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MECHANISMS FOR NEURAL SIGNAL ENHANCEMENT IN THE BLOWFLY COMPOUND EYE

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Summary

In the blowfly *Calliphora vicina* visual signals are enhanced by amplification and antagonism as they pass from the site of phototransduction in the retina to second-order neurones (LMCs) in the first optic neuropile, the lamina. The mechanisms responsible for amplification and antagonism were investigated, using current-clamp techniques, to examine the conductance mechanisms generating LMC responses. LMCs responded Ohmically to injected current. Voltage-sensitive conductances and feedback mechanisms driven by the potential of a single LMC played a minor role in shaping responses. The LMC's response to an increment in illumination, a transient hyperpolarization, was generated by a large and transient conductance increase with a reversal potential close to the maximum response amplitude (30–40 mV below dark resting potential). The depolarization of the LMC in response to a decrement in light intensity was partially generated by a reduction in direct synaptic input from the photoreceptors. Changes in depolarizing conductances with positive reversal potentials played a secondary role, contributing to large-amplitude responses to dimming or light-off, and to the slow decay of the LMC response to steady illumination. Antagonism, including lateral antagonism, operated principally by shutting down the direct photoreceptor input, presumably by presynaptic regulation. The results of dye injection suggested that the identified large monopolar cell L2 is more strongly affected by lateral antagonism than the similar cells L1 and L3. We conclude that LMCs are essentially passive integrators of a well-regulated direct input from the photoreceptors. This suggests that the intrinsic properties of photoreceptor–LMC synapses and presynaptic interactions are primarily responsible for amplification and antagonism.

Introduction

In the fly's compound eye, photoreceptor signals are enhanced to protect them

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from contamination by intrinsic noise as they are transmitted synaptically to second-order neurones. Enhancement involves removal, by antagonism, of the redundant signal components that correspond to the local mean level of illumination (Srinivasan *et al.* 1982), and amplification of the remainder, which corresponds to useful pictorial detail. Antagonism and amplification are optimized to promote coding efficiency (for a review, see Laughlin, 1987). In this paper we study the mechanisms responsible for these two processes by measuring the conductance changes associated with the responses of the second-order neurones, the large monopolar cells or LMCs.

Present evidence, in particular the relationship between presynaptic photoreceptor signals and postsynaptic responses (Laughlin *et al.* 1987), suggests that amplification is performed by the array of high-gain chemical synapses connecting photoreceptors to LMCs (Shaw, 1979, 1981, 1984). The photoreceptor neurotransmitter, probably histamine (Hardie, 1987), produces an increase in chloride conductance in the postsynaptic LMC (Zettler & Straka, 1987). Antagonism suppresses LMC responses to sustained stimuli so that transient hyperpolarizations are generated when light intensity suddenly increases, and transient depolarizations occur when intensity suddenly drops. The simplest explanation for this transient behaviour is that the photoreceptor–LMC synapses respond transiently to step changes in presynaptic membrane potential, acting as high-pass filters with biphasic impulse responses (Hayashi *et al.* 1985; Laughlin *et al.* 1987).

Nonetheless, a number of observations suggest that additional mechanisms shape the LMC response. The LMC OFF response, elicited following a brief light-pulse delivered to the dark-adapted eye, contains a rapid depolarizing component that is probably generated in the medulla (Zettler & Järvilehto, 1973; Guy & Srinivasan, 1988). There is pharmacological and electrophysiological evidence for additional depolarizing synaptic inputs to LMCs (Zimmerman, 1978; Hardie, 1987; Wang-Bennett & Glantz, 1987). Moreover, the dark-adapted synaptic impulse response is monophasic, in accordance with the observation that antagonism declines at low intensities (Laughlin & Hardie, 1978; Srinivasan *et al.* 1982). Thus the synaptic mechanisms driving LMCs are modified by the mean level of photoreceptor input. In addition, LMCs are subjected to lateral antagonism, as well as to the antagonism generated within one cartridge (Zettler & Järvilehto, 1972; Dubs, 1982). In crayfish LMCs, lateral antagonism acts presynaptically, suppressing a tonic synaptic drive from photoreceptors (Wang-Bennett & Glantz, 1987), but the synaptic mechanisms mediating lateral antagonism in fly have not been determined.

In this study we examine the roles played by LMC conductance mechanisms in amplifying responses and mediating antagonism, by injecting current into LMCs and measuring response waveforms, reversal potentials and changes in input resistance. We pay particular attention to the transient responses to either a sudden brightening or a sudden dimming of the stimulus because these LMC transients represent the interplay between the amplification and antagonism.

Materials and methods

Animals and recording techniques

Female *Calliphora vicina* R-D. (previously *erythrocephala*) were taken from a regularly refreshed laboratory culture. A small hole (approx. 100 μm in diameter) was cut in the cornea of an intact restrained fly and sealed with silicone grease to allow for insertion of the micropipettes. Intracellular recording electrodes were filled with 0·6 mol l^{-1} potassium sulphate and 5 mmol l^{-1} potassium chloride, and connected to the amplifier head stage by a chloridized silver wire. The electrodes had resistances of 120–250 MΩ when first inserted into the retina, but they often (and conveniently) broke when passing from retina to lamina, lowering the resistance to 70–120 MΩ. Recordings were digitized, averaged, stored and later analysed using a microcomputer system (Cambridge Electronic Design 1401 interface; BBC Master).

Identification of recording site

Unless stated otherwise, large monopolar cells (LMCs) were identified on the basis of their hyperpolarizing response to light (Autrum *et al.* 1970). A recording site in the lamina was characterized by prominent light-induced extracellular depolarizations (Mote, 1970) and by penetrations of neighbouring receptor axons. Recordings in the chiasm were characterized by an alternation of LMC-receptive fields from frontal positions to more posterior positions, in passing from cell to cell. For a minority of cells the recording position could not be established by these criteria.

In several of the experiments examining lateral antagonism, the recorded cell was identified by injecting it with Lucifer Yellow. After marking just one cell, the retina and optic lobes were dissected free, and processed and examined using standard procedures. The marked cells were identified on the basis of the stratification of dendrites in the lamina (L1 and L2 cf. L3) and the level of termination in the medulla (L1 cf. L2), as described by Strausfeld & Nässel (1981).

Measurement of the response to injected current

A discontinuous current clamp (Axoclamp-2A, Axon Instruments, Burlingham, CA, USA) was used to examine reversal potentials of the LMC response, and to determine LMC current–voltage relationships. The amplifier headstage was switched between current injection and recording mode. Current injection lasted for 30 % of the duty cycle and the membrane potential was recorded by a sample-and-hold amplifier at the end of the cycle, immediately preceding the next period of current injection. The response of the headstage to current injection was continuously monitored (e.g. Fig. 1A), allowing for optimal adjustment of the capacity compensation control and the switching rate, which was set between 3·5 and 5·0 kHz, depending upon the particular cell and electrode.

When using high-resistance electrodes on cells with low input impedances (such as LMCs), the results obtained from switching clamp are prone to artefact. When

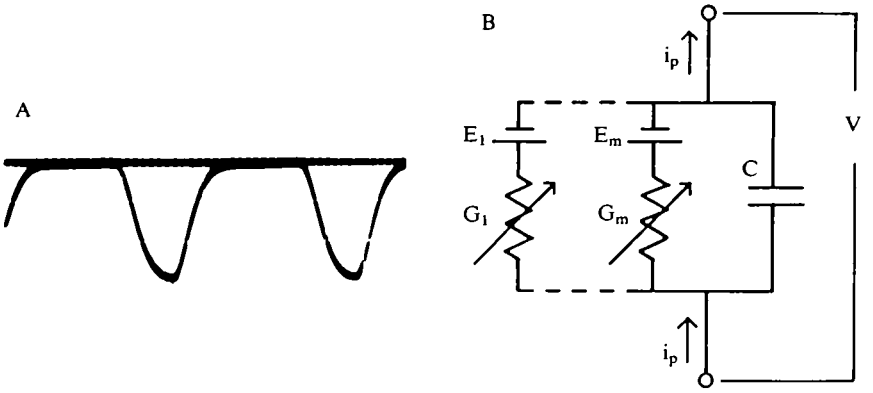

Fig. 1. (A) The voltage recorded across the headstage during discontinuous clamping. The electrode (resistance 90 MΩ) was inserted in the lamina extracellular space. Negative current was injected in the first part of the duty cycle (negative deflection). The potential was measured by a sample-and-hold amplifier just prior to the initiation of the current pulse, by which time the voltage across the headstage had returned to the baseline. The current injected was −1·0 nA, averaged over the cycle, and the switching rate 4·5 kHz. Major graticule divisions = 100 mV and 50 μs. (B) The simple circuit used to model the response of a cell to current clamp consists of a number of conductances, G_n, each with its own reversal potential, E_n, and a capacitor, C. A constant current, i_p, is applied across this circuit and the voltage, V, measured. See text for further details.

the time constant of the electrode is at least 10- to 20-fold less than the time constant of the cell membrane, the potential recorded at the end of the switching cycle approximates the true membrane potential. When the electrode time constant is more than one-tenth of the LMC time constant the potential recorded is contaminated by a series resistance component from the LMC. Thus the polarization recorded from injected current will be increased, leading to an artefactual increase in the apparent input resistance of the cell. This artefact will also tend to linearize the response of the membrane to injected current. In the fly lamina, the resolution of this switching technique is limited by the high electrode resistance required to record reliably from the 2–3 μm diameter LMCs. For measurement of current–voltage relationships and reversal potentials, the lowest electrode resistances compatible with obtaining high-quality impalements (70–100 MΩ) were chosen. Capacitance effects were reduced by using a driven shield and by inserting the electrode into the eye through a silicone grease seal. This seal prevented electrolyte from creeping up the outer surface of the electrode and increasing the capacitance. Nonetheless, the electrode capacitance increased as it was advanced deeper into the tissue and electrode tracks were employed that minimized the depth of insertion into the tissue. Under optimal conditions, effective (maximally compensated) electrode time constants were approximately 30 μs. The LMC membrane time constant, in the synaptic zone in the lamina, and in the dark-adapted state and the steady-light adapted state, was of the order of 0·5–1·0 ms. This value was measured using a balanced bridge circuit or the

switching clamp to determine the response to small 20 ms current pulses, averaged over 400–1000 repetitions. With this value of membrane time constant, the measurements made in the dark-adapted cell fall within the limits of satisfactory performance. For each cell we checked for satisfactory operation of the discontinuous clamp by comparing the recorded response of a cell to a 0·2 or 0·5 nA current injection with the response elicited using a balanced bridge circuit.

