Two-Channel Polarization Analyzer in the Sustaining Fiber-Dimming Fiber Ensemble of Crayfish Visual System

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Polarization sensitivity (PS) was examined in two classes of neurons, sustaining fibers and dimming fibers, in the medulla externa (second optic neuropile) of the crayfish, Pacifastacus leniusculus. Visual responses were recorded intracellularly and extracellularly. The influence of e-vector orientation (θ) was probed in steady-state responses, with brief flashes and with a rotating polarizer. The results indicate that the entire sustaining fiber population appears to be maximally sensitive to vertically polarized light. Although the evidence is less complete for dimming fibers, they appear to be maximally inhibited by vertically polarized light and excited by horizontally polarized light. Thus the sustaining fibers and dimming fibers form a two-channel polarization analyzer that captures the main features of the polarization system established in photoreceptors and lamina monopolar cells. The available evidence suggests that this two-channel system has the same characteristics across most or all of the retinula. Lateral inhibition in sustaining fibers is differentially sensitive to θ. Inhibition is substantial at θ = 90° (horizontal) and essentially absent at θ = 0°. The details of the sustaining fiber polarization response closely follow features established in more peripheral neurons, including the magnitude of PS, enhanced responsiveness to a changing e-vector, and modest directionality to a changing e-vector in ~40% of the cells.

INTRODUCTION

A notable feature of many arthropod and cephalopod visual systems is the sensitivity to the e-vector orientation (θ) of polarized light (Shashar and Cronin 1996; Waterman 1981, 1984; Wehner 1989). This capacity arises from photoreceptors structurally specialized for the discrimination of θ (Shaw 1966, 1969; Waterman 1981). Arthropod retinula may contain two to four distinct e-vector channels (Marshall et al. 1991; Shaw 1966; Wehner 1983). Furthermore, polarization analysis may be extended by rotation of the retinula via head and eyestalk movements.

Polarization sensitivity (PS) is expressed in a wide range of behaviors, including navigation (Wehner 1989), the detection of specular reflections (Schwind 1991), and optomotor reflexes (Schöne and Schöne 1961; von Philipsborn and Labhart 1990; Wolf et al. 1980). The neuronal mechanisms that encode polarization-related signals are only partially understood. In crayfish, the retinular cells and lamina monopolar neurons form the basis of two parallel pathways that signal orthogonal θs (Glantz 1996a; Nässel and Waterman 1977; Sabra and Glantz 1985; Waterman and Fernandez 1970). A subsequent stage consists of “tangential cells,” which are components of the lamina–medulla externa (second optic neuropile) circuitry (Strausfeld and Nässel 1981; Wang-Bennett and Glantz 1987b). In one subclass of these cells PS is enhanced by an opponency mechanism, and the second subclass is directionally selective for a changing e-vector (Glantz 1996b). Tangential cells modulate the visual signals of the sustaining fibers (tonic ON neurons) (Wang-Bennett and Glantz 1987b), which in turn synapse on optomotor neurons (Glantz and Nudelman 1988; Glantz et al. 1984). The sustaining fibers and dimming fibers (tonic OFF neurons) are the principal output neurons of the medulla externa (Kirk et al. 1982; Wiersma and Yamaguchi 1966, 1967).

Previous studies of PS in sustaining fibers of shrimp (Yamaguchi et al. 1976) and the crayfish Procambarus clarkii (Yamaguchi et al. in Waterman 1984) indicate strong sensitivity to a changing e-vector. PS was not examined in the dimming fibers. This study describes the stationary and dynamic polarization response of sustaining fibers and dimming fibers in Pacifastacus leniusculus. The results indicate a stationary PS profile with a single peak near the vertical for the entire population of sustaining fibers. Conversely, dimming fibers are inhibited by vertically polarized light and discharge maximally at a horizontal θ.

