our sample remained stable for some 15–45 min after impalement, whereas the spike discharge often deteriorated or was lost with time. Hence, quantitative measurements of the contrast response were confined to the EPSP.

Figure 11, A–C, shows representative contrast/response curves for measurements of normalized EPSP amplitude [maximum amplitude varied from cell to cell: 10.9 ± 2.6 (SE) mV]. Cells showed some clear differences in contrast dominance across the population sampled. A few showed considerably larger responses to positive contrast (A), some were relatively symmetrical or balanced (B), whereas others showed maximum responses to negative contrast (C). The mean contrast gains for the EPSPs were high: \( G_P = 15.5 ± 2.0\% \) and \( G_N = 20.9 ± 2.7\% \), and the difference in the gains was statistically significant \( (P < 0.01) \). Figure 11D shows a representative contrast/latency plot for the EPSP evoked by contrast onset. Measurements of latency differences \( (L_p - L_n, \text{as defined earlier in the text for amacrine cells}) \) over all contrast levels for a total of 10 cells showed, on average, that the negative contrast latency was shorter by 25.1 ± 1.2 (SE) ms than that for the equivalent positive contrast. This difference
was highly significant \((P = 0.001)\), providing strong support for the conclusion that for contrast steps of comparable magnitude, the response to negative contrast arises earlier than that to positive contrast. The minimum asymptotic latencies of the EPSPs to contrast onset were: \(L_{\text{min}} = C_{0.02} = 76.3 \pm 5.0 \text{ ms}, C_{0.05} = 58.0 \pm 4.3 \text{ ms}, \) a highly significant difference \((P < 0.001)\). However, 1 cell of the 15 in our sample showed the reverse result, i.e., positive contrast latency shorter than negative contrast latency. This result also was found previously for a very small minority of ON-OFF cells in extracellular recordings (Burkhardt et al. 1998).

DISCUSSION

Although the light-evoked responses of ON-OFF amacrine cells have been described in various species since the first report of Werblin and Dowling (1968), this report supplies new information by providing a quantitative analysis of contrast processing in the light-adapted retina. Most of the cells in our sample showed evidence for a strong suppressive surround, so it useful to emphasize that all the following discussion applies to stimuli designed to optimally stimulate the center of the receptive field.

**Dynamic range and the reflectance of objects in natural environments**

In natural environments, virtually all objects are seen by reflected light. Reflectance (percent of light reflected) typically covers a range from \(\sim 5\%\) for black objects to \(\sim 85\%\) for white objects, with midgray at \(\sim 22\%\) (Goldstein 1998). If it is assumed that the background corresponds to the midgray reflectance, then white and black objects (85 and 5% reflectances), respectively, will correspond to contrasts of about \(\pm 0.60\). These limits are shown in Fig. 6 (---). The distribution of dynamic ranges in Fig. 6 is thus sufficient to encode this range but also includes some cells with exceedingly small ranges, particularly for negative contrast. The latter cells are well designed to detect very dim gray objects but are ill-equipped to represent the full range of contrasts in natural environments. Recent measurements (Vu et al. 1997) suggest that the contrast of most objects in natural scenes falls within a range of about \(\pm 0.25\) log contrast, thus covering about half of the \(\pm 0.60\) range shown in Fig. 6. With respect to the extreme case, i.e., a cell adapted to an object of either extreme reflectance (i.e., 85 or 5%) and subsequently exposed to the other, the resulting contrast step would be 1.2 log units, which is within the dynamic range of a subset of cells in Fig. 6.