Analysis of the response to injected current

The principles and limitations of current-clamp techniques may be illustrated by reference to a simple circuit model of an isopotential cell membrane (Fig. 1B), with a capacitance C and m species of conductance, G_n, each with a reversal potential E_n. The membrane is polarized by an applied current i_p. Under this condition:

$$i_p = \sum_{n=1}^{n=m} i_n + i_c, \tag{1}$$

where i_n is the current flowing across the nth conductance and i_c is the current flowing across the capacitor. For each conductance:

$$i_n = (V - E_n)\, G_n \tag{2}$$

where V is the membrane potential at any instant. In the steady state, no current flows across the capacitor and the membrane potential, V, is given by combining equations 1 and 2:

$$V = \frac{i_p + \sum_{n=1}^{n=m} (E_n\, G_n)}{\sum_{n=1}^{n=m} G_n} \tag{3}$$

which reduces to:

$$V - V_0 = i_p / G, \tag{4}$$

where V_0 is the steady-state potential recorded without applied current and G is the total conductance. Thus, in the steady state, or in a purely resistive circuit, the polarization produced by the current clamp is inversely proportional to the total conductance, i.e. it is proportional to the input resistance.

Continuous monitoring of cell input resistance

It is useful to be able to inject current and monitor the amplitude and the time course of changes in conductance and resistance that accompany a cell's physiological response. The simplest approach is to compare the amplitude of a response obtained under current clamp with the normal response elicited without current and assume a purely resistive circuit (e.g. Baylor & Fuortes, 1970;

Laughlin, 1974*b*; Guy & Srinivasan, 1988). Furthermore, by neglecting capacitance, equation 4 can be applied to potentials that vary in time, V(t), to give the total conductance as a function of time, G(t). Thus:

$$V(t) - V_0(t) = i_p/G(t), \tag{5}$$

where V(t) is the time course of membrane potential changes measured with constant injected current, i_p, and $V_0(t)$ is the time course measured without applied current. This method has been successfully applied to photoreceptors by subtracting the average response waveforms obtained with and without small applied currents (van Hateren, 1986*a*).

Because capacitance slows the rate of change of potential, its effect is equivalent to a slowing in the rate of change of conductance in a purely resistive circuit. It follows that by neglecting capacitance one observes a low-pass filtered version of the true conductance changes (van Hateren, 1986*a*). We have confirmed this by numerical simulation of the flow of charge in a two-conductance version of the circuit modelled above (equations 1,2; Fig. 1B). The time course of the response of the model to a step change in conductance was determined both with and without the application of a small polarizing current. We then applied equation 5 to the voltage responses modelled with and without applied current to deduce the time course of the apparent conductance change. As predicted (van Hateren, 1986*a*), the apparent conductance rises exponentially to its true value, in parallel with the change in potential. Thus the resolution of conductance changes is limited by the cell's time constant which, in LMCs recorded in the lamina, varies between 0·5 and 1·0 ms. However, LMC conductance changes are primarily driven by the photoreceptor synapses. The receptors limit the signal to lower frequencies that are relatively little affected by the time constant of the LMC response (Laughlin *et al.* 1987). This means that the majority of synaptically driven conductances will change relatively slowly, and the effects of capacitative currents upon the continuous measurements of these conductance changes can be disregarded. A second, and potentially serious, complication to analysis with current clamp, the presence of voltage-sensitive conductances, has little effect on our measurements. Small currents, usually less than 0·2 nA, were used to measure resistance changes. More importantly, our measurements show that, under most conditions, voltage-sensitive conductances play a negligible role in shaping the LMC response.

We injected current and measured the time course of conductance changes as follows. Digitization of the record was initiated 100 ms after the onset of the injection of a small constant current. This current was usually injected *via* a bridge circuit because, with small currents, this is less noisy. The light stimulus was then delivered at a fixed interval from the onset of digitization. Shortly after the cell's response had returned to the steady-state level, digitization of this first record was stopped and the current switched off. Following another 100 ms delay, there was a second period of digitization and stimulation, identical to the first. This cycle of digitizing records with and without injected current was repeated 100–600 times and the two records averaged separately. Note that by interleaving the records

obtained with and without current, one controls against many of the artefacts generated by changes in recording quality during the lengthy period of averaging. Furthermore, the coupling of the stimulus to the onset of digitization ensured that the data points in the two records corresponded exactly, so eliminating artefacts due to jitter between the timing of sampling and stimulation. Each data point in the record elicited without current, $V_0(t)$, was subtracted from the corresponding data point obtained with current, $V(t)$, and the difference divided by the current to give the resistance (equation 5). Because we are interested in the changes in conductance that accompany a response, and bridge techniques are not suitable for measuring absolute resistances over lengthy periods, we usually plotted the change in resistance relative to the initial value in the record (e.g. Fig. 5). To check the validity of this method, its results were compared with resistance changes measured by injecting 10 ms current pulses at different times during the LMC response, and there was an excellent correspondence.

Stimuli

To study lateral antagonism we combined three independent stimuli, a central LED, an annulus of six equally spaced LEDs, each 3° from the centre, and a widefield background light. The LEDs were mounted on a Cardan arm so that they could be centred on a particular unit's optical axis. The LEDs were selected to give identical outputs (±5%) for a given current. The intensities of the centre and the surround were independently controlled by separate current amplifiers. The widefield adapting background was presented by means of a half-silvered mirror interposed between the LEDs and the eye. The fly viewed the LEDs through the half-silvered mirror, together with the reflection of the 20° diameter adapting field. This uniform adapting field was generated using a quartz–halogen lamp and the field intensity was controlled by neutral-density filters. The effective intensities of these three stimuli were calibrated by presenting them to dark-adapted photoreceptors and counting quantum bumps. All the data presented here concerning the receptive fields of light-adapted adapted cells were collected at the maximum background intensity, which corresponded to 2×10^4 effective photons receptor^{-1} s^{-1}.

Small movements of the retina occur in *Calliphora* and these make it difficult to control precisely the effective intensities of the centre and surround. To minimize eye-movement artefacts and to check the optics, the deep-pseudopupil (Franceschini, 1975) was observed before the experiment, and the stability of a unit's optical axis continuously checked throughout an experiment. We did not use animals whose deep-pseudopupils moved, and we rejected data from cells whose receptive fields drifted by more than half a degree.

In the experiments that did not analyse lateral antagonism, the stimulus was either a point source (an LED) centred on the optical axis or a diffuse source (a diffusing screen mounted close to the eye and illuminated by an LED). The point source was always centred on a unit's optical axis and was only employed for work

on dark-adapted cells. When working with light-adapted cells, the mean effective intensity was of the order of 10^6 effective photons receptor^{-1} s^{-1}.

Results

LMC responses were elicited in both light-adapted and dark-adapted states. We examined the changes in membrane potential and response waveform resulting from applied current, and the dependence of response waveform and cell input resistance on the position of the stimulus within an LMC's receptive field. Interpretation of the data is simplified by the favourable geometry of LMCs. Each cell is essentially a cylinder, with a small cell body connected by a narrow dendrite at the distal end. The input synapses from the photoreceptors and other lamina elements are restricted to the distal 60 μm of this cylinder. The remainder of the cell is a long axon (200–1200 μm) which is apparently devoid of synapses, and terminates in the medulla neuropile. Cable modelling suggests that the length constants of the axons of fly (van Hateren, 1986*b*; Guy & Srinivasan, 1988) and crayfish LMCs (Wang-Bennett & Glantz, 1987) allow for the efficient electrotonic propagation of signal from lamina to medulla, but the low impedance of the lamina synaptic region severely attenuates signals propagating in the reverse direction, from medulla to lamina. Thus an electrode placed in the lamina synaptic region principally records signals generated in the lamina. When current is injected into an LMC in the lamina, the medulla terminal polarizes by an amount that is estimated to be about 70% of the value recorded at the site of injection (van Hateren, 1986*b*).

The electrical properties of LMCs in darkness and in continuous light

In darkness, the LMC membrane potential can be between −40 and −75 mV, relative to the retinal extracellular space. The potentials recorded in the extracellular space of the lamina, relative to the retina, vary over approximately the same range (for a review see Laughlin, 1981*a*). This variability prevents measurement of absolute values of LMC membrane potential, so in this paper membrane potentials are usually measured relative to the steady dark-level. Also, in darkness, tonic synaptic activity generates appreciable noise in LMCs (Laughlin, 1973; Wang-Bennett & Glantz, 1987; Laughlin *et al.* 1987) of up to 5 mV peak to peak amplitude. Inaccuracy in measurement, resulting from this noise, was reduced by signal averaging.

To investigate the electrical properties of LMCs, dark-adapted cells were polarized by injecting current *via* the recording electrode, using the discontinuous current clamp. The apparent membrane potential was recorded (Fig. 2), giving an estimate of the current/voltage relationship (I/V curve) of the LMC (Fig. 3). I/V curves were obtained for 10 dark-adapted cells, using electrodes with resistances of less than 100 MΩ. The maximum response amplitudes of these cells fell within the range 35–48 mV, indicating a reasonable quality of impalement. In all cases the I/V curves were approximately linear (e.g. Fig. 3), suggesting that the dark

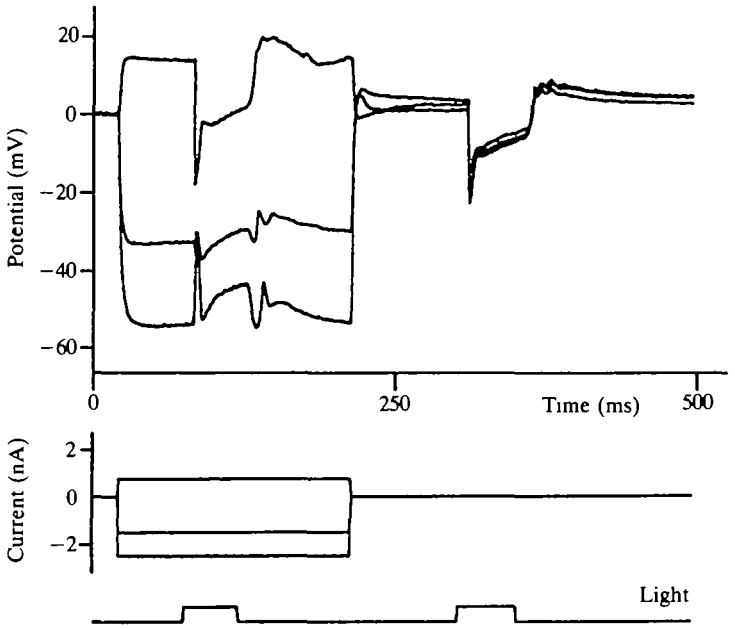

Fig. 2. Response of an LMC to injected current pulses, and to retinal illumination during and after polarization by current. Note the following: (1) the hyperpolarizing ON transient reverses at a potential close to its normal maximum amplitude; (2) depolarization increases the amplitude of all phases of the LMC response, including the OFF transient; (3) the OFF transient becomes biphasic when reversed. Each trace is averaged 10 times and voltage is measured relative to the dark resting potential at the beginning of the experiment.

