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THE TRANSFER OF SIGNALS FROM PHOTORECEPTOR CELLS TO LARGE SECOND-ORDER NEURONES IN THE OCELLAR VISUAL SYSTEM OF THE LOCUST *LOCUSTA MIGRATORIA*

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Summary

The operation of the first synapse in the ocellar pathway of the locust *Locusta migratoria* has been studied by making simultaneous intracellular recordings from photoreceptors and large, second-order L-neurones.

1. The transfer curve for the synapse, obtained by plotting the amplitudes of the initial peak responses by the two cells to pulses of light against each other, shows that L-neurones are extremely sensitive to changes in photoreceptor potential and that the connection is tonically active in darkness.

2. Postsynaptic current in an L-neurone, produced when pulses of light are delivered from a dark background, saturates at a slightly brighter light intensity than does the postsynaptic potential.

3. The signal-to-noise ratio improves with increases in light intensity in both cells, but the reduction in noise as signals are transmitted from photoreceptors to L-neurones is less than would be expected from the number of photoreceptors that probably converge on each L-neurone.

4. In both cells, in the presence of different intensities of background illumination, the slope of the intensity–response curve is maintained as the curve moves along the light intensity axis. Adaptation is relatively slow so that, at least for several minutes after an increase in background illumination, both cells maintain a sustained response and the responses to stimuli of increased illumination are reduced in amplitude. During sustained background illumination, the transfer curve for the synapse between a photoreceptor and an L-neurone shifts along both axes without a change in its maximum slope.

5. The slope of the synaptic transfer curve depends on the speed as well as the amplitude of changes in light.

6. In response to injection of depolarising pulses of current into a photoreceptor, an L-neurone generates brief, hyperpolarising responses. The amplitude of the responses depends on the strength and speed of the depolarising stimuli. After an initial response by an L-neurone, subsequent responses are reduced in amplitude for 200 ms.

7. The amplitude of L-neurone responses to electrical stimulation of a photoreceptor increases when the hyperpolarising constant current is injected into the photoreceptor.

Key words: synapse, retina, adaptation, noise, insect, locust, *Locusta migratoria*.

Introduction

Large second-order neurones of the ocelli (L-neurones) of the locust have both very extensive visual fields and a high sensitivity to point sources of light (Wilson, 1978*a*). Compared with the corresponding second-order neurones of the compound eyes, the large monopolar cells (LMCs), L-neurones are more sensitive to small spots of light by at least 2 log units, and their peak hyperpolarising responses would saturate if an ocellus were directed at a full moon (Wilson, 1978*a*). It is unlikely that ocellar photoreceptors are significantly more sensitive to light than are compound eye photoreceptors, because the response range of ocellar photoreceptors spans at least 5 log units and they give peak responses in excess of 40 mV (dragonflies, Chappell and Dowling, 1972; locusts, Patterson and Goodman, 1974). A more probable reason for the high sensitivity of L-neurones to light is that the synapses linking them with photoreceptors operate with a high gain. This gain has not been measured, but previous work on ocellar neurones of dragonflies suggests that it is high (Chappell and Dowling, 1972; Simmons, 1982*b*), and the gain of the synapses between photoreceptors and LMCs in the compound eye of the blowfly is high relative to that of other synapses (Laughlin *et al.* 1987). In a locust ocellus, there is considerable scope for convergence of photoreceptors onto L-neurones, because each ocellus contains about 1000 photoreceptors (Goodman *et al.* 1979) and L-neurones arborize over a wide area of the retina (Goodman *et al.* 1979; Simmons, 1982*a*). Besides ensuring that an L-neurone has a wide receptive field, contact with many photoreceptors should enhance the signal-to-noise ratio in an L-neurone compared with that in a photoreceptor. In the blowfly compound eye,

each LMC receives synapses from six photoreceptors sharing the same optical axis (Nicol and Meinertzhagen, 1982), and this convergence is important in reducing the noise at low light intensities caused by the random capture of light in photoreceptors and by transduction (Laughlin *et al.* 1987).