METHODS

This study is based on intracellular and extracellular recordings in the crayfish Pacifastacus leniusculus. The animals were prepared as in Glantz (1996b). The eyestalks were glued in their sockets with cyanoacrylate adhesive. The blood was replaced with oxygenated saline during a 1-h perfusion at 8°C. The optic lobe was exposed by excision of the dorsal cuticle of the eyestalk. The animal was clamped in a Plexiglas chamber (containing 2 frosted glass windows, 2 cm on a side) and bathed in oxygenated saline at 20°C. One of the eyes was centered in front of a window at 15 mm from the frosted glass.

Recording procedures

Intracellular recordings from sustaining fibers were made with micropipettes filled with 3.0 M potassium acetate (tip resistances of 100–200 MΩ) and led to an Axoclamp B1 amplifier. The signals were stored on magnetic tape. All cells were impaled in the medulla externa (Kirk et al. 1982). The same procedures were also used to characterize dimming fibers and medullary amacrine neurons (Waldrop and Glantz 1985).

Extracellular recordings from sustaining fiber axons in the optic nerve were made with tungsten electrodes, sharpened electrolytically to 5.0 μm, and coated with an epoxy-based insulating varnish. The signals were led to an AC amplifier and stored on magnetic tape. For these recordings animals were suspended from a dorsal
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FIG. 1. Response of sustaining fiber 019 to a changing e-vector orientation ($\theta$) [clockwise (CW) polarizer rotation] and to stationary $\theta$. Membrane resting potential is $-65$ mV and is indicated by zero on the left-hand ordinate. Bottom trace monitors polarizer rotation. $\theta = 0^\circ$ (vertical) is up, and orientation is read from the right-hand ordinate.

clamp that could be rotated relative to the optical axis of the stimulus system.

**Stimulus system**

Visual stimuli were generated by two optical channels alternately accessible via a 45° mirror. One channel, consisting of a 7.0-mW helium-neon laser and directed by galvanometer controlled mirrors, was used to locate the receptive field. The sustaining fibers were identified on the basis of receptive field location, after the methods of Wiersma and Yamaguchi (1967). The stimulus was controlled with an electromagnetic shutter and neutral density wedge.

The second channel, used to deliver linearly polarized light, consisted of a 300-W quartz iodide lamp, heat filter, electromagnetic shutter, neutral density wedge, focusing optics, and polarizer mounted on a motorized rotation device. All optical elements were aligned on a single axis. A diaphragm controlled the spot size from 3.0 to 15 mm ($11.0^\circ$). The focusing lens was mounted on a manipulator to control the target spot location. An optical baffle restricted the illumination of the frosted glass plate to the optic axis and blocked reflected light. The stimulus was monitored with a photodiode covered with vertically oriented polarizer film.

The stimulus consisted of white light, and the unattenuated power was 1.2 mW/cm², over the wavelength range of 450–650 nm. For most experiments, the diaphragm was set to deliver a 3.0-mm diameter spot, with total maximum power of 0.34 mW.

The optical system was calibrated with a silicon photodiode and polarization analyzer. With the diode placed at the normal position of the crayfish eye, the degree of polarization exceeded 99%, and stray polarization was <0.5%. Stray polarization was measured by rotating the polarizer and measuring the photodiode response with the polarization analyzer removed.

**Experimental protocols**

Because previous reports (Leggett 1976; Waterman 1984; Yamaguchi et al. 1976) indicated that crustacean visual neurons are insensitive to the e-vector angle of a stationary flash, two protocols were developed to explore this issue. These procedures examine the steady-state and transient responses to variations in e-vector angle.

In the contrast method the eye is continuously exposed to polar-
Polarized light of constant intensity, and the e-vector angle is varied stepwise. Each \( \theta \) is presented for 8–10 s, and the polarizer is then rotated to another angle. Each rotation is associated with a transient burst of \(~1.0\) s in duration. The \( \theta \)-dependent variations in mean rate were assessed for the interval from 3.0 to 8.0 s after the establishment of each \( \theta \). The responses to each \( \theta \) were measured 10 times.

Transient responsiveness to \( \theta \) was measured with a scanning procedure. Responses were elicited with brief (0.5–0.7 s) pulses of illumination, and the polarizer was rotated to successive angles in steps of 12–22°. For synaptic potential measurements, the responses to four to six stimulus cycles were averaged. For impulse trains, the responses to 20 cycles were averaged to form poststimulus time histograms (PSTs) at each e-vector angle. To avoid saturation, these experiments were performed at intensities <10% of the saturation intensity.