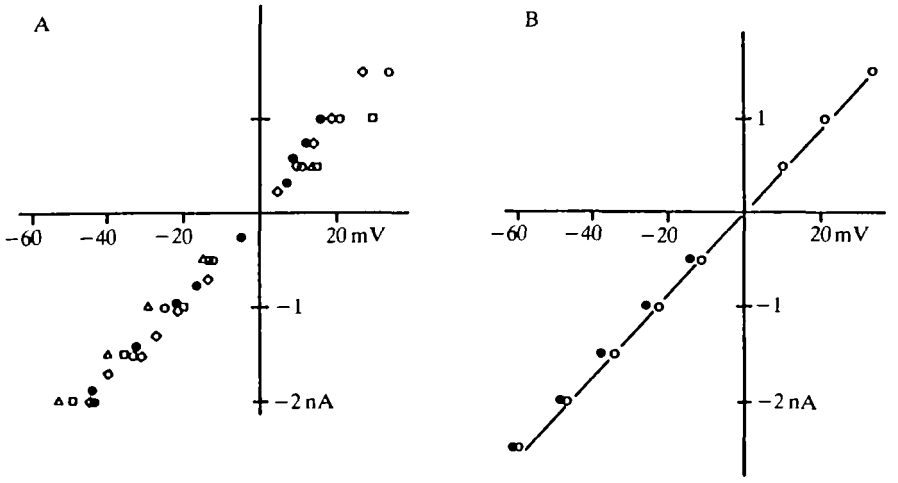

Fig. 3. I/V curves (plots of applied current against membrane polarization) for (A) five dark-adapted LMCs and (B) a single LMC when dark- (●) and light- (○) adapted. In A each type of symbol represents a different cell.

adapted LMCs are Ohmic over the physiological response range of −40 to +30 mV (measured relative to the dark resting potential). Recordings of apparent membrane potential made using the discontinuous clamp are contaminated by a component derived from the resistance of the electrode (Materials and methods). This artefactual component is presumably Ohmic, and will contribute to the linearity of the measured I/V curve. However, as discussed in the Materials and methods section, precautions were taken to minimize this artefact, and it will not obscure non-linearities that are large enough to produce changes in the I/V curve that have a significant effect upon signal amplification and coding. As an additional control, linearity was confirmed by injecting current through a balanced bridge circuit. Moreover, linearity of I/V curves has been independently established for LMCs in the dragonfly *Hemicordulia* (Laughlin, 1974*b*), the hoverfly *Eristalis* (Guy & Srinivasan, 1988) and the crayfish (Wang-Bennett & Glantz, 1987) using single-electrode techniques. In *Musca* LMCs, linearity of I/V curves has been established using double-barrelled electrodes (R. C. Hardie, personal communication). We found that the input resistances of *Calliphora* LMCs varied over a wide range, from 14 MΩ, recorded in the lamina, to 39 MΩ, recorded in the chiasm. Our data suggest that LMC input resistances are lower in the lamina ($20{\cdot}0 \pm 4{\cdot}4$ MΩ, $N = 5$) than in the chiasm ($29{\cdot}3 \pm 8{\cdot}2$ MΩ; $N = 3$). Thus *Calliphora* LMCs appear to conform to the pattern established in *Eristalis* by Guy & Srinivasan (1988), with a low-resistance synaptic zone in the lamina and a high-resistance axon spanning the chiasm. The I/V curves of light-adapted LMCs are also linear, and LMC input resistances are either identical to, or slightly less than, the dark value (Fig. 3B).

The linearity of the I/V curves suggests that voltage-sensitive conductances play a minor role in shaping the waveform of LMC responses, in both the dark-adapted and the light-adapted states. This suggestion is consistent with the observation that, although the LMC was transiently hyperpolarized by a sustained photoreceptor depolarization, it is tonically hyperpolarized by applied negative current (Fig. 2). Similarly, applied positive current produced a tonic depolarization. When injecting current, two voltage-sensitive effects were seen. The first was a difference between the time courses of polarizations of equal amplitude but opposite polarity. The membrane depolarized more rapidly than it hyperpolarized (Fig. 4A) but, at any one time, the responses to injected current differed by less than 10%. Following the release from hyperpolarization there was a small depolarization with a time course that was similar to the difference in rates of polarization just described (Fig. 4B). These two effects are consistent with the behaviour of a potassium or chloride conductance that is inactivated by hyperpolarization, either directly or through a feedback circuit. The second voltage-sensitive effect was a rapid depolarizing transient that followed the cessation of large hyperpolarizations (Fig. 4C). This rapid transient was not seen in every cell and, in agreement with Guy & Srinivasan's (1988) conclusion that this component is generated at the proximal terminal of the LMC, it was more prominent when recording in the chiasm (see also Zettler & Järvilehto, 1973).

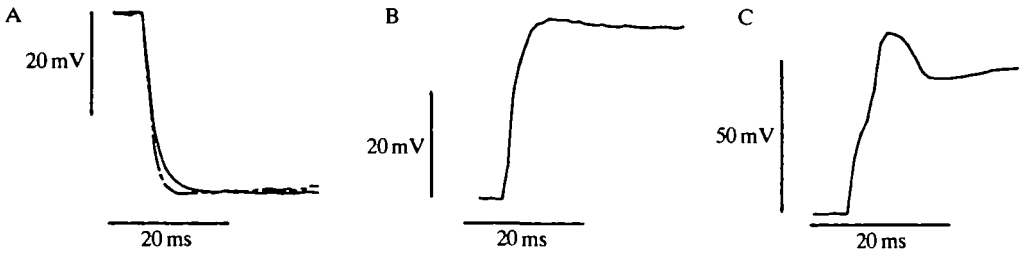

Fig. 4. Voltage-sensitive effects elicited in LMCs by applying current. (A) In a given cell the response to hyperpolarizing current of −1·5 nA (——) rises slightly more slowly than the equivalent response to a 1·5 nA depolarizing current (– – –). The depolarizing response has been inverted for the purposes of comparison. (B) Following release from hyperpolarization, a slow transient depolarization is seen: current = −1·5 nA, same cell as A. (C) A rapid voltage-sensitive polarization is elicited by releasing the cell from hyperpolarization, current = −2·0 nA. This effect is not seen in all cells. All records are the average of 10 repetitions.

The dark-adapted LMC response

An initially dark-adapted LMC responded to a prolonged pulse of light with a rapid and transient hyperpolarization. This ON response was followed by a plateau phase, during which the membrane potential decayed more slowly towards the dark level (Fig. 5). When the light was turned off, the LMC transiently depolarized. The waveform of this depolarizing OFF response depends critically upon the state of retinal adaptation. The OFF response is not seen when a brief and weak stimulus is applied to a dark-adapted retina (Dubs, 1982). When the retina is light-adapted the amplitude of the OFF response increases with background intensity and exceeds the ON response at high background light levels (Laughlin & Hardie, 1978; Srinivasan *et al.* 1982).

The light response of the dark-adapted LMC was accompanied by large and rapid changes in cell input resistance (Fig. 5). A transient decrease in resistance accompanied the ON transient. During the plateau phase there were small changes in resistance that varied in polarity and time course from cell to cell. During the OFF response the input resistance first rose above the dark level, and then fell below it. Because voltage-sensitive conductances play a minor role in the LMC response, these changes in resistance must be driven by the synaptic mechanisms that generate the response. These mechanisms were investigated in greater detail by examining the relationship between response waveform and applied current.

Conductance mechanisms generating the transient ON response

To examine the effects of applied currents on the LMC response waveform, light stimuli were presented during the polarization of the LMC, and, as a control, following recovery from polarization (Fig. 2). The amplitude of the ON transient was increased by depolarizing currents, and decreased or reversed by hyperpolarizing current (Figs 2, 6). This dependency demonstrates that the ON transient is

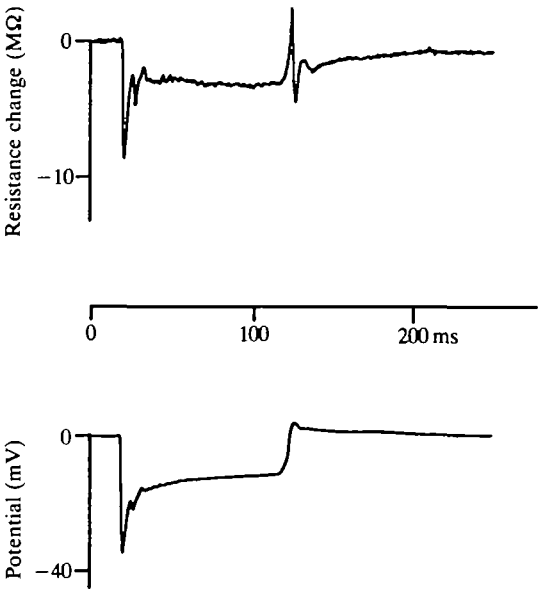

Fig. 5. The change in LMC input resistance (upper trace) that accompanies a saturated response (lower trace) to a 100 ms light pulse. Resistance measurements are made by comparing responses elicited with and without the injection of a small (0·2 nA) current (see Materials and methods for more details). The ON transient is accompanied by a correspondingly transient decrease in resistance. During the later part of the plateau phase of the response, the LMC depolarizes slightly while resistance decreases. The OFF transient is initiated by a rapid resistance increase, followed by a rapid decrease. The responses were averaged from 200 records. LMC input resistance and membrane potential were measured relative to stable dark level.

generated by an increase in a conductance, or set of conductances, with a reversal potential well below the dark resting level. Intracellular chloride injection is known to decrease the ON transient amplitude, implicating a chloride conductance increase (Hardie, 1987; Zettler & Straka, 1987). In pilot experiments, we also observed that chloride injection apparently reduced the reversal potential of the hyperpolarizing response, thus necessitating the use of sulphate-filled electrodes.

The change in cell input resistance associated with the response can be estimated by plotting response amplitude against applied current (Baylor & Fuortes, 1970; Laughlin, 1974*b*; Guy & Srinivasan, 1988). The resistance change is given by the slope of the linear relationship between applied current and response amplitude (Fig. 6). This slope is unaffected by the artefactual potential induced across the electrode by applied current. Thus the data obtained using discontinuous clamp and bridge techniques were pooled, irrespective of either the electrode time constant or the quality of bridge balance. In dark-adapted LMCs, the saturated ON responses were associated with a mean decrease in input resistance of 16·1 ± 7·52 MΩ; $N = 15$. Smaller responses to dimmer stimuli were

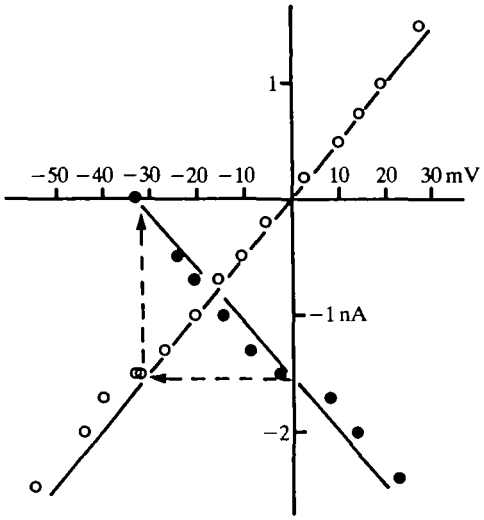

Fig. 6. The effect of hyperpolarizing current on the LMC membrane potential (○) and on the amplitude of the hyperpolarizing ON response (●). The linear decline in hyperpolarizing response amplitude indicates that the response is associated with a decrease in cell input resistance of 20 MΩ. The reversal potential of −32 mV is taken from the I/V curve at the value of current required to abolish the response, as illustrated by the arrows.

associated with smaller resistance changes. This value of the resistance change is probably an overestimate because of another artefact associated with the discontinuous current-clamp technique. When a cell's input resistance falls, one can expect its time constant to reduce in proportion. Thus, at the peak of the LMC response, when the resistance is reduced, the cell's membrane will have partially discharged by the time the voltage is sampled by the amplifier. The effect of this artefact is to displace the recorded value towards the membrane potential recorded when no current is injected. Thus, when injecting current with the switching clamp, the potential recorded at the peak of the LMC's hyperpolarizing response will be displaced towards the value recorded without current injection. This artefact will lead to an apparent increase in the change of response amplitude induced by current injection, giving a larger apparent change in resistance. It will also displace the apparent reversal potential of the response towards the true reversal potential. Comparisons with responses elicited when the same current was injected using a bridge circuit showed that the error introduced by this artefact was small.