When the mean background intensity of light changes, the intensity–response function for ocellar L-neurones maintains its slope and shifts along the intensity axis (locusts, Simmons, 1993; dragonflies, Simmons, 1982*b*; cockroaches, Mizunami *et al.* 1986). In the compound eyes of a blowfly and a dragonfly, the same observation has been made for photoreceptors as well as for second-order LMCs (Laughlin and Hardie, 1978). Such adaptation allows the visual system to respond to stimulus contrast over a wide range of mean light intensities (Laughlin, 1989) and it appears to be achieved in many peripheral visual systems at the level of output synapses made by photoreceptors. At these synapses, a sustained response to background illumination is subtracted from responses to changes in light intensity. In the locust ocellus, this subtraction is incomplete, because a sustained response to a change in background illumination is maintained in L-neurones and their postsynaptic targets for at least several minutes (Simmons, 1993). A comparison of the responses of LMCs and of photoreceptors of the blowfly compound eye to identical stimuli shows that, when background intensity is changed, the slope of the curve describing the transfer of potentials between these two cell types is unaltered, but the curve shifts along the photoreceptor axis (Laughlin *et al.* 1987). The same kind of shift occurs in the transfer curve for the first synapse in the median ocellar pathway of a barnacle, *Balanus nubilus*, where adaptation can be mimicked by injection of sustained depolarising or hyperpolarising current into the photoreceptor cells (Hayashi *et al.* 1985). The most likely mechanism for this shift involves local changes in membrane potential and current close to the presynaptic terminals, brought about by an interplay between voltage-activated calcium channels and calcium-sensitive potassium channels (Hayashi *et al.* 1985).

The aims of this paper are to describe the transfer curve for the synapse between a photoreceptor and an L-neurone of the locust ocellus, in order to measure its gain, and to determine the types of changes that accompany adaptation to different levels of background illumination. The method employed is to insert a pair of microelectrodes simultaneously into a photoreceptor and an L-neurone, in order to record responses either to light stimuli or to the injection of current into one of the cells. Previous work has concentrated on the electrophysiological properties of the output synapses made by locust L-neurones. Most of these are excitatory, and the transfer curves show no changes in response to either sustained polarisation of the presynaptic neurone (Simmons, 1981, 1982*a*) or to sustained changes in light intensity (Simmons, 1993). In contrast, at some other synapses, which are inhibitory, transmission decrements extremely rapidly (Simmons, 1982*a*, 1985, 1986). In the dragonfly, recordings have been made simultaneously from ocellar photoreceptors and L-neurones, and it has been shown that depolarising current pulses injected into a photoreceptor elicit brief, hyperpolarising responses in L-neurones (Simmons, 1982*b*).

Materials and methods

Experiments were performed on 39 male and female *Locusta migratoria*, taken from our laboratory stock. A lateral ocellus and its nerve were exposed as previously described (Simmons, 1985) and their perineuria were softened by application of 1 % protease (Sigma, type XIV) in saline for 3 min. The depth of saline bathing the ocellus and its nerve during an experiment was minimised in order to reduce capacitive coupling between microelectrodes.