PS was estimated from the response functions by determining the difference in stimulus intensity at \( \theta_{\text{max}} \) necessary to generate comparable responses at \( \theta_{\text{max}} \) and \( \theta_{\text{min}} \). To determine the dependence of PS on stimulus intensity \((I)\), the response-intensity functions were measured at \( \theta_{\text{max}} \) and \( \theta_{\text{min}} \). For any response magnitude \( \text{PS} = I(\theta_{\text{max}})/I(\theta_{\text{min}}) \). For each neuron the average magnitude of PS was determined for the intensity range from 0.5 log units above threshold to just below saturation intensity. The PS of dimming fibers was measured in seven cells from the size of the light-elicited inhibitory postsynaptic potential (IPSP).

The response to temporal variations in \( \theta \) was examined with a rotating polarizer. The influence of both the rate and direction of change was examined in 30 preparations. The responses were evaluated from the phase and amplitude of the oscillatory membrane potential and from variations in the impulse rate relative to \( \theta \).

For studies of lateral inhibition the stimulus system was reconfigured such that two optical pathways, separated by a visual angle of 90°, converged on the eye. Each path consisted of a tungsten lamp, shutter, neutral density wedge, focusing optics, and polarizer. The excitatory stimulus was a slowly rotating polarizer. The stimulus to the inhibitory surround was a constant illumination at fixed \( \theta \). Baseline firing rates were measured with a digital counter that provided indications of drift in excitability or the occurrence of an excited state (Wiersma and Yamaguchi 1967). During excited states measurements were suspended until the control baseline rate resumed.

Data acquisition and analysis

Data acquisition was performed with a Data Translation A/D card and a PC computer. Intracellular recordings were digitized at 500 Hz. For extracellular recordings the action potentials were isolated with a time-amplitude window discriminator and digitized at 300 Hz, which is the maximum discharge rate.

Data analysis was performed with programs written in MATLAB. For intracellular recordings a Butterworth low-pass filter \((\tau = 0.03\) s) separated action potentials from the compound excitatory postsynaptic potential (EPSP). The filtered signals were then averaged with the stimulus trace as a temporal reference. The action potential sequences were converted into a train of unitary events.
FIG. 3. Sustaining fiber (01) polarization response profiles to stationary flashes at varied e-vector angle. 

A: subthreshold responses. Log $I = -4.7$. Membrane resting potential is $-50 \text{ mV}$ and indicated by zero on the left-hand ordinate. Bottom trace: e-vector angle (right-hand ordinate) CW rotation. 

B: poststimulus time histograms (PSTs) of suprathreshold impulse trains elicited at $15^\circ \theta$ intervals. $\theta_{\text{max}}$ is $15^\circ$. Each PST is based on 20 responses to 0.5-s flashes, and $\theta$ was varied from $-40$ to $120^\circ$. The peak transient discharge of each PST is aligned with the stimulus $\theta$ on the abscissa. 

C: PSTs at $\theta = 0^\circ$ for five stimulus intensities. PS for the responses in B are determined from the ratio of intensities required for equal magnitude responses at $\theta_{\text{min}}$ and $\theta_{\text{max}}$. 

D: polarization response profile of another cell (sustaining fiber 014). Note peaks at $\theta$s of 20 and 100°. Data are organized as in B.
FIG. 4. Response-intensity functions of sustaining fiber EPSP at $\theta = 0$ and 90°. A: signal-averaged EPSP of sustaining fiber 019 for log intensities $-4.0$ to $-1.0$ in 0.5 log unit steps at $\theta = 0°$. Flashes indicated by bar at bottom of figure and were 0.4 s in duration. Each trace is the average of 5 responses. Impulses were removed with a low-pass filter before signal averaging. B: as in A for $\theta = 90°$. C: transient response amplitude vs. intensity at $\theta = 0°$ (---) and $\theta = 90°$ (-- --). D: plateau phase amplitude at $\theta = 0°$ (-----) and 90° (---). E: mean impulse rate vs. log intensity at $\theta = 0°$ (-----) and 90° (---). Vertical bars are ±1.0 SD.

and used to derive mean impulse rates and to form PSTs. Temporal variations in the impulse rate were derived from the impulse probability divided by the PST binwidth. Extracellular recordings were evaluated from their mean impulse rates and from PSTs.