To estimate the reversal potential of the LMC ON transient response, the current necessary to abolish the response was derived from the intercept of the current–response curve with the current axis. The corresponding membrane polarization was then determined from a particular cell's I/V curve (Fig. 6). This estimate of reversal potential is affected by artefactual potentials induced across the electrode. Taking the most reliable data, obtained with the discontinuous

current clamp and the lowest-resistance electrodes, the mean reversal potential of the ON transient was $-35 \pm 5{\cdot}4$ mV; $N = 5$ (measured relative to the dark resting potential, and recording in the lamina). Because of the changes in LMC time constant occurring during the response, the reversal potential will tend to be underestimated. However, responses were reversed by similar currents irrespective of whether they were injected by a bridge or by the discontinuous clamp, suggesting that this error was small. Indeed, such small errors are to be expected when the genuine reversal potential is close to the peak amplitude of the response, as our data suggest. For one cell recorded in the chiasm, closer to its proximal terminal, the ON transient reversed 72 mV below the dark level. This lower apparent reversal potential is to be expected when the recording site is in the higher-resistance axon, at some distance from a synaptic zone of lower resistance (Guy & Srinivasan, 1988). By comparison, the lower reversal potentials recorded in the lamina synaptic zone support the suggestion that the transient hyperpolarizing response of LMCs is generated by a chloride conductance mechanism, activated by the photoreceptor synapses (Hardie, 1987; Zettler & Straka, 1987). For this reason we will refer to this hyperpolarizing mechanism as a chloride conductance but, in the absence of exact measures of membrane potentials and reversal potentials, and without knowledge of intra- and extracellular chloride concentrations, it should be borne in mind that other species of ions may be involved.

Given that the corresponding photoreceptor input is sustained (Laughlin & Hardie, 1978), what mechanisms are responsible for producing a transient hyperpolarizing response in an LMC? Two classes of mechanism could repolarize the LMC. The first class of mechanism would produce a transient activation of the chloride conductance. The second would repolarize the LMC by activating additional conductances on the LMC membrane that would tend to depolarize it, i.e. they would have reversal potentials that are considerably more positive than that of chloride. Because the ions responsible for such depolarizing effects have not been identified we will refer to this mechanism as a depolarizing conductance. The actions of these two classes of mechanism, transient chloride conductance activation and the activation of a depolarizing conductance, may be distinguished by examining the waveforms of the light responses elicited during current injection and by measuring the resistance changes associated with light responses.

When an LMC was sufficiently hyperpolarized by injected current, the ON response reversed but remained transient (Figs 2, 7; see also Djupsund *et al.* 1987). When using the discontinuous clamp, the waveform of the reversed ON response is subject to artefacts produced by the accompanying changes in LMC time constant. However, there can be little doubt that the reversed response is truly transient. Transient reversed responses have previously been observed in insect LMCs, using bridge techniques (Laughlin, 1974*b*; Guy & Srinivasan, 1988) and we confirmed this for *Calliphora*, again using a bridge to inject current. If depolarizing conductances were responsible for repolarizing the LMC, a less transient, or even tonic, response would be expected for the following reason. When the chloride

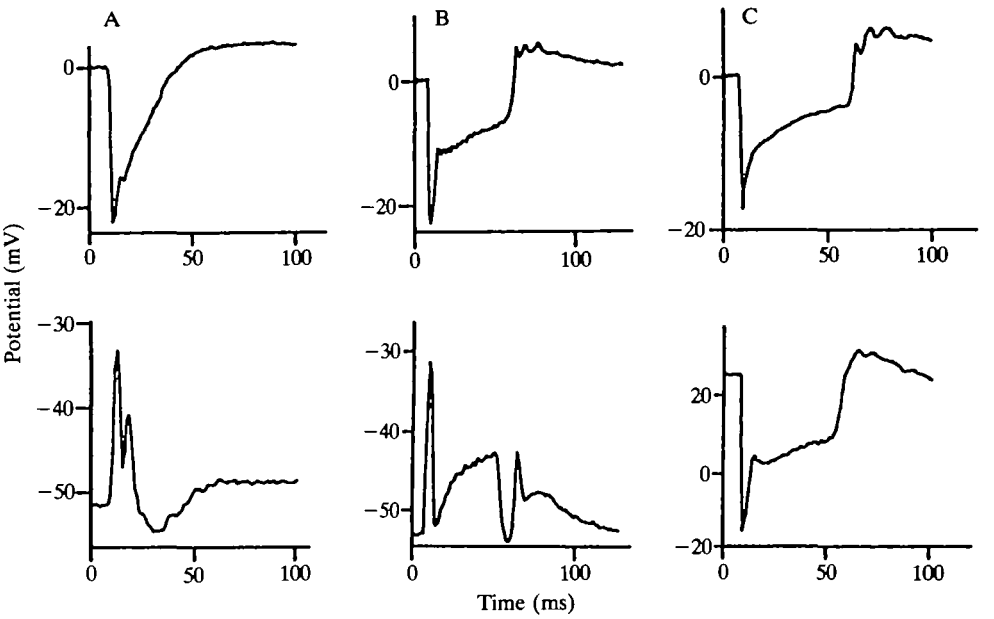

Fig. 7. The effect of polarizing current on the waveforms of the responses of dark-adapted LMCs. In each case the upper record is a control response without current, and the lower record is the response elicited in the presence of current. Current and light pulses were delivered in the sequence shown in Fig. 2 with records averaged 10 times. The membrane potential is measured relative to the dark resting potential, and is plotted against time following the onset of the light pulse. (A) Response to a brief (1 ms) flash when the initial transient is reversed by the injection of −2·0 nA current. Note the shorter duration of the initial transient, the larger oscillations and the slower hyperpolarizing wave in the reversed response. (B) Reversed responses to a 50 ms light pulse. Note the stronger and more rapid cut-back of the ON transient and the more pronounced depolarization that follows. The initial phase of the OFF response is inverted but is followed by an enhanced depolarization. Injected current = −2·50 nA. (C) Response elicited in the presence of a depolarizing current of 1·50 nA. Note the reduced cut-back in the plateau and the larger OFF transient.

response is reversed, the cell will depolarize at light ON, as observed, and it will then continue to depolarize as the depolarizing conductances are activated. Thus, the transient waveform of the reversed ON response rules out a major role for depolarizing conductances, and suggests that the chloride conductance is transiently activated.

The transient action of the chloride conductance was confirmed by continuously monitoring the changes in LMC input resistance that occurred during the response. During a transient ON response the input resistance first fell (Fig. 5), as the chloride conductance was activated and the cell hyperpolarized. After the hyperpolarizing response had reached its peak value, the LMC rapidly repolarized. During this phase of the response, the input resistance rapidly rose, suggesting that the chloride conductance was being shut down (Fig. 5). Were the

repolarization of the LMC membrane to be produced by the activation of an antagonistic depolarizing conductance, the input resistance would have continued to fall. Thus, on the basis of these two pieces of evidence, we conclude that the transient nature of the ON response results primarily from the transient activation of the chloride conductance. A similar observation has been made in crayfish LMCs, albeit for a slower response (Wang-Bennett & Glantz, 1987).

However, the action of the chloride conductance cannot account for all aspects of the LMC response. There are secondary mechanisms at work, shaping the LMC response waveform. The effects of such mechanisms may be seen in the waveforms of reversed responses and the relationships between input resistance and response amplitude. If all phases of the LMC response were to be generated by varying a single type of conductance, e.g. chloride, then (1) the reversed response would be an exact inversion of the normal response; (2) when the response amplitude is plotted against the input resistance, all measurements would fall on approximately the same curve, irrespective of when during the response a particular combination of amplitude and resistance was measured.

Under most conditions, neither of these two expectations is fulfilled. Consider first the waveforms of responses with ON transients that have been reversed by the injection of hyperpolarizing current (e.g. Fig. 7A,B). These reversed responses are not a simple inversion of the normal responses but differ in a number of respects and vary from cell to cell. A common observation is that the reversed ON transient has a faster time course and decays more rapidly than the normal response (Fig. 7A,B). In addition, oscillations or small notches in the ON response are enhanced. Following the ON transient there is, in the normal response, a slower plateau phase of repolarization (e.g. Fig. 7B,C). In cells hyperpolarized beyond the reversal potential of the ON transient, this depolarizing phase is accentuated (Fig. 7B), suggesting that a component is due to the progressive activation of a depolarizing conductance. This interpretation is supported by the observation that, when an LMC is depolarized by current injection, the amplitude of this slower repolarization is reduced (Fig. 7C).