Microelectrodes were filled with 2 mol l^{-1} potassium acetate and had d.c. resistances of either 40–50 MΩ, for recording from L-neurones, or 140–160 MΩ, for recording from photoreceptors. The two electrodes were connected to an Axoclamp-2A amplifier (Axon Instruments). In most experiments, recordings were made from a photoreceptor and from an L-neurone using the two channels of the amplifier in the bridge balance configuration, so that current could be passed through the recording electrodes. Usually, photoreceptors were penetrated in the distal region of the ocellus, where their cell bodies are located, and L-neurones were penetrated near to the junction of the ocellar nerve with the brain. Stable recordings were maintained from L-neurones for several tens of minutes, but photoreceptors were much more fragile, and the amplitudes of responses to light often declined within a few seconds of penetration, particularly when current was injected or when the ocellus was illuminated constantly. Recordings from photoreceptors were only used when the amplitude of the response to switching on a microscope light exceeded 25 mV and when both the dark resting potential and the amplitudes of responses to test pulses of light remained stable for at least 4 min. A further indication of good-quality recordings, from both photoreceptors and L-neurones, was that responses to low levels of illumination consisted of bumpy changes in membrane potential. Voltage-clamp experiments on L-neurones were performed using the two-electrode clamp configuration. The voltage recording electrode, which was closest to the ocellus, was shielded by applying conductive paint along its shank to within 200 μm of the tip. The paint was grounded at the amplifier headstage and insulated with nail varnish. L-neurone potential was held at dark resting potential during voltage-clamp. Recordings were stored and analyzed using a CED computer interface with Spike 2 Software (Cambridge Electronic Design) and with SigmaPlot v.4.1 (Jandel). All measurements of membrane potential were made relative to dark mean resting potential.

In preliminary experiments, the light source was a green- or a blue-light-emitting diode, but in all the experiments presented in this paper, the ocellus was illuminated by an electrostatic *x*,*y* display with a green phosphor (Kikusui, COS1611). The screen was placed 80 mm from the ocellus, and a circular patch of light subtended an angle of 35° at the eye.