RESULTS

PS in the response to a stationary e-vector

Rotating a polarizer elicits transient responses, as in Fig. 1 (first 25 s), synchronized to the changes in e-vector angle. As $\theta$ approaches 0°, the membrane depolarizes, and there is a burst of impulses. As $\theta$ approaches 90°, the membrane repolarizes, and the impulse rate declines toward a minimum. Modest changes in e-vector angle (10–30°), produce changes in membrane potential and in mean impulse rate that persist for ~20 s, as shown in Fig. 1. At each stepwise change in $\theta$ between 0 and 60° the membrane potential and the impulse rate decline. Conversely, successive steps between 60 and 180° produce depolarization and increased impulse rates. Figure 2A shows the mean rate (subsequent to the transient burst) for each of 6 $\theta$s. At $\theta_{\text{max}}$ (between 150 and 0°) the mean rate was 3.6 times that at $\theta_{\text{min}}$ (60°), and PS was 7.0.

For purposes of comparison, the variations in impulse rate elicited with a continuously rotating polarizer (at the same intensity) are shown in Figs. 1 (1st 25 s) and 2B. These data exhibit $\theta_{\text{max}}$ and $\theta_{\text{min}}$ near 150° (●) and 60°, respectively, and a 3.4-fold difference in responsiveness. Thus continuous rotation augments the discharge, but the ratio of impulse rates at $\theta_{\text{max}}$ and $\theta_{\text{min}}$ are similar. The similarity between $\theta_{\text{max}}$ measured at stationary e-vector angles and the phase of the peak response elicited with a slowly rotating polarizer was observed for nearly all neurons so examined (as in Fig. 2C).

Thus, when a crayfish rotates in a field of polarized light, the elements of the sustaining fiber ensemble will each discharge at a rate proportional to the proximity to $\theta_{\text{max}}$.

The stationary polarization response profile was also measured with the scanning method, as shown in Fig. 3. In Fig. 3A the data were derived with subthreshold light pulses, and the EPSPs vary from 4.5 mV at $\theta_{\text{max}}$ (0°) to 2.0 mV at $\theta_{\text{min}}$. At suprathreshold intensities the discharge is evaluated with the PST, and PS is interpolated from the intensity-response function. Figure 3B shows $\theta$-dependent variations in firing rate. The peak impulse rate at $\theta_{\text{max}}$ (15°) is about 2.2 times that at $\theta_{\text{min}}$ (105°), and PS is 4.0 as determined by the variations in the intensity dependence of the peak rate in Fig. 3C. At moderate intensities, 3 of 33 cells tested exhibited a two-peak sensitivity profile, as in Fig. 3D, with the peaks spaced ~90° apart. An additional five cells exhibited this pattern at higher intensities. Thus there is evidence for convergent input from the orthogonal channels in 10–25% of the cells.

The intensity dependence of PS can be evaluated from intensity-response functions at $\theta_{\text{max}}$ and $\theta_{\text{min}}$, as shown in
Fig. 4. In this cell PS increases with stimulus intensity. The responses in Fig. 4, A and B, are averaged compound EPSPs at $\theta_{\text{max}}$ and $\theta_{\text{min}}$. At the lowest intensities, both the transient and plateau responses exhibit only modest PS ( $\approx 2.0$, as in Fig. 4, C and D). At higher intensities however the response to flashes at $\theta = 0^\circ$ is saturated yet remains 30–50% greater than the response to flashes at $\theta = 90^\circ$. This result implies an intensity dependence in PS. The discontinuity at log $I = -3.0$ may reflect a high-threshold opponency mechanism. Sustaining fibers are subject to intensity-dependent lateral inhibition (Glantz and Nudelman 1976; Waldrop and Glantz 1985). At high stimulus intensities, the EPSP traces in Fig. 4, A and B, exhibit a dip in the membrane potential (arrow in Fig. 4A) between the peak and plateau phases of the response. A similar dip in the PST has been previously shown (Glantz and Nudelman 1976) to reflect a delayed action from the inhibitory surround. Furthermore, the dip occurs over the same range of stimulus intensities associated with the divergence of the response functions at $\theta_{\text{max}}$ and $\theta_{\text{min}}$ (in Fig. 4, C and D). More direct evidence for PS in the surround inhibitory mechanism is described below.