**Contrast sensitivity**

The contrast sensitivity of many ON-OFF amacrine cells was remarkably high. Contrast gain, an index of the small signal response, often ranged as high as 20–35%. Thus 20–35% of the cells’ total response could be evoked by a contrast of only 1%. Furthermore a contrast of \(< 1\%\) was sufficient evoke a response of \(\geq 10\%\) of the maximum \((C_{10})\) in more than half of the cells in our sample. The rather remarkable sensitivity of these cells can be appreciated from the observation that a simple contrast step of \(\sim 1\%\) is just detectable (i.e., the threshold contrast) when applied to the fovea of the human retina under favorable conditions (Burkhardt et al. 1987; Westheimer et al. 1999). The mean contrast gains, \(\sim 13\) and \(17\%\) of the maximum response, for \(C_{10} \text{ and } C_{100}\) respectively, correspond to signals in the 1.6- to 2.0-mV range, signals that would be large enough to often be detectable even in the face of the physiological and electrode noise in our recordings. \[The corresponding signals in cones (Burkhardt and Fahey 1998) are in the range of 0.13 and 0.07 mV for positive and negative steps, respectively.\] Because the slope of the contrast/response curve typically remained quite steep for relatively large responses (Fig. 3), \(C_{50}\) the contrast required to evoke a response 50% of the maximum, was remarkably low in many cells. Thus 24 of 25 cells had a \(C_{50}\) value of \(\leq 0.10\) (i.e., \(\leq 10\%\) contrast) for one contrast polarity and for 12 of 25 cells, \(C_{50}\) was \(\leq 0.10\) for both contrast polarities.

The observed differences between cells in contrast sensitivity and dynamic range seemed unrelated to differences in maximum response, latencies, resting membrane potential, age of the preparation, or other factors that might be indicative of compromised cells or abnormal retinal function. Thus it seems likely that the observed differences in the contrast response are largely due to normal physiological differences within the amacrine cell population.

**Contrast rectification and the effects of contrast polarity**

The degree to which negative and positive contrast generate similar responses depends on the cell and response measure in question. For very small signals, it might be expected that the response would approximate linearity and thus the contrast gain would be independent of contrast polarity. Although some cells in Fig. 4 do show similar contrast gains, many clearly do not and of the latter, more show higher gains for negative contrast. Over all cells, a regression analysis showed that only 43% of the variance in contrast gain was dependent on contrast polarity. For the half-maximal contrast \((C_{50})\), some cells fall near the 45° locus for equivalence in Fig. 5 but more than half do not. These departures are bidirectional and so large (note that the scale in Fig. 6 is logarithmic) that, statistically, they swamp out the symmetrical cases. Consequently, the overall correlation between \(C_{50n}\) and \(C_{50p}\) is exceedingly low \((r = 0.02)\). The results for contrast gain and \(C_{50}\) indicate that the departure from contrast equivalence increases as one goes from the small to the larger signal domain. This generalization also applies for the dynamic range results of Fig. 6. Thus the \(C_{10p}\) and \(C_{10n}\) values are relatively similar, whereas the \(C_{90}\) values show some striking differences as a function of contrast polarity. For contrast dominance based on response maxima, many cells were relatively balanced, but across the population ratios as large as 2 to 1 in both directions were found (Fig. 3). We also have described clear cases (see Fig. 1 and text) in which the response waveform is strongly dependent on the contrast polarity. In sum, our findings show that some ON-OFF amacrine cells respond in a relatively similar fashion independent of the contrast sign, but many show variable degrees of bias in favor of positive or negative contrast. Thus “contrast rectification” rarely holds exactly and the consequent variation in contrast dominance provides a distributed parameter across the ON-OFF amacrine population.
Relations between the contrast response of amacrine and bipolar cells

Although the details are not established, it is widely believed that the response of the ON-OFF amacrine cells is shaped by interaction between synaptic inputs from the hyperpolarizing (Bh) and depolarizing (Bd) bipolar cells. Our results highlight some new aspects of this issue because the contrast responses of bipolar cells recently have been studied under identical conditions (Burkhardt and Fahey 1998). Figure 12 summarizes some of the measurements made here for amacrine cells (Am) along with a new analysis of our previous work on hyperpolarizing (row Bh) and depolarizing (row Bd) bipolar cells. In addition, mean results for cones (Burkhardt and Fahey 1998) are shown by - - - in the Bh panels. Data for bipolar cells and cones are new plots of results reported in Burkhardt and Fahey (1998). A and B: contrast gain and C50, the contrast required for a half-maximal response. Data are plotted in octave steps for positive contrast (□) and negative contrast (■). - - - at ±0.60 correspond to the range of contrasts associated with objects of 5% and 85% reflectance (see text). C: histograms of \( \frac{P_{\text{max}}}{P_{\text{max}} + N_{\text{max}}} \), where \( P_{\text{max}} \) and \( N_{\text{max}} \) are the maximum responses evoked by positive and negative contrast, respectively. D: histograms of the difference in latency of responses to positive and negative contrast. Sample sizes: Bh = 89; Bd = 92; Am = 192. Positive values refer to the case where the latency to negative contrast is shorter than the latency to positive contrast (see text).