Now consider the relationship between response amplitude and input resistance. For small amplitudes (Fig. 8A), the data points relating amplitude and resistance at corresponding times fall predominantly on a single curve, irrespective of whether they were gathered on the initial hyperpolarizing phase of the response (open circles) or during the subsequent repolarization (filled circles). Thus the relationship between resistance and response amplitude is approximately the same, irrespective of the phase of response, and the same mechanism, or set of mechanisms, must be responsible for both. For larger-amplitude responses (Fig. 8B) this simple correspondence breaks down. During the initial hyperpolarization and the subsequent phase of rapid repolarization, the relationship between resistance and response amplitude is approximately the same. This observation confirms that the early decay of the ON transient results primarily from shutting down the chloride conductance. However, during the later period of slow repolarization the relationship between response amplitude and resistance

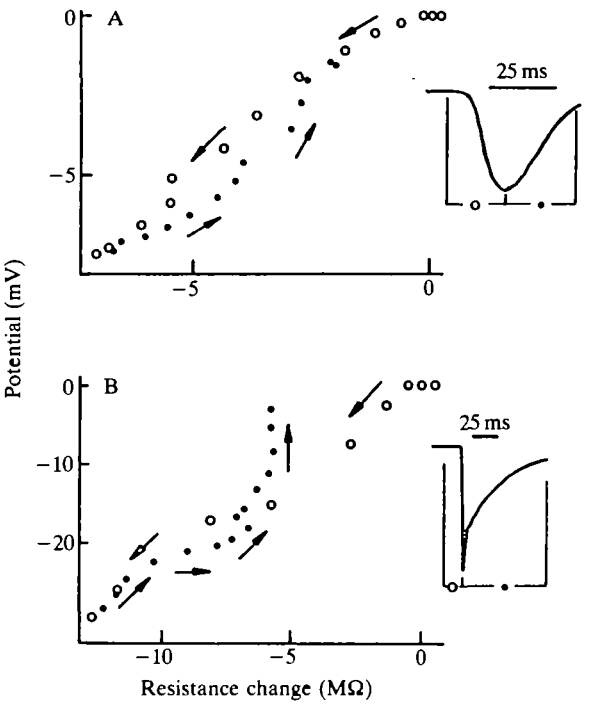

Fig. 8. The change in LMC membrane potential plotted against the corresponding change in input resistance, measured during the responses shown in the insets. The data are derived by plotting the amplitude of response (e.g. a point selected from lower record in Fig. 5) against the change in input resistance at the same instant (e.g. from upper record in Fig. 5). As shown in the insets, (○) represent points measured during the initial hyperpolarizing phase of the response and (●) represents points measured during the subsequent repolarization. The arrows show the sequence of measurements, starting in each case at the top right-hand corner. (A) During the response of a dark-adapted LMC to a weak light pulse the amplitude and resistance follow approximately the same linear relationship during both the hyperpolarizing (○) and the repolarizing (●) phases of response. (B) During the larger response (of a different cell) the points measured during the ON transient follow an approximately linear relationship. However, the last points, measured during the plateau phase of the response, show that the LMC repolarizes with little change in input resistance. Data are averaged from 200 records.

changes dramatically (Fig. 8B). The cell repolarizes with little change in input resistance. It follows that this depolarization cannot be due to the further shutdown of the chloride conductance. Additional mechanisms must be operating. The simplest explanation is that as the chloride conductance decreases, a depolarizing conductance increases. As a result, the input resistance changes little but the cell depolarizes.

Conductance mechanisms underlying the OFF transient

For dark-adapted LMCs, the waveform and amplitude of the depolarizing OFF

response are variable. Our results indicate that three components can contribute. The first is a depolarization generated by reducing the chloride conductance at light off. The second is an increase in depolarizing conductance, i.e. the activation of conductances with reversal potentials that are more positive than the dark resting potential. The third, and most variable component, is the activation of the voltage-sensitive depolarization that was generated by releasing the LMC from hyperpolarization (Fig. 4C). This latter component has already been described and will not be discussed further. The combined effects of the chloride conductance and the depolarizing conductances are demonstrated by measurements of changes of conductance during the response (Fig. 5). The small OFF response illustrated in Fig. 5 is accompanied by an early resistance increase followed by a decrease. We interpret this as follows. Immediately the light is extinguished the photoreceptors hyperpolarize and neurotransmitter release should be transiently reduced. This reduction would result primarily in a decrease in LMC chloride conductance; consequently the LMC would start to depolarize and its input resistance rise. However, our data do not exclude the possibility that additional conductance mechanisms are also shut down. This first phase of a resistance increase is followed by the activation of an unidentified conductance for ions with a more positive reversal potential. This leads to a fall in input resistance. However, this change in conductance is accompanied by little further depolarization of the LMC. We suggest that this is because the chloride conductance is starting to rise again as photoreceptor synaptic activity is restored to the tonic dark level. Observations of the light responses that have been reversed by injecting hyperpolarizing current confirm this suggestion. In these hyperpolarized LMCs, the OFF response was split into two components (Fig. 7B). The first phase was a hyperpolarization that, under the condition of chloride reversal, corresponded to a shutting down of the chloride conductance. The second phase was an enhanced depolarization, compatible with the later activation of both the depolarizing conductance and the chloride conductance. The waveform of this second phase suggests that it may be split into fast and slow components, but no further attempt at an experimental separation has been made. The complicated behaviour of the OFF transient when reversed under discontinuous current clamp cannot be attributed to artefacts associated with changes in the LMC time constant. Just before the OFF transient was initiated, the LMC input resistance was close to the dark level (Fig. 5) and under this condition clamping was good (Materials and methods). The input resistance then increased, improving clamp quality, and the subsequent decrease in resistance below the dark level (Fig. 5) was not sufficient to introduce substantial artefacts.

Conductance mechanisms in light-adapted LMCs

On light adaptation, the primary hyperpolarization in the LMCs accelerated, both in gain per unit contrast and in time course. Both these adaptation effects can be accounted for by changes in photoreceptors, without invoking modifications in lamina processing (Laughlin *et al.* 1987). Light adaptation also enhances the

transient nature of the LMC response (Laughlin & Hardie, 1978). In particular, the depolarizing components of the LMC response become more marked. Hyperpolarizing transients cut back more rapidly and depolarizing transients become larger in amplitude and faster in time course. For small signals this change is equivalent to an increase in a delayed depolarizing component in the synaptic impulse response (Laughlin *et al.* 1987).

The mechanisms responsible for generating these transients follow the pattern established in the dark-adapted cell. Plots of response amplitude against polarizing current (Fig. 9A) show that the hyperpolarizing response to brightening is dominated by the activation of a conductance or set of conductances with a negative reversal potential. This component is undoubtedly dominated by the chloride conductance and measurements of resistance changes during the response confirmed that it is transiently activated (Fig. 10A). At present we are not certain why the small-amplitude ON responses (● in Fig. 9A) reversed with less applied current than the saturating response (○ in Fig. 9A). One possibility is the artefact induced during discontinuous clamping by changes in LMC time constant, as discussed above. Another is that unidentified conductance mechanisms are activated in parallel with the chloride conductance, to an extent that depends upon the intensity of the light increment. When the light is dimmed, the LMC transiently depolarized. Small- and intermediate-amplitude depolarizations, generated by dimmings of low contrast, were associated with a decrease in conductance to ions with a negative reversal potential, similar to that for small hyperpolarizing responses (▲ and △, cf. ● in Fig. 9A). Note that the LMC input resistance was rising during these responses, therefore the cell's time constant was increasing. Because the input resistance of light-adapted LMCs is close to the dark-adapted value (Fig. 3B) and clamping is satisfactory under these conditions, the changes in time constant will have no significant effect on our recordings of these depolarizing potentials.

Continuous measurements of LMC input resistance (Fig. 10B) showed that the conductance decrease accompanying dimming had a similar time course to the voltage response. In addition, both the depolarization and the conductance change were, at least to the peak of the response, mirror images of responses to intensity increments of a similar amplitude (Fig. 10B). The similar reversal potentials and time courses, and the opposite response polarities and resistance changes, suggest that both the small depolarizing responses to intensity decrements and the hyperpolarizing responses to intensity increments were generated by the same mechanism. Again this mechanism is likely to be dominated by the direct synaptic input from photoreceptors. Intensity increments depolarize photoreceptors, and this should lead to a transient elevation in transmitter release above a tonic level and a corresponding increase in LMC chloride conductance. Conversely, intensity decrements hyperpolarize the photoreceptors, and transiently depress transmitter release, so generating a transient decrease in chloride conductance and a depolarization of the LMC.

Our findings indicate that the larger depolarizations evoked in LMCs by stimuli

of high contrast are generated both by shutting down the chloride conductance and by activating a depolarizing conductance. The interplay between these two conductance mechanisms is illustrated by hyperpolarizing the LMC beyond the

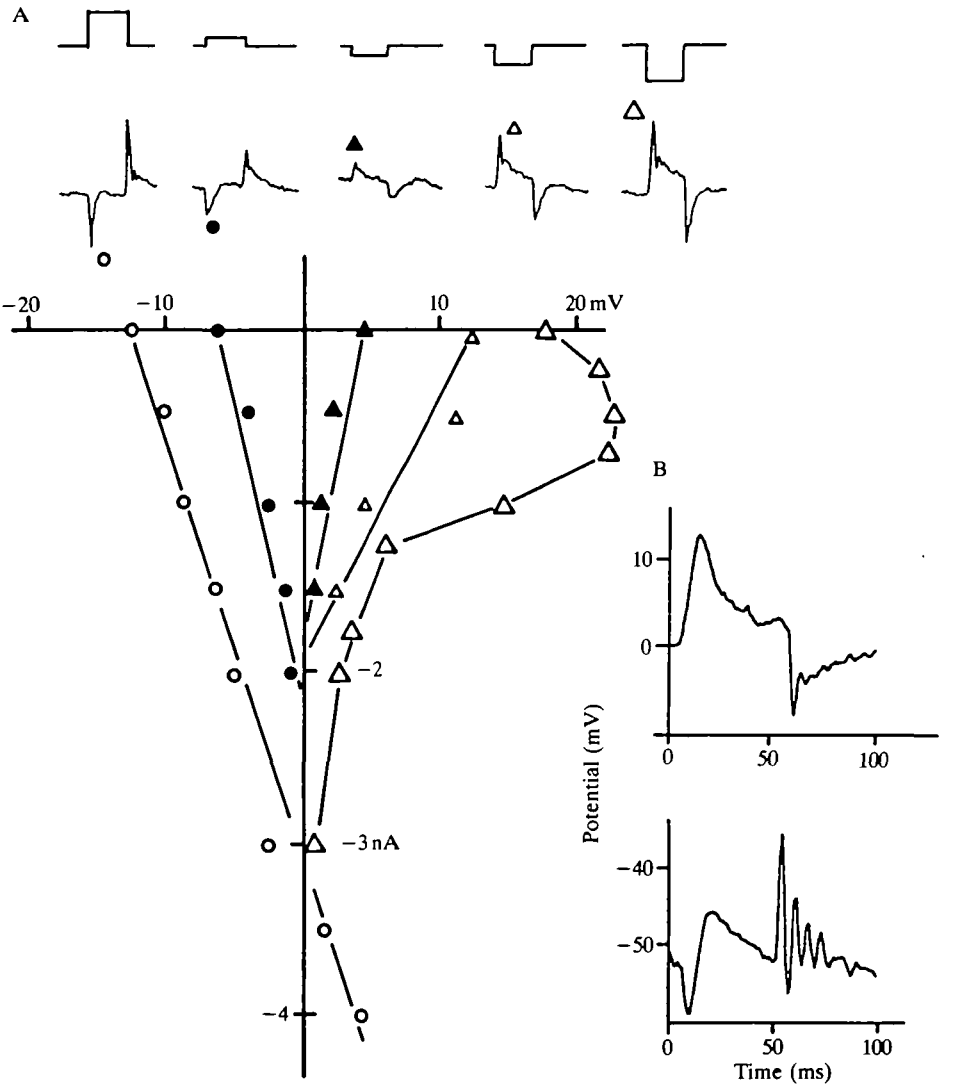

Fig. 9. The effects of injected current on the responses of light-adapted LMCs. (A) The dependence of amplitude on polarizing current for five different types of response elicited from a single LMC. The symbols for the appropriate curves are shown. From left to right these are: (○) the amplitude of the transient hyperpolarizing response to a large increment; (●) the amplitude of the hyperpolarizing response to a small increment; (▲) the amplitude of the depolarizing response to a small decrement, (△) an intermediate decrement and (△) a large decrement. (B) The waveform of a response to a stimulus decrement of high contrast, before and after reversal by applied current of −2·50 nA. Potential was measured relative to the dark resting potential. The time scale starts at stimulus onset; stimulus duration 50 ms.