The impulse rates between threshold, log $I = -3$, and saturation (as in Fig. 4E) exhibit an average PS of 5.6 ± 2.0 (SD). Intensity-response functions at $\theta_{\text{max}}$ and $\theta_{\text{min}}$ were examined in 12 neurons and revealed an average PS of 4.8 ± 3.0, which is comparable with the PS of receptors and lamina monopolar cells (Glantz 1996a). The subthreshold response to polarizer rotation at 76 and 146$^\circ$/s in the clockwise (CW) and counterclockwise (CC) directions. The oscillations in synaptic potential are modestly saturated yet remains 30–50% for CC rotations and at the higher velocity. Directionality and sensitivity to rates of change were examined in 30 neurons. Thirteen cells exhibited a consistent directional bias similar to that shown in Fig. 6 and with responses in one direction exceeding that in the opposite direction by 40–100%. Most cells were also sensitive to the rate of change of the e-vector, with a maximum response at 100–180$^\circ$/s and diminished responses at lower and higher rates of change (data not shown).

**Distribution of $\theta_{\text{max}}$ in sustaining and dimming fibers**

The $\theta_{\text{max}}$ of sustaining fibers clusters near the vertical as previously inferred by Yamaguchi et al. (1976) from dynamic responses. The distribution of $\theta_{\text{max}}$ values for 48 cells measured with a stationary polarizer is shown in Fig. 5A. The data sample includes 9 of 14 sustaining fiber subtypes (receptive field locations) identified by Wiersma and Yamaguchi (1967). When these results are segregated by receptive field location (dorsal vs. ventral, anterior vs. posterior) the result is similar for each subgroup and similar to Fig. 5A (data not shown). A striking feature of the distribution is the paucity of $\theta_{\text{max}}$ observations near 90$^\circ$. Only 2 of 48 cells exhibited $\theta_{\text{max}}$ values within ±15$^\circ$ of the horizontal. This distribution contrasts markedly with those of receptors and lamina monopolar cells (Glantz 1996a) in which both orthogonal $\theta_{\text{max}}$ values are well represented in the cell populations. The preference for a vertical e-vector is quite robust. When the stimulus is centered in the inhibitory surround such that only the internally reflected light reaches the excitatory region, the distribution of $\theta_{\text{max}}$ remains clustered about the vertical. An interesting feature of PS in dimming fibers is that the distribution of $\theta_{\text{max}}$ for inhibition, Fig. 5B, is similar to that for excitation in sustaining fibers.

**Sustaining fiber response to a changing e-vector**

The sustaining fiber response to temporal variations in $\theta$ reflects both the stationary PS profile and the direction and rate of change of $\theta$ (as in Figs. 1 and 2). Figure 6 shows the subthreshold response to polarizer rotation at 76 and 146$^\circ$/s in the clockwise (CW) and counterclockwise (CC) directions. The oscillations in synaptic potential are modestly larger (30–50%) for CC rotations and at the higher velocity. Directionality and sensitivity to rates of change were examined in 30 neurons. Thirteen cells exhibited a consistent directional bias similar to that shown in Fig. 6. and with responses in one direction exceeding that in the opposite direction by 40–100%. Most cells were also sensitive to the rate of change of the e-vector, with a maximum response at 100–180$^\circ$/s and diminished responses at lower and higher rates of change (data not shown).