**FIG. 12.** Summary of contrast/response measurements for 25 amacrine cells (Am), 20 depolarizing bipolar cells (Bd), 24 hyperpolarizing (Bh) bipolar cells, and 8 cones. Mean results for cones are shown by - - - in the Bh panels. Data for bipolar cells and cones are new plots of results reported in Burkhardt and Fahey (1998). A and B: contrast gain and C50, the contrast required for a half-maximal response. Data are plotted in octave steps for positive contrast (□) and negative contrast (■). - - - at ±0.60 correspond to the range of contrasts associated with objects of 5% and 85% reflectance (see text). C: histograms of \( \frac{P_{\text{max}}}{P_{\text{max}} + N_{\text{max}}} \), where \( P_{\text{max}} \) and \( N_{\text{max}} \) are the maximum responses evoked by positive and negative contrast, respectively. D: histograms of the difference in latency of responses to positive and negative contrast. Sample sizes: Bh = 89; Bd = 92; Am = 192. Positive values refer to the case where the latency to negative contrast is shorter than the latency to positive contrast (see text).
Amacrine cells show much lower C50 values than do cones (Fig. 12B), indicating a substantial transformation in suprathreshold contrast processing from cones to amacrine cells. Moreover, Fig. 12B suggests that the C50 distributions for amacrine and bipolar cells differ. This question was evaluated with a nonparametric test (Wilcoxon signed rank) because C50 was of indeterminate value (i.e., $>2.0$) for a good number of Bh and Bd cells, thus precluding parametric statistical tests. The signed-rank test showed that Bh $n$ and Am $n$ differed greatly ($P < 0.001$). When all measurements were combined across both bipolar cells types and contrast polarities, it also was found that the composite bipolar distribution differed from the composite amacrine population ($P < 0.001$). The origins of these differences seem evident on inspection of Fig. 12B because the amacrine cells show a relatively greater proportion of very low C50 values and fewer cases of high, indeterminate C50 values. Thus the C50 results suggest that, on average, the suprathreshold contrast response of amacrine cells is somewhat enhanced relative to that of bipolar cells. Possible mechanisms for this enhancement might involve contributions of voltage-sensitive conductances of amacrine cells (Barnes and Werblin 1986; Cook and Werblin 1994; Werblin 1977) and/or signal shaping by the amacrine-bipolar feedback synapse (Lukasiewicz and Werblin 1994; Yu and Miller 1996; Zhang et al. 1997).

A comparison of the contrast dominance distributions for response maxima (Fig. 12C), show that both cones and Bh cells are positive contrast dominant. Thus the means for cones (0.65) and Bh cells (0.60) were both much higher ($P < 0.01$) than would be expected for a perfectly balanced distribution with a mean of 0.50. The amacrine cells were, on average, slightly negative dominant (mean = 0.46) and clearly differed from cones and Bh cells ($P < 0.001$). Bd cells fell between the two extremes, with a mean of 0.53 that approached but did not quite reach statistical significance when evaluated against either Bh cells ($P = 0.19$) or amacrine cells ($P = 0.12$). In sum, Fig. 12 suggests that contrast dominance tends to shift in the direction of negative contrast as signals are transmitted from cones to bipolar to amacrine cells.

Although the latency of Bh and Bd cells may differ slightly (see next paragraph), both cell types tend to show a fundamentally similar relation between contrast and latency; this may be summarized: for contrast steps of equal magnitude but opposite polarity, positive contrast evokes a shorter latency than negative contrast (Burkhardt and Fahey 1998). Thus the latency differences, $L_p - L_n$ (as defined in RESULTS), tend to be negative in sign in Fig. 12D, top and middle. The means for both Bh and Bd cells are negative in value and differ statistically from the null (0 difference case): $P = 0.014$ for Bd cells and $P < 0.001$ for Bh cells. Just the opposite results were found here for amacrine cells (Fig. 7, B and C). Thus the differences in Fig. 12D are predominantly positive in sign. When measured over all contrasts, the mean latency difference of the distribution for amacrine cells (Fig. 12D) was 28.5 ± 1.6 ms ($n = 182$), a value significantly more positive ($P < 0.001$) than that expected for the null, zero difference case. Moreover, the amacrine distribution differs very significantly from those of Bh and Bd cells ($P < 0.001$ for both cases). Thus the comparisons in Fig. 12D strongly support the conclusion that, in the latency domain, there is a striking transformation occurring between the bipolar and ON-OFF amacrine cells. This might be a general feature of inner retinal neurons, regardless of response polarity and type because it also was observed for the hyperpolarizing and sustained neurons of Figs. 8 and 9.