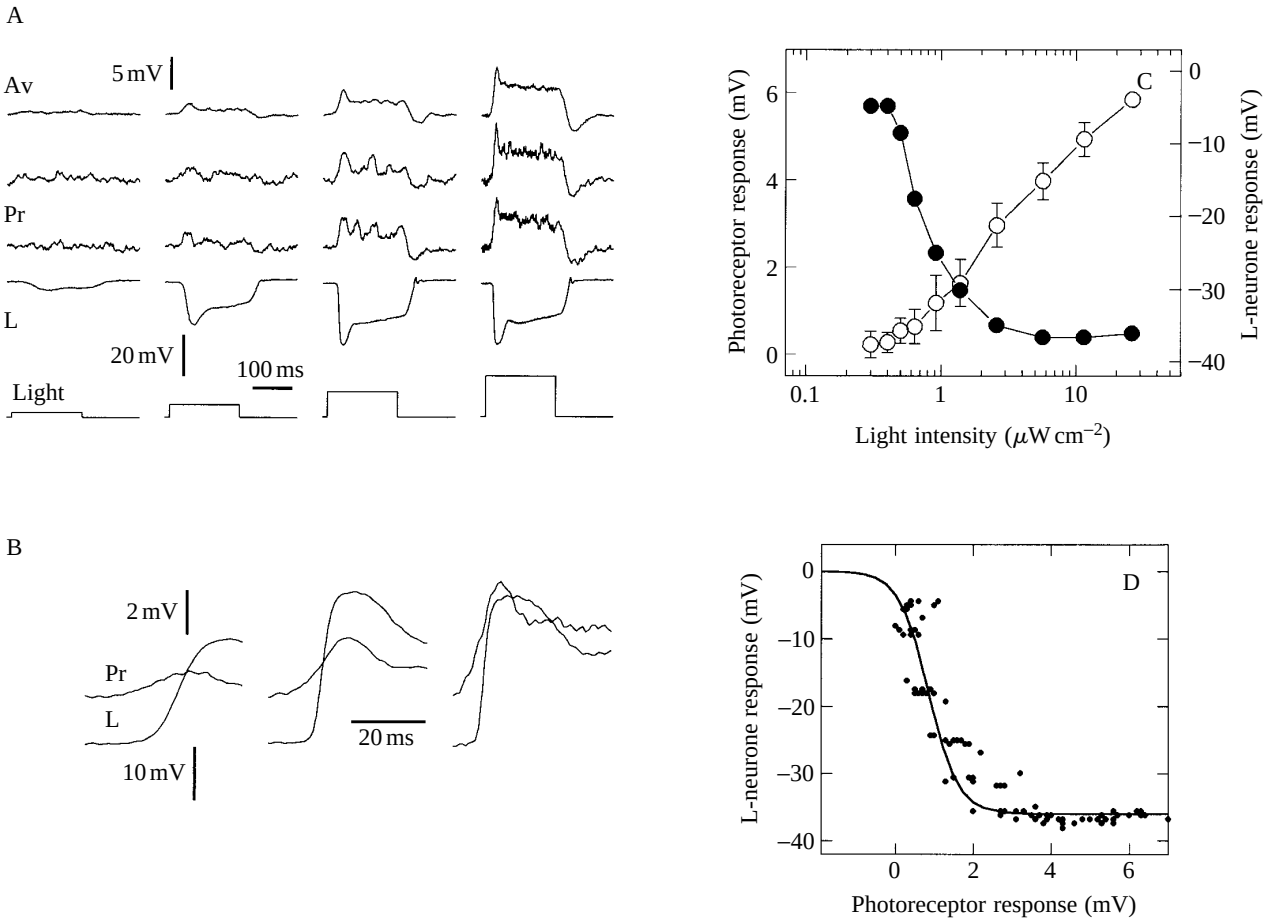


Fig. 1. Responses of a photoreceptor and an L-neurone to pulses of light delivered in the absence of sustained background illumination. (A) Responses to four intensities of light pulse (from left to right, 0.3, 0.64, 1.4 and 26 μW cm^{-2}, bottom traces). For the photoreceptor, the average of the ten responses to each stimulus (Av, top traces) and two single responses (Pr) are shown. Each L-neurone response (L) is to a single stimulus. (B) Comparison of the time courses of responses in the two cells to the onset of single light stimuli of three intensities (from left to right, 0.92, 5.7 and 26 μW cm^{-2}). The responses of the L-neurone are inverted to aid comparison, and light-on was 15 ms before the start of each recording. (C) Plots of the amplitudes of the initial peak responses to light by the photoreceptor (open circles, mean ± S.D. of 10 repetitions) and by the L-neurone (filled circles, means of 10 repetitions; S.D. smaller than each symbol on the graph). (D) Transfer function for the connection. Individual peak responses by the photoreceptor and the L-neurone are plotted as dots and the line is the exponential function fitted to the dots, as described in the text.

Stimuli were controlled by a microcomputer fitted with a VSG2 visual stimulus generator and RG2 raster generator (Cambridge Research Systems). Interference from the screen was reduced (but not entirely eliminated at high light intensities) by placing a grounded, conductive translucent sheet in front of the screen and by shielding electrodes with aluminium foil. A range of light intensities spanning almost 3 log units could be delivered without the need for manipulating neutral density filters. Maximum intensity was about 10^{12} photons cm^{-2} s^{-1} (green light); this is about 1 log unit more intense than the green light from the sky at twilight (Dusenbery, 1992) or than the ultraviolet plus green light from a full moon (Wilson, 1978*a*). Light was calibrated using a radiometer (Ealing) with its sensor placed at the position of the ocellus.

Results

Peak responses to pulses of light delivered in darkness

In response to light, photoreceptor cells depolarised from their dark resting potential and L-neurones hyperpolarised with an initial peak that decayed to a more sustained potential (Fig. 1). The initial peak response was briefer in a photoreceptor than in an L-neurone (Fig. 1A,B), and the onset of the response of the L-neurone was delayed by approximately 4 ms relative to that of the photoreceptor (Fig. 1B). The time taken for potential to reach the initial peak was similar in the two neurones although, at the highest light intensities used, the response rose to its peak slightly more rapidly in the L-neurone (Fig. 1B). L-neurones generated relatively smooth response waveforms at light intensities where responses by