**PS in the inhibitory surround**

The intensity dependence of PS described in Fig. 4 and the insensitivity of $\theta_{\text{max}}$ to stimulus location may have a common basis in sustaining fiber surround inhibition (Wul}
FIG. 6. Sustaining fiber 01 subthreshold response to a rotating polarizer. The shutter is opened at $t = 2.2$ s (bottom trace) eliciting a transient depolarization that decays to a plateau phase (top trace). The membrane resting potential was $-60$ mV, and it is indicated by zero on the left-hand ordinate. Polarizer rotation commences at $t = 12$ s in the CW direction at $76^\circ$/s, followed by counterclockwise (CC) rotation at $76^\circ$ and $146^\circ$/s and CW rotation at $146^\circ$/s. Bottom trace is stimulus monitor, and e-vector angle is indicated on the right-hand ordinate.

Drop and Glantz 1985; Wiersma and Yamaguchi 1967). If the inhibitory mechanism is polarization sensitive with $\theta_{\text{max}}$ near $90^\circ$, the PS profiles for net excitation would be shifted toward $0^\circ$. The results of two types of experiments suggest that the sustaining fiber inhibitory mechanism is polarization sensitive and that $\theta_{\text{max}}$ for inhibition is $\sim 90^\circ$.

In one procedure, $\theta_{\text{max}}$ for excitation was assessed with a rotating polarizer ($30\text{–}60^\circ$/s) and with the light beam focused on the excitatory field. After a control measurement (as in Fig. 7A) the inhibitory field was illuminated with constant polarized light from a second beam at $\theta = 0^\circ$ or $\theta = 90^\circ$, as seen in Fig. 7, B and C, respectively. When the inhibitory stimulus is polarized to $\theta = 0^\circ$, there is little or no evidence of inhibition. When the inhibitory field was illuminated at $\theta = 90^\circ$, both the peak and mean rates, elicited by the excitatory stimulus, were substantially diminished. Similar results were obtained in three of four experiments. An inhibitory stimulus at $\theta = 90^\circ$ was $20\text{–}50\%$ more effective than one at $0^\circ$. The fourth cell revealed no evidence of inhibition at any $\theta$.

In a second experiment, the PS of the cells that mediate sustaining fiber lateral inhibition (Fig. 12), the medullary amacrine cells (Waldrop and Glantz 1985), was examined with a stationary or slowly rotating polarizer as in Fig. 8. In this cell, $\theta_{\text{max}}$ was at $78^\circ$, as shown in Fig. 8A. Three of six cells exhibited a $\theta_{\text{max}}$ near $90^\circ$, and only one revealed a $\theta_{\text{max}}$ near $0^\circ$. The $\theta_{\text{max}}$, evaluated from the response to a rotating polarizer, depends on the e-vector the inhibitory mechanism is polarization sensitive with $\theta_{\text{max}}$ rate of change. A $\theta_{\text{max}}$ near $90^\circ$ obtains at rates of up to $90^\circ$/s, the PS profiles for net excitation would be shifted $120^\circ$/s (as in Fig. 8B) but not at higher rates (as in Fig. 8, C and D).

**PS in dimming fibers**

Dimming fibers are spontaneously active in the dark and exhibit an OFF response to intensity decrements. Increments of illumination elicit a graded hyperpolarization and a pause in the discharge, as shown in Fig. 9. With polarized light, the hyperpolarizations elicited by pulses at $\theta = 0^\circ$ (as in Fig. 9, right column) are larger than those obtained with pulses at $90^\circ$ (as in Fig. 9, left column), and the $\theta$-dependent difference generally increases with stimulus intensity, as in Fig. 9G.