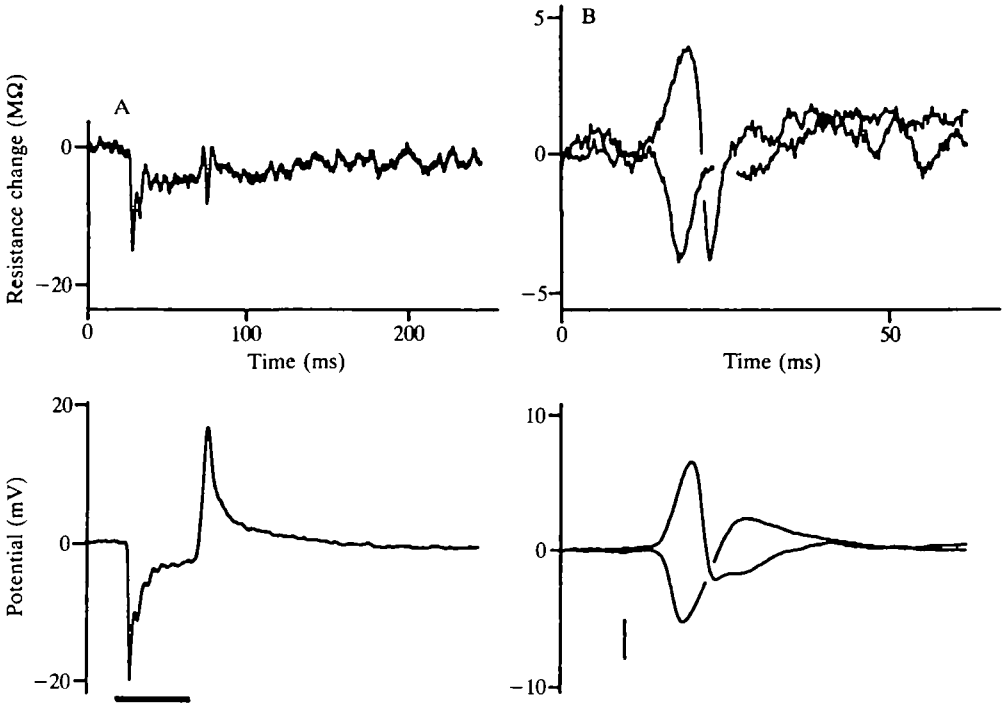

Fig. 10. Resistance changes (upper traces) measured during the responses (lower traces) of light-adapted LMCs. Resistances and voltages are measured relative to constant background level. (A) Response to a large pulse increment of 50 ms duration, indicated by the bar beneath the response. (B) Impulse responses to a brief intensity decrement and brief increment, of 1 ms duration (time of delivery shown by lower vertical bar). The responses are superimposed to illustrate their symmetry. In response to a decrement, the LMC initially depolarizes and resistance rises, whereas for an increment the cell hyperpolarizes and resistance falls.

reversal potential of the hyperpolarizing mechanism. As found for the OFF responses of the dark-adapted LMC, only the initial phase of the response reversed (Fig. 9B), showing that an initial reduction in chloride conductance is followed by the activation of a depolarizing conductance mechanism. The relationship between response amplitude and applied current (Fig. 9A) provides further evidence for the involvement of two conductance mechanisms. Small hyperpolarizations of the LMC membrane increased the amplitude of the largest response to dimming, suggesting that a depolarizing conductance mechanism is involved. However, further hyperpolarization of the LMC membrane by injected current reduced the amplitude of the response to dimming, suggesting that the shut-down in chloride conductance is also occurring. In summary, stimuli of low constrast are coded almost exclusively by modulation of the chloride conductance, as expected from the action of the photoreceptor synapses (Fig. 10B). As in the dark-adapted cell, the larger ON responses are dominated by the transient ctivation of the chloride conductance. Larger OFF responses contain a com-

ponent generated by an unidentified depolarizing conductance in the LMC membrane.

Effects of stimulus position on ON response

Another method of dissecting the components of the LMC response is to exploit the dependence of the response upon the position of the stimulus. LMCs exhibit lateral antagonism (Zettler & Järvilehto, 1972) that tends to be stronger in the horizontal than the vertical axis (Dubs, 1982), an observation that we confirmed but did not study further. This lateral antagonism narrows LMC receptive fields, relative to receptors' fields (Zettler & Järvilehto, 1972), and produces a low-frequency roll-off in the spatial frequency response (Dubs, 1982). The response waveform also changes with stimulus position. As a point source is moved away from the centre of the LMC receptive field (the axis), the saturated amplitude of the hyperpolarizing response decreases, and depolarizing response components become more prominent. With appropriate off-axis stimuli a purely depolarizing response to light is observed (Zettler & Weiler, 1976; Dubs, 1982; Shaw, 1981).

The effects and origins of lateral antagonism were studied using a simple centre-surround stimulus. The relative intensities of the centre and the 6° annulus were adjusted to give equal responses in a dark-adapted photoreceptor or LMC when the unit's optical axis (i.e. the point of maximum sensitivity in its receptive field) was aligned with the centre. Under this condition the total energy of the annulus was 12–30 times that of the centre, depending principally upon the interommatidial angle in the retinal area being stimulated (Hardie, 1985), and to a lesser extent upon the quality of the optics. Since we found that the photoreceptor angular sensitivities did not change measurably over the range of adapting intensities used (up to 2×10^4 effective photons receptor^{-1} s^{-1}), the changes in the LMC receptive fields seen on light adaptation are likely to have been due to signal processing in the lamina itself. In addition, under light-adapted conditions, stimuli of low contrast were used to avoid saturating the LMCs that looked directly at points on the annulus.

LMC responses to centre and surround

The nature of the LMC response to the surround is determined by the adaptation state of the eye (Dubs, 1982). In the dark-adapted state, LMC impulse responses are monophasic to flashes containing fewer than about 10 effective photons receptor^{-1}, and are similar in waveform for both centre and surround stimuli. However, as reported by Dubs (1982), there are small differences. We found that the responses to off-axis stimuli peaked slightly later and decayed more slowly and we also observed a similar difference in photoreceptors and their terminals in the lamina, as have Weckström *et al.* (1988). These observations support Dub's (1982) contention that such differences are genuine physiological effects, and not artefacts (Smakman & Stavenga, 1987). Changes in membrane resistance during small responses (<5 mV) in dark-adapted LMCs showed a conductance increase of similar duration to the LMC depolarization and peaked a

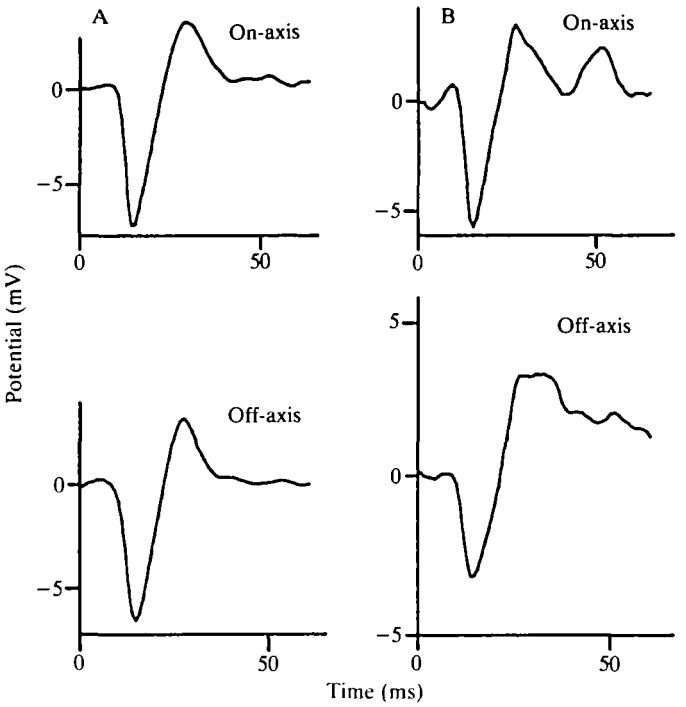

Fig. 11. Changes in the waveforms of impulse responses with stimulus position in identified light-adapted cells. Stimulus onset is at time zero. (A) The responses of a marked L1 cell to on- and off-axis illumination are similar in waveform. Contrast of centre stimulus = 0·5, energy in 6° annulus = 24 × centre energy. Each waveform is from 100 averaged records. (B) The responses of a marked L2 cell to on- and off-axis stimuli differ: the relative amplitude of the depolarizing component is increased off-axis. Stimulus as in A.

the same time. The coincident timing of the conductance and the voltage peaks in this primary hyperpolarization implies that a single mechanism, presumably the photoreceptor synapse, is responsible for generating both the centre response and the slightly different surround response.

Light adaptation reduced the sensitivity of LMCs to off-axis stimuli relative to on-axis ones, and we found that there was no concomitant change in photoreceptor angular sensitivity. However, there were marked differences between individual LMCs. In some cases the waveforms of the responses to the on- and the off-axis stimuli were very similar (Fig. 11A), whereas in others the relative amplitude of depolarizing components was greatly increased off axis (Fig. 11B). The identification of nine cells by dye injection suggested that these differences were correlated with cell type. The two L2 cells that we marked showed the pronounced increase in depolarizing components with off-axis stimulation, whereas the five L1 cells and two L3 cells did not. However, no marked cell gave a pure depolarizing response to an off-axis intensity increment of the type seen in Fig. 12. It is

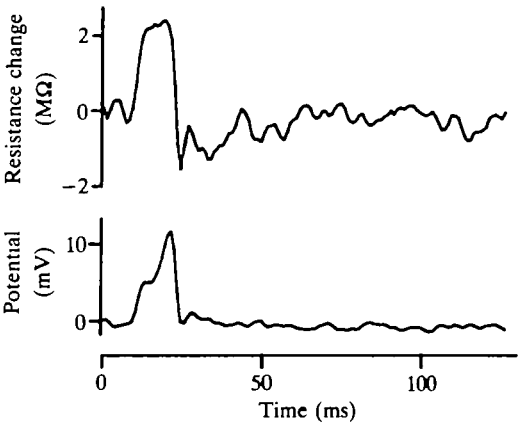

Fig. 12. The change in input resistance (top trace) and the voltage response (lower trace) induced by illumination of the 6° annulus in a light-adapted LMC. Note that the depolarizing response to brightening of the annulus is associated with an increase in input resistance. The voltages and resistances are measured relative to the steady-state levels found with the adapting background alone. The stimulus was 10 ms brightening of surround with a contrast of 0·5.

important to note that the strongly depolarizing responses to off-axis light were associated with a marked conductance decrease (Fig. 12), as also found in crayfish (Wang-Bennett & Glantz, 1987). Furthermore, depolarizations of this type were also recorded in the chiasm. These two observations show that depolarizations are a genuine component of the intracellular LMC signal which would probably be seen by the medulla terminals.