photoreceptors were difficult to discern, even when several responses by the photoreceptor to the same, repeated stimulus were averaged (Fig. 1A, left-hand recordings). At light intensities of about 1 μW cm^{-2}, recordings of the responses by photoreceptors to individual light stimuli showed a series of depolarising potentials (Fig. 1A, second and third recordings), and for the peak response in the photoreceptor, the mean had about the same amplitude as the standard deviation (Fig. 1C). At higher light intensities, there was less variation in the amplitude of the initial peak response by a photoreceptor and the sustained plateau depolarisation that followed was much smoother (Fig. 1A, right-hand recordings; Fig. 1C). Between light intensities of approximately 2 and 30 μW cm^{-2}, the amplitude of the response by photoreceptors to light increased by 3 mV for a tenfold increase in light intensity (Fig. 1C, open circles). The light used as a stimulus in these experiments was not sufficiently intense to saturate the response of photoreceptor cells, and the relatively small responses recorded might, in part, have been due to damage to these cells during an experiment. However, in a few experiments, it was established that the peak responses to rapid increases in light provided by a microscope light and controlled by a shutter exceeded 45 mV, which is similar to the peak amplitudes of responses recorded from other insect photoreceptors. Responses by L-neurones spanned a range of light intensities of less than 1 log unit, considerably narrower than the range spanned by photoreceptors, with the response saturating at about −36 mV and the steepest part of the intensity–response curve having a slope of −52 mV per tenfold change in light intensity (Fig. 1A, and filled circles in Fig. 1C). Standard deviations for the responses by L-neurones were covered by the symbols on the graph (Fig. 1C).

The relationship between the amplitudes of the peak responses in the photoreceptor and in the L-neurone is plotted in Fig. 1D. For each of eight different intensities of light pulse, ten responses are shown. The relationship between pre- and postsynaptic potentials is generally considered to be determined by an exponential relationship between presynaptic potential and the rate at which transmitter is released and to be limited by the driving force for the postsynaptic potential, which declines as the postsynaptic potential approaches its reversal potential. The line drawn in Fig. 1D is based on an equation previously used by Laughlin *et al.* (1987) describing transfer across the synapse between a photoreceptor and a large monopolar cell in the fly compound eye:

$$L = \frac{L_{\mathrm{r}} ka \exp(bP)}{1 + ka \exp(bP)}, \qquad (1)$$

where L is L-neurone potential, L_{r} is the reversal potential for the response by the L-neurone to light, P is photoreceptor potential and k, b and a are constants. The line in Fig. 1D suggests that the photoreceptor releases transmitter tonically in darkness. The constant b is the reciprocal of the change in photoreceptor potential that accompanies an e-fold change in L-neurone potential over the steepest part of the relationship and has been measured from recordings. In the experiment illustrated in Fig. 1, each e-fold change in L-neurone potential was accompanied by a change in photoreceptor potential of 0.38 mV (equivalent to a value of 2.6 mV^{-1} for b). In eight different experiments, the mean value for the change in photoreceptor potential accompanying an e-fold change in L-neurone potential was 0.48 mV, range 0.38–0.67 mV. If L_{r} is taken as the amplitude of the initial peak saturating response to light, in the same eight experiments its mean value was −35.9 mV (range −32.0 to −39.5 mV). This value is less negative than the reversal potential, as measured by two-electrode current-clamp (Wilson, 1978*b*; Ammermüller and Zettler, 1986; Simmons and Hardie, 1988), probably because of a decrement in the postsynaptic potential as it travels from relatively fine postsynaptic processes into the axon. Values for k and a were generated by the curve-fitting program; for the experiment in Fig. 1, k was 0.46, and a was 0.23; mean values from eight experiments were 0.38 for k (range 0.23–0.60) and 0.20 for a (range 0.12–0.30).