Under identical conditions as used here, the latency of Bh cells was found to be ~20 ms shorter than that of Bd cells (Burkhardt and Fahey 1998) in qualitative agreement with past work in mudpuppy and turtle (Frumkes and Miller 1978; Kim and Miller 1993; Marchiafava and Torre 1978; Nelson 1973). Thus the faster response of amacrine cells to negative contrast steps might be explained if it is assumed that Bh and Bd cells provide the dominant input, respectively, for negative and positive contrast. This seems consistent with well-known pharmacological evidence that Bd and Bh cells mediate, respectively, the ON and OFF responses of amacrine cells (Miller 1994; Slaughter and Miller 1981). However, we can provide no further evidence for this hypothesis, and alternative possibilities, including differences in rise and peak times in bipolar cells and/or voltage-sensitive conductances in amacrine cells, might be proposed. Indeed, several observations suggest that the amacrine response arises from operations more complex than simple polarity inversion and algebraic summation of input from Bh and Bd cells (Marchiafava and Torre 1978; Miller 1979; Sakuranaga and Naka 1985b).

Contrast responses of ON-OFF amacrine and ON-OFF ganglion cells

Our finding that the contrast responses of the EPSPs of amacrine and ganglion cells are quite similar suggests that the bipolar cells may drive both ON-OFF ganglion and amacrine cells by very similar synaptic mechanisms. With respect to the view that ON-OFF amacrine provide a strong inhibitory input to ganglion cells (Frumkes et al. 1981; Morgan 1990; Werblin 1977; Wunk and Werblin 1979; Yu and Miller 1996; Zhang et al. 1997), our results suggest that the inhibitory input may be, apart from the difference in polarity, quantitatively similar to the ganglion cell’s excitatory input.

Evidence that the latency to negative contrast is shorter than that for positive contrast previously was seen in the extracellular impulse discharge of ON-OFF ganglion cells (Burkhardt et al. 1998). The present results clearly suggest this finding is a direct consequence of latency differences in the ganglion cell EPSP (Fig. 10) and show that the difference first appears earlier, at the level of the ON-OFF amacrine cells (Figs. 1 and 7). At the offset of contrast steps, the extracellular recordings showed that the latency difference usually was reversed, i.e., the latency to positive contrast was shorter than that for negative contrast (Burkhardt et al. 1998). This somewhat unexpected result is confirmed and perhaps explained by the present results (Figs. 1 and 10). Both ganglion cells and amacrine cells exhibit an initial hyperpolarization at the offset of negative contrast that may delay the appearance of the subsequent depolarizing response.
Distributed encoding in amacrine cells

It now is recognized widely that the amacrine population of vertebrate retinas is diverse, encompassing a number of morphological and functional classes (Ammermuller and Kolb 1995; Kolb et al. 1992; Morgan 1990; Yang et al. 1991), so it seems evident that there is distributed encoding of visual information across the amacrine cell population as a whole. However, the present paper focuses on only one general class of amacrine cell, the transient, depolarizing ON-OFF type. Our results (Figs. 3–6 and 12) show that ON-OFF cells vary substantially in dynamic range, contrast dominance, half-maximal contrast (C50), and contrast gain. Whether the distributions are continuous or composed of identifiable functional subtypes (Yang et al. 1991) remains to be established. In either case, our findings suggest that differences across the depolarizing ON-OFF amacrine cells could provide a substrate for distributed encoding. Thus differential activation of cells within the population could enhance discrimination between the varied permutations of contrast polarity and magnitude found across objects in the environment. Cells with preferential responses to negative contrast might be critical for detecting shadows produced by predators overhead. Cells with high contrast gain and narrow dynamic ranges seem all the more important when it is realized that high-contrast objects in the environment end up as low-contrast images on the retina whenever the eye is out of focus or light is scattered by fog, rain, or snow or by turbidity underwater.

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