The time course of light adaptation

Support for the idea that at least three distinct physiological mechanisms act directly or indirectly upon LMCs (the direct photoreceptor input, a lateral antagonism of this photoreceptor input and a separate set of depolarizing mechanisms) comes from observing the time course of light adaptation. A dark-adapted LMC was stimulated by 5 ms flashes, delivered to the centre and the surround of the receptive field, and adjusted to give approximately equal responses (Fig. 13). The cell was then light-adapted by turning on a widefield background. The responses to both centre and surround flashes changed rapidly. The response to the surround converted from a hyperpolarization to a depolarization within 100 ms (Fig. 13). Because the intensity of the surround stimulus was set to a level that did not saturate the surround response, the rapid onset of the depolarization was a true reflection of the rapid onset of lateral antagonism with light adaptation. By comparison, the ON and OFF transients of the centre response adjusted more slowly to light adaptation, increasing over a period of 5 s (observed in five experiments). This difference in the time course of adaptatio

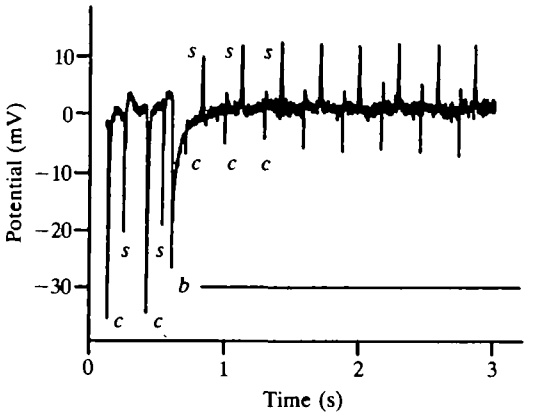

Fig. 13. The time course of the potentiation of both intra-cartridge and lateral antagonism in a fly LMC. The cell is alternately stimulated by 5 ms light pulses delivered first to the centre (*c*) and then the surround (*s*). These pulses saturate the dark-adapted LMC but were adjusted in intensity to produce equal responses in a photoreceptor centred on the stimulus. After two cycles of presentation in the dark, the full background is switched on, producing the response indicated by (*b*) and is sustained for the rest of the record, as shown by the horizontal line. Background illumination immediately reverses the polarity of responses to the surround. The responses to the centre are smaller than before and develop a depolarizing OFF transient over the course of 2 s.

again indicates that inter-cartridge and intra-cartridge antagonism are activated by different sets of adaptation processes.

Discussion

Measurements of the resistance changes associated with both the hyperpolarizing and the depolarizing responses of LMCs allow us to examine the underlying conductance mechanisms. First, we will discuss the nature of these conductance mechanisms, and then assess the role they play in the neural enhancement of the photoreceptor signal. Finally, we will examine the design principles that govern the generation and transmission of signals in LMCs.

LMC conductance mechanisms

LMC responses are generated by a hyperpolarizing conductance mechanism, with a reversal potential that is at least 30–40 mV negative to the dark resting potential and a depolarizing mechanism (or mechanisms) which, although not reversed experimentally, has been inferred to have a more positive reversal potential. The LMC's transient hyperpolarizing response to brightening is generated by the transient activation of the hyperpolarizing conductance mechanism. The sensitivity of the reversal potential of the hyperpolarizing transient to injected chloride suggests that the hyperpolarizing mechanism is a chloride

conductance (Zettler & Straka, 1987; Hardie, 1987). This chloride conductance is thought to be activated by histamine, released from the synaptic terminals of the photoreceptors (Hardie, 1987). Moreover, large quantities of histamine are present in the retina (Elias & Evans, 1983) and in the terminals of the photoreceptors R1–6 (Nässel *et al.* 1988). Thus, it is reasonable to attribute much of the hyperpolarizing conductance to the activation of chloride conductances by the release of histamine at photoreceptor–LMC synapses. Our data support the evidence that the photoreceptor–LMC synapses are tonically active in the light (Laughlin *et al.* 1987). When the light dims we find that the LMC chloride conductance decreases and the cell depolarizes. The dimming of the light hyperpolarizes the photoreceptors (e.g. Laughlin *et al.* 1987). We suggest that this hyperpolarization reduces neurotransmitter release at the photoreceptor–LMC synapses, leading to a reduced LMC chloride conductance. Our data indicate that this reduction makes a substantial contribution to the LMC's depolarizing response to dimming. Thus, our measurements of conductance changes show that the direct input from the photoreceptor synapses dominates both ON and OFF phases of the LMC response, a proposal that is supported by the observation that this class of synapses outnumber all other inputs to LMCs by approximately 50:1 (Shaw, 1984).

The depolarizing conductance mechanisms are weaker in action, and are observed under three conditions. Following the release of the LMC from hyperpolarization a rapid transient depolarization is generated. This rapid depolarization is larger in amplitude and faster in time course when recorded in the chiasm (Zettler & Järvilehto, 1973; Laughlin & Hardie, 1978; Guy & Srinivasan, 1988), suggesting that the depolarization is generated at the medulla terminals of the LMC. A similar response component is observed in the analogous second-order ocellar neurones of insects (Wilson, 1978) and barnacle (Oertel & Stuart, 1981), and pharmacological evidence suggests that, in cockroach ocellar neurones, this response is a calcium spike generated at the output synapses (Mizunami *et al.* 1987). In LMCs, the origin of this depolarizing component has not been resolved. A slower depolarizing conductance mechanism is observed during the OFF response of light-adapted LMCs, as seen in barnacle ocellar neurones (Oertel & Stuart, 1981) and crayfish LMCs (Wang-Bennett & Glantz, 1987). In fly LMCs there is also a steady build-up of a depolarizing conductance during the plateau response to sustained illumination. The different conditions under which these three depolarizing effects are observed, and their individual time courses, suggest that they could be driven by independent neural pathways, such as the cell L4 and the small-field centrifugal neurones (Shaw, 1984; Strausfeld, 1984). However, our evidence does not exclude the possibility that a single presynaptic cell could provide all synaptic depolarizing inputs to LMCs and that the differences could reflect the way in which this one cell is driven.

The uncertain identity of the depolarizing components emphasizes that several inputs to LMCs have yet to be explained. For example, what are the origins of the oscillations in LMC response (Laughlin & Hardie, 1978; van Hateren, 1987)?

Moreover, a critical LMC conductance mechanism has not been resolved. In darkness, and in steady light, the photoreceptor synapses are tonically active (Wang-Bennett & Glantz, 1987; Laughlin *et al.* 1987), yet the membrane potential is unusually high. The LMC is depolarized to a level about 40 mV positive to the chloride reversal potential. It follows that a sustained depolarizing conductance must oppose the tonic synaptic input to maintain the LMC in this partially depolarized state, a state that is essential if the LMC is to be further hyperpolarized by intensity increments. Given that we find no evidence for powerful voltage-sensitive conductances in LMCs, is the sustained depolarization generated by spontaneously active channels in the LMC membrane or by synapses? Zimmerman (1978) suggested, on the basis of the response to bicuculline, that γ-aminobutyric acid (GABA) tonically depolarizes LMCs in darkness and this action of GABA has been demonstrated directly (Hardie, 1987). The small-field centrifugal fibre, C2, is presynaptic to LMCs L1 and L2 (Strausfeld, 1984) and antibody staining suggests that it is GABAergic (Meyer *et al.* 1986; Datum *et al.* 1986). However, structural considerations suggest that this GABAergic neurone can only provide a small fraction of the depolarizing input that opposes the chloride conductance. The small number of C2 synapses are restricted to the very top of the LMC dendritic region (Strausfeld, 1984) and are unlikely to be effective in antagonizing the large numbers of photoreceptor synapses distributed over the full length of the synaptic zone. If a tonic depolarizing synaptic input to LMCs were modulated by changes in light level, it could help account for the changes in depolarizing conductance that have been inferred during the plateau phase of the response and the OFF transient. In addition, a depolarizing synaptic input would contribute to signal amplification if it were transiently decreased by light and transiently increased by dimming.

Mechanisms that amplify the LMC signal

The amplification of the signal, as it passes from photoreceptor to LMC, is an essential component of signal enhancement. Amplification promotes coding efficiency by expanding the visual signal to fill the LMC dynamic response range (Laughlin, 1981*b*). The amplified signal is thus rendered more resistant to noise contamination. Moreover, synaptic amplification can improve the signal-to-noise ratio for synaptic transmission (Laughlin *et al.* 1987). At the receptor–LMC synapse amplification is optimized by matching its gain to the statistics of natural signal levels. This matching is implemented by the sigmoidal characteristic curve that relates the presynaptic photoreceptor response amplitude to the postsynaptic LMC response (Laughlin *et al.* 1987). The fact that this measured characteristic curve follows a function associated with transmission at a chemical synapse (Falk & Fatt, 1972; Shaw, 1979, 1981) suggests that amplification can be 'matched' to signal statistics, by operating a single type of chemical synapse with an appropriate high sensitivity for transmitter release (Laughlin *et al.* 1987). Our measurements of conductance provide a direct test of this hypothesis. If, in the light-adapted LMC, a single type of chemical synapse mediates all transient responses, then both the

depolarizing responses to dimming and the hyperpolarizing responses to brightening will be associated with a single conductance mechanism.

We find that both hyperpolarizing and depolarizing responses are generated by transient changes in chloride conductance that can be attributed to the direct action of photoreceptor synapses. This conductance changes rapidly by large amounts, reducing LMC input resistance by as much as 75 % in a few milliseconds (e.g. Fig. 5). Thus, our evidence strongly supports the hypothesis that amplification results from the direct action of high-gain photoreceptor synapses (Shaw, 1979). However, the larger OFF transients generated in light-adapted LMCs contain a contribution from one or more depolarizing conductance mechanisms. Our data on the role played by this depolarizing component in such OFF transients are equivocal. On the one hand, the amplitudes of such responses are boosted by injecting small amounts of negative current (Fig. 9A), suggesting a contribution from a depolarizing mechanism. On the other hand, both the continuous measurements of conductance and the waveforms of reversed responses indicate that a decrease in hyperpolarizing conductance dominates the peak of the voltage response. Further analysis of this early phase of large OFF responses is required to determine the roles played by depolarizing conductances in signal amplification.