The relationship between the amplitude of responses by L-neurones and light intensity was further investigated by comparing potential changes with the postsynaptic currents, measured under voltage-clamp, produced in response to the same series of light stimuli (Fig. 2). When the voltage-clamp was applied, membrane potential changes were reduced to less than 5 % of their amplitude in the unclamped neurone (compare middle and lower traces, Fig. 2A). In this neurone, a saturating response in membrane potential of −35 mV was achieved at a light intensity of just over 1 μW cm^{-2} (Fig. 2B, filled circles), but the postsynaptic current saturated at about 4 μW cm^{-2} (Fig. 2B, open circles). This indicates that the amplitude of the saturating voltage response by the L-neurone to pulses of light delivered in darkness was limited by the postsynaptic reversal potential rather than by the rate of transmitter release. The similarity in the time courses of the current and voltage responses (Fig. 2A) indicates that passive membrane properties of L-neurones do not play a significant role in shaping the voltage signal. In the fly compound eye, LMCs also behave passively within the range of membrane potentials normally caused by light signals (Laughlin and Osorio, 1989).

Responses to sustained increases in light intensity

Following the initial peak response to light-on, the repolarisation of membrane potential towards a sustained response was more gradual and had a more complex waveform in L-neurones than in photoreceptors (Fig. 3A). In response to a moderate or intense step increase in light intensity (second and third recordings in Fig. 3A), the initial depolarising response in a photoreceptor was followed within 10 ms by a slow, smooth repolarisation towards a sustained depolarisation from the dark resting potential. In an L-neurone, a smooth repolarisation towards a sustained potential began about 100 ms after the initial peak hyperpolarisation and was often interrupted by notches with the appearance of small rebound spikes (Fig. 3A, arrows).

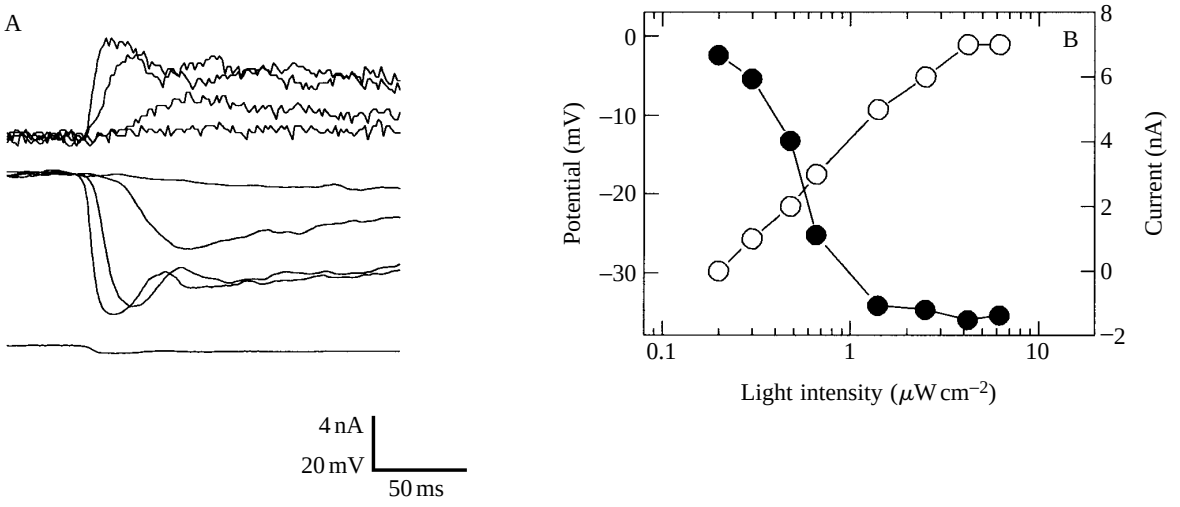


Fig. 2. Postsynaptic potentials and currents produced in an L-neurone in response to pulses of light. (A) Recordings of currents (top traces) and potential changes (middle traces) in response to the onset of light stimuli, at intensities 0.3, 0.7, 1.4 and 6.2 μW cm^{-2}, which occurred at the start of each recording. The bottom trace is the potential change in response to the most intense light stimulus, recorded during voltage-clamp. (B) Plots of the peaks of the postsynaptic potential (filled circles) and postsynaptic current (open circles) against the intensities of light stimuli.