The dimming fiber polarization response profile (as in Fig. 10A), is a continuous function of $\theta$ and $\theta_{\text{max}}$ for inhibition is $\sim 0^\circ$. The IPSP at $\theta = 0^\circ$ is $\sim 60\%$ larger than that at $90^\circ$, and the response varies as a $\cos^2\theta$ function, as in Fig. 10B. The average PS of dimming fibers was $4.9 \pm 3.0$ ($n = 7$). $\theta_{\text{max}}$ for inhibition was determined with the scanning method, as in Fig. 10, or from the response to a slowly rotating polarizer, as in Fig. 11. In 12 of 14 cells so tested, $\theta_{\text{max}}$ was within $\pm 15^\circ$ of $0^\circ$, as in Fig. 5B. Thus
dimming fibers are inhibited, and sustaining fibers are excited at the same $\theta_{\text{max}}$. For a changing e-vector, inhibition at $\theta = 0^\circ$ hyperpolarizes the cell as $\theta$ approaches $0^\circ$, and the cell depolarizes (recovers from inhibition) as $\theta$ approaches $90^\circ$, as shown in Fig. 11. Although the $\theta$-dependent variation in membrane potential is a modest $1.0 - 3.0$ mV (as in Fig. 11, $B$ and $C$), it is sufficient to maximize the impulse rate as $\theta$ approaches $90^\circ$.

![Diagram of Fig. 7](image)

**Fig. 7.** Dependence of lateral inhibition on $\theta$. $A$: control response of sustaining fiber 038 (left-hand ordinate) to CW polarizer rotation (bottom trace, right-hand ordinate). Mean rate is $10.9 \pm 0.1$ impulses/s. $B$: as in $A$ but with continuous illumination of the inhibitory surround at $\theta = 0^\circ$. Mean rate is $9.7 \pm 1.1$ impulses/s. $C$: as in $B$ but illumination of inhibitory field is at $\theta = 90^\circ$. Mean rate is $5.3 \pm 2.3$ impulses/s.

![Diagram of Fig. 8](image)

**Fig. 8.** Response of nonspiking medullary amacrine neuron to CW polarizer rotation at $53^\circ/\text{s}$ ($A$), $120^\circ/\text{s}$ ($B$), $218^\circ/\text{s}$ ($C$), and $300^\circ/\text{s}$ ($D$). Note at the lowest velocity the maximum depolarization obtains at $\theta = 78^\circ$, and phase lag increases at successively higher rates of e-vector change. Each trace is the average of 5 ($A$) or 10 ($B - D$) responses. Membrane resting potential is $-50$ mV, indicated by 0 on left-hand ordinate. Broken traces are from the stimulus monitor and scaled on the right-hand ordinate.
FIG. 9. Dimming fiber IPSPs elicited with 2.0-s pulses of light at $\theta = 90^\circ$ (left column) and $0^\circ$ (right column) and at log intensity $-3$ (A and B), $-2$ (C and D), and $-1$ (E and F). Note that the IPSP increases progressively with intensity at $\theta = 0^\circ$ but not at $\theta = 90^\circ$. Lower bar indicates stimulus timing. G: IPSP amplitude vs. intensity at $\theta = 0^\circ$ (——) and $\theta = 90^\circ$ (-----). Circles are at ±1.0 SD.

**Discussion**

The principal purpose of this study was to examine the transfer of polarization-related information from the more distal nonspiking visual neurons to the sustaining fibers and dimming fibers. Toward this end, aspects of PS, previously measured in lamina monopolar cells and tangential cells, were examined with the same procedures in sustaining and dimming fibers. Both monopolar cells and tangential cells make indirect functional connections to sustaining fibers (Wang-Bennett and Glantz 1987a,b).

The monopolar cells exhibit an average PS of 4.5 (Table 1); they provide separate pathways for horizontal and vertical $\theta$s (Fig. 12) and enhanced responsiveness to a changing e-vector. The sustaining fiber polarization response is similar to that of only one class of lamina monopolars (with $\theta_{\text{max}} \sim 0^\circ$, as in Table 1). The absence of a horizontal e-vector channel in sustaining fibers implies a highly selective pattern of innervation. Because the dimming fiber $\theta_{\text{max}}$ at $90^\circ$ results from inhibition at $\theta = 0^\circ$, the dimming fiber input must also come from a subset of columnar cells with $\theta_{\text{max}} = 0^\circ$. Excitation of sustaining fibers is mediated by glutamate (Pfeiffer-Linn and Glantz 1991), but inhibition of dimming fibers requires acetylcholine (Pfeiffer and Glantz 1989). Thus the two synaptic actions, which have similar $\theta$ dependence, must be mediated by different transmedullary cells, as shown in Fig. 12. It is likely however, that these cholinergic and glutamatergic transmedullary cells are excited by common lamina monopolar neurons (M3 in Fig. 12). Another inference from these studies is that the glutamatergic transmedullary cells (Pfeiffer-Linn and Glantz 1991), which are presynaptic to sustaining fibers (TM-GL, in Fig. 12), are likely to exhibit a $\theta_{\text{max}}$ near $0^\circ$. 