Antagonistic mechanisms in the lamina

Antagonism is the second essential component of signal enhancement, allowing for the amplification of incremental and decremental signals over a wide range of background intensities. The high-gain synapses have a much smaller dynamic range than the excursion of photoreceptor membrane potential produced by normal background intensities (Laughlin & Hardie, 1978). As described in barnacle ocellus (Hayashi *et al.* 1985), antagonism keeps the characteristic curves for synaptic transmission centred on the mean level of photoreceptor depolarization, so allowing for the high-gain transmission of small incremental and decremental signals over a wide range of backgrounds (Laughlin & Hardie, 1978; Laughlin *et al.* 1987). The decay in LMC response to a step change in intensity represents the resetting of the characteristic curve to the new steady-state level of receptor response. By generating such transient responses, antagonism gives transmission the properties of a high-pass filter, with synaptic gain increasing with frequency (Järvilehto & Zettler, 1971), and this behaviour is simply described by a biphasic synaptic impulse response. A small and momentary receptor depolarization results in an LMC response consisting of a rapid hyperpolarization followed by a slower antagonistic response of opposite polarity. This simple impulse response accounts for small hyperpolarizing and depolarizing LMC transients (Laughlin *et al.* 1987), suggesting again that LMC signals are primarily generated by regulating the output of tonically active photoreceptor synapses.

What mechanisms mediate antagonism? A simple model of synaptic transmission from receptors to LMCs (Laughlin *et al.* 1987) suggests that antagonism could act in several ways: (1) by activating shunting and/or depolarizing conductances in the LMC membrane; (2) by desensitizing the response to synaptic

transmitter; (3) by reducing the amount of transmitter released by a given receptor input; and (4) by subtracting a steady voltage component at the photoreceptor terminals (Laughlin *et al.* 1987). We can now eliminate or qualify a number of these possibilities. The cut-back in hyperpolarizing response is accompanied by a rapid restoration of input resistance; consequently, the activation of a depolarizing postsynaptic conductance cannot be responsible for this rapid component of antagonism. Such a depolarizing conductance appears to play a minor role in repolarizing the LMC during the later plateau phase of the response. The desensitization of the postsynaptic binding sites or channels is unlikely to be important because ionophoretically applied histamine produces a tonic hyperpolarization of the LMC (Hardie, 1987). We conclude that the major components of antagonism act presynaptically to regulate transmitter release from photoreceptors.

How is this hyperpolarizing photoreceptor input regulated? Two further classes of presynaptic effect must be considered. The first possibility is an intrinsic mechanism within the photoreceptor–LMC synapse, of the type investigated in barnacle, where transmitter release appears to be restored to a tonic rate following a change in potential (Hayashi *et al.* 1985). LMCs respond reliably and consistently to rapidly repeated flashes, arguing against a role for transmitter depletion in the presynaptic terminal. The inactivation of calcium channels has been suggested as a means of generating transient responses (Simmons, 1985) but this probably requires a population of channels, activating and inactivating over different parts of the range of presynaptic depolarizations (Hayashi *et al.* 1985). For barnacle it has been suggested that the voltage generated by the presynaptic calcium conductance is opposed by a calcium-activated potassium conductance, operating locally at the presynaptic terminal (Hayashi *et al.* 1985). At present there is no evidence for this mechanism operating in fly photoreceptors.

The second possibility is that antagonism opposes the voltage signal driving the photoreceptor synapses by depolarizing the extracellular space surrounding the photoreceptor terminals and/or by activating synaptic inputs onto photoreceptor terminals (for a review, see Shaw, 1984). There is evidence that current from the photoreceptor terminals is forced to flow across high-resistance barriers between the retina and lamina (Shaw, 1975), and between lamina cartridges (Shaw, 1984). The resulting depolarizations of the extracellular space are well placed to mediate antagonism by subtracting directly from the potential at the receptor terminal (Laughlin, 1974*a*). This extracellular mechanism readily accounts for the observation that the background component of the receptor response is removed without reducing the gain for the transmission of rapid changes in intensity because it is subtractive (Laughlin & Hardie, 1978). Moreover, lateral current flow in the lamina could mediate lateral antagonism (Shaw, 1975, 1981; Dubs, 1982). As in crayfish (Wang-Bennett & Glantz, 1987), we find that lateral antagonism is associated with an increase in LMC input resistance. This observation supports, but by no means proves, the hypothesis that extracellular current flow mediates lateral antagonism, because it is consistent with the suppression of transmitter

release from the photoreceptor terminals (Wang-Bennett & Glantz, 1987). Our measurement of a resistance change also resolves Shaw's (1979, 1981) well-founded question as to whether the depolarizations indicative of lateral antagonism are of functional significance. Any mechanism that acts directly upon the synaptic output of photoreceptors must determine the passage of information from retina to lamina. However, our observations raise a new problem. If a major component of lateral antagonism acts presynaptically on the photoreceptor terminals, how can L2 exhibit a more pronounced lateral antagonism than L1 and L3, when these three cells are adjacent postsynaptic elements at tetradic synapses (for a review, see Shaw, 1984)? One possibility is that the differences in amplitude of depolarizing response used to distinguish the marked cells reflect differing additional contributions to lateral antagonism from depolarizing conductance mechanisms.

Our experiments provide no clear evidence for the action of the feedback synapses made onto the photoreceptor terminals (Shaw, 1981, 1984), although these could act subtractively, providing that they opposed rather than shunted the current flowing into the terminals from the retina. A powerful negative feedback from LMCs to photoreceptor terminals should show up when LMCs are hyperpolarized by current injection. For example, hyperpolarization might drive the LMC output synapses below their operating range, so breaking the feedback loop. Alternatively, by reversing the LMC response, a negative feedback loop would be converted to a positive one, again eliminating the transient response. In fact, the reversed LMC response retains its transience. Moreover, were a feedback loop powerful enough to produce the ON transient, negative current injected into an LMC would have produced an equivalent transient polarization by stimulating the feedback system to shut down the tonically active receptor–LMC synapses. We find that LMCs respond remarkably passively to injected current. However, our results do not eliminate the possibility that the identified feedback synapses play a significant role in shaping LMC responses (Shaw, 1981, 1984). Synaptic inputs to receptor terminals, such as those provided by the alpha and centrifugal fibres (Shaw, 1984; Strausfeld, 1984), are probably unaffected by current injection into LMCs.

We now turn to the relationship between antagonism and adaptation state. Antagonism removes the background component from the LMC response. At low intensities, photon noise seriously contaminates the photoreceptor signals. To ensure that the components removed by antagonism are a reliable (noise-free) estimate of the background signal, the antagonistic mechanisms must be adjusted so that they average signals over wider areas of space and time. In other words, antagonism is weakened at low light levels and its space and time constants are increased (Srinivasan *et al.* 1982). Because both the level of tonic response and the input resistance of LMCs are relatively little affected by background light, it is unlikely that the signal responsible for regulating the level and extent of antagonism comes directly from the LMC membrane potential. These regulatory mechanisms, like antagonism itself, are more likely to reside in the photorecepto-

terminals, or cells other than LMCs that are postsynaptic to receptors. However, if antagonism continually resets the synaptic output of the photoreceptor terminals to the dark level, what remains to regulate antagonism as a function of mean luminance? The most likely possibility is that antagonism does not completely reset the level of synaptic activity. Instead, the sustained mean activity of photoreceptor synapses slowly increases with background intensity. In support of this hypothesis, the saturated LMC ON response amplitude decreases with light adaptation whereas the OFF response increases (Laughlin & Hardie, 1978). A corresponding shift of operating point down the characteristic curve is also observed, together with an increase in high-frequency synaptic noise levels (Laughlin *et al.* 1987). In addition, the offset in hyperpolarizing input may be greater than is indicated by the maximum ON transient amplitude because the slow tonic depolarization counteracts the input from photoreceptor synapses. How, then, might this tonic increase in receptor input act on the lamina to enhance transience and to increase the strength of lateral inhibition? An interesting possibility (Shaw, 1984) is that the synapses connecting photoreceptors to glial cells regulate antagonism by changing the resistances of the extracellular barriers.

The structure and function of LMCs

The large monopolar cells L1, L2 and L3 are essentially cables with four sections, a passive cell body and neurite, the synaptic zone in the lamina, a cable crossing the chiasm to the medulla and the medulla terminal (Guy & Srinivasan, 1988). Our data support the view that most components of the LMC signal are generated in the lamina and transmitted passively to the medulla. The response of the lamina synaptic zone is dominated by the synaptic drive from photoreceptors. With over 1200 synapses impinging on each LMC in this narrow zone it is not surprising that a saturated hyperpolarizing response is associated with a decrease in input resistance of 75 %. The remarkable property of signal generation in this zone is that the LMCs appear to act as passive integrators of synaptic drive. Our evidence suggests that the two dominant operations of signal enhancement, amplification and antagonism (including lateral effects), are primarily executed presynaptically by the regulation of the array of high-gain photoreceptor synapses. Voltage-sensitive conductances and feedback from LMCs to photoreceptors are apparently of little significance, although we cannot exclude the possibility that electrode impalement has introduced an artefactual low-resistance shunt that reduces voltage-sensitive effects. Moreover, the LMC response is dominated by the hyperpolarizing input from photoreceptor synapses: depolarizing inputs appear to be of secondary importance. Note that presynaptic processing has the advantage of economy: it tends to stabilize the photoreceptor transmitter flux and the postsynaptic LMC response. Given the large number of synapses in a cartridge, this stabilization of output may simplify the problems associated with neurotransmitter turnover and with ionic homeostasis in the restricted extracellular space and in LMCs.

Cable modelling suggests that the signals generated in the lamina can be

passively conducted to the medulla (van Hateren, 1986*b*; Guy & Srinivasan, 1988). Thus, both the hyperpolarizing LMC responses generated by increased activity of the photoreceptor synapses and the depolarizing responses generated by decreased receptor input are generated in the lamina and can be transmitted to the medulla. In addition, a component of the OFF response in dark-adapted cells is generated in the medulla. This finding led to the proposal that LMC responses are generated by spatially distinct mechanisms, with hyperpolarizing ON signals generated in the lamina and depolarizing OFF signals generated in the medulla (Guy & Srinivasan, 1988). We find that, for light-adapted cells, both hyperpolarizing and depolarizing components are generated by the photoreceptor synapses in the lamina. The function of the medulla response component in light-adapted cells has not been determined.

Finally, we consider the coding of information in parallel channels in the fly lamina. Previous work (for a review, see Laughlin, 1987) has suggested that the LMCs are designed to optimize the transmission of information from retina to medulla, so raising a paradox as to why there should be a replication of channels, with L1–3 receiving similar synaptic inputs and generating similar responses. Two of these three channels appear to be redundant and the fractionation of signal within these cells (e.g. a division into ON and OFF pathways) would improve signal transmission by increasing the effective response range. A plausible resolution to this paradox is that the different levels of LMC termination in the medulla (Strausfeld & Nässel, 1981) reflect inputs to different medulla subsystems, as described for locust (Osorio, 1987*a*,*b*). The requirements of each subsystem may engender the need for different receptive field shapes and response time courses in LMCs, as seen in the responses of L1 and L2 to off-axis light. In addition, the local processing of signals executed at the medulla terminals may differ between subsystems and, in graded potential cells of this type, it would be difficult to prevent interference between subsystems if the two types of local processing operated close together in the one neurone.

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