For a particular photoreceptor or L-neurone, the ratio between the amplitude of the initial peak response and the amplitude of the response to a continuing light stimulus was fairly constant for different light stimuli (Fig. 3B). In Fig. 3B, the sustained response was measured as the mean membrane potential over 2.5 s, starting 2 s after light-on. Each peak response to light was a single measurement, and for the photoreceptor (Fig. 3B, open circles), the standard deviation of the membrane potential during the sustained response is shown. The ratio of the amplitudes of the sustained plateau responses to the peak responses, given by the slope of the regression line drawn to fit the data points, is 0.48, a value close to that found in other experiments. In the L-neurone, the peak response to light saturated at −36 mV, with a light intensity of 1.5 μW cm^{-2}, but the sustained response did not saturate. For sub-saturating peak responses, the ratio between the amplitudes of sustained plateau responses to peak responses was 0.38 (Fig. 3B, filled circles). This ratio varied in different experiments between 0.25 and 0.45, possibly because of the activity of efferent neurones, which has previously been reported to affect the amplitude of sustained responses by locust L-neurones (Rotzler, 1989). The relationship between the sustained potentials in the photoreceptor and in the L-neurone is plotted in Fig. 3C. The ranges of measurements for each light intensity, over a 2.5 s period starting 2 s after light onset, are enclosed in an ellipse. For comparison, peak responses are also plotted (filled circles, dotted line). For low intensities of light, the relationship between photoreceptor and L-neurone responses was similar for the peak and for sustained responses, but at higher intensities the relationship was less steep for the sustained responses.

During a sustained response to light, the amplitude of noise was generally greater in a photoreceptor than in an L-neurone (Fig. 3D). For each cell, standard deviations from the mean of membrane potential, measured at 1 ms intervals over 2.5 s, are given in Fig. 3D. Signal-to-noise ratios (the mean deviation from the dark resting potential divided by the standard deviation from this mean over 2.5 s) at light intensities of 0.07, 0.78 and 12.5 μW cm^{-2} were: for the photoreceptor, 2.1, 4.9 and 8.0; and for the L-neurone, 7.0, 32.7 and 63.2. The signal-to-noise ratio for both cells, therefore, increased as light intensity increased, ninefold in the L-neurone and fourfold in the photoreceptor.

Responses to changes in light from a constant background illumination

The effects of different intensities of sustained illumination on responses to changes in illumination were studied by subjecting an ocellus to 200 ms long stimuli, delivered every 1.5 s. Initially, following dark adaptation for 2 min after cell penetration, 11 different light intensities were delivered in darkness. Usually, three different backgrounds of illumination were employed, presented in order of increasing intensity: 0.66, 3.57 and 14.92 μW cm^{-2}. After a background had been turned on, 10 s was allowed for adaptation before the ocellus was challenged with stimuli, alternating between increased and decreased light. The duration of these experiments was limited by the difficulty in maintaining stable recordings from photoreceptors when they were illuminated continuously. In locust L-neurones, sensitivity continues to increase with dark adaptation for hours (Wilson, 1978*a*,*c*) and adaptation to bright background illumination continues for at least several minutes (Simmons, 1993).

As background intensity increased, compression occurred in the range of response amplitudes by both cells (Fig. 4). This compression was particularly marked for responses to increases in light from different background intensities, either to the maximum light intensity (Fig. 4A, and Fig. 4E,F, filled circles) or to intensities only slightly greater than background (Fig. 4B and Fig. 4E,F, open symbols). The amplitude of the

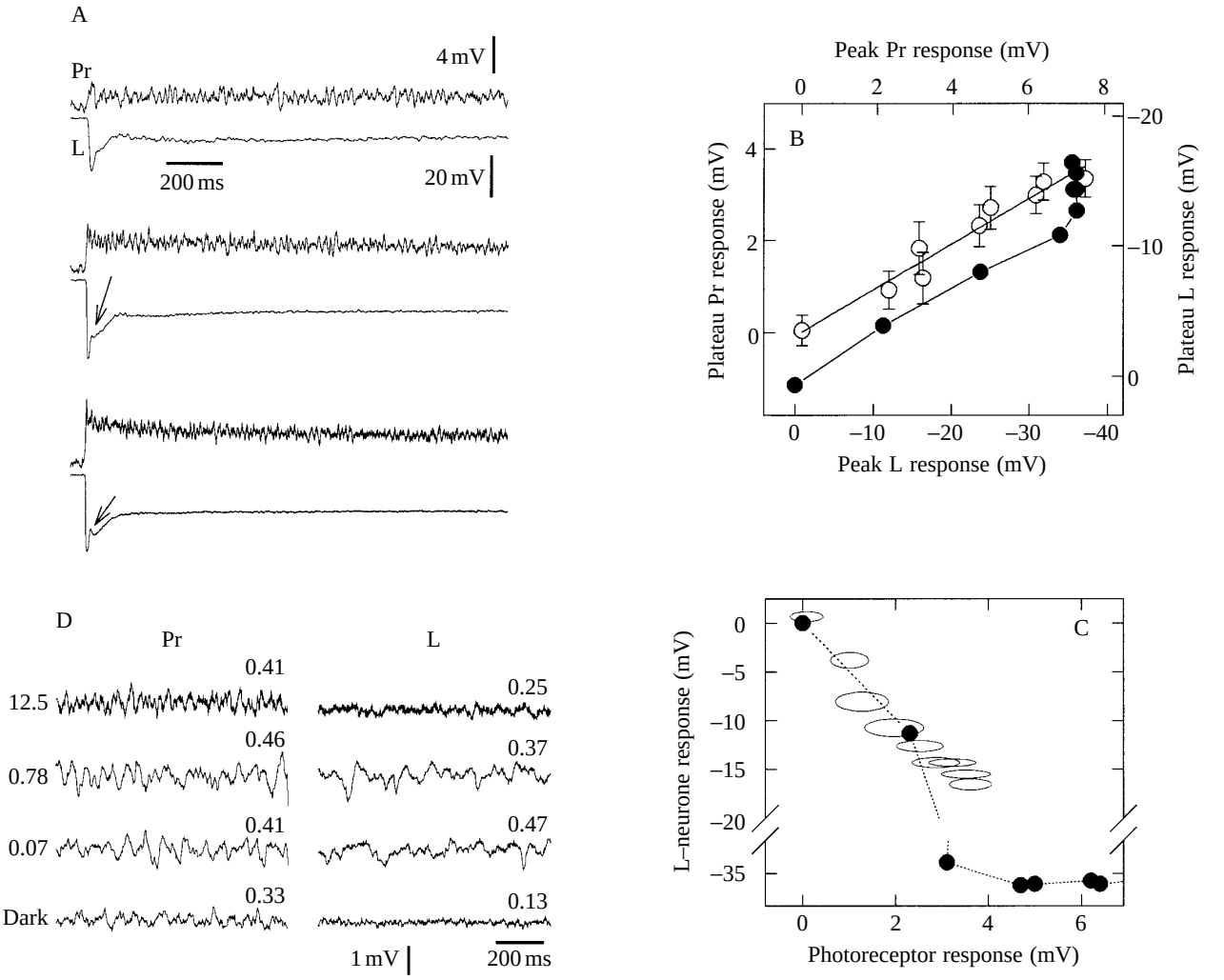