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Sustaining fiber PS also resembles that in one class of tangential neuron (type I), which exhibits modest directionality in the response to a changing e-vector. Thus sustaining fibers appear to capture the essential features of a subset of lamina monopolar neurons and the type I tangential cells.

The results indicate that the output side of the medulla externa contains two pathways with orthogonal e-vector sensitivity. The signals are transmitted to the brain via their axons in the optic nerve (as in Fig. 12). The essential features of the photoreceptor-lamina monopolar PS system are all contained in the sustaining–dimming fiber ensemble. These include the stationary PS profiles of two orthogonal channels, enhanced responsiveness to a changing e-vector, the fidelity of dynamic phase and stationary
θ_{\text{max}}$, and the representation of PS for the entire panoramic visual field.

The function of the PS system for crayfish behavior is less clear. One problem is that all the neurons of the polarization-sensitive afferent pathway are also sensitive to the local contrast of unpolarized light. Thus a change of θ in a local area will effect particular sustaining and dimming fibers in a manner that is indistinguishable from a change in local intensity. There are several possible resolutions to this problem, and each requires assumptions about the behavioral context. One possibility is that, in a restricted environment, local intensity and θ may be correlated. Alternatively, the PS in this system may enhance contrast detection (caused by reflected polarized light) in circumstances in which intensity contrast is absent (Bernard and Wehner 1977; Leggett 1976). In this context it may be immaterial whether the source of the contrast is a difference in θ or local intensity.

Previous studies described PS in neurons of the crab medulla externa (Leggett 1976). The cells are insensitive to the stationary e-vector angle but highly sensitive to e-vector change and/or the direction of change. In contrast to the crayfish sustaining and dimming fibers, however, the crab neuron’s response is not modulated with variations in θ. The opposite extreme is found in the cricket medulla, which contains three classes of polarization-sensitive neurons with θ_{\text{max}} separated by ~60° (Labhardt 1988). Stationary PS is enhanced by an opponency mechanism (Labhardt and Petzold 1993). It is notable that three arthropods analyze polarization signals in homologous structures (the second optic neuropile) but with very different strategies.

An important difference regarding PS in crayfish sustaining and dimming fibers and that in several insect species is the distribution of polarization analyzers across the retina. In crayfish PS is broadly distributed across the retina, and there are orthogonal e-vector analyzers at all or most locations. In bees, ants, and crickets (Wehner 1989) PS is

### TABLE 1. Comparison of the polarization-sensitive properties in four classes of neurons

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<th>N_{\text{PS}}*</th>
<th>PS</th>
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<td>13/30</td>
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</tbody>
</table>

Values are means ± SE. N represents the number of cells satisfying a minimal criterion (e.g., PS ≥ 2.0) relative to the size of the tested population; PS is based on the e-vector orientation [θ dependence of the inhibitory postsynaptic potential (IPSP, n = 7)]; θ_{\text{max}} is taken as 90° to the θ for maximum IPSP or it is based on the θ dependence of the peak impulse rate; N(0°)/N(90°) is the number of observations of θ_{\text{max}} within ±15° of 0 and 90°, respectively; NA, not available.
largely restricted to the ommatidia on the dorsal face of the compound eye, and the distribution of analyzers is consistent with a matched filter for skylight polarization. This organization provides a basis for navigation by polarized skylight. The more homogeneous distribution of PS in crayfish eye may indicate a different function such as contrast enhancement in an aquatic environment.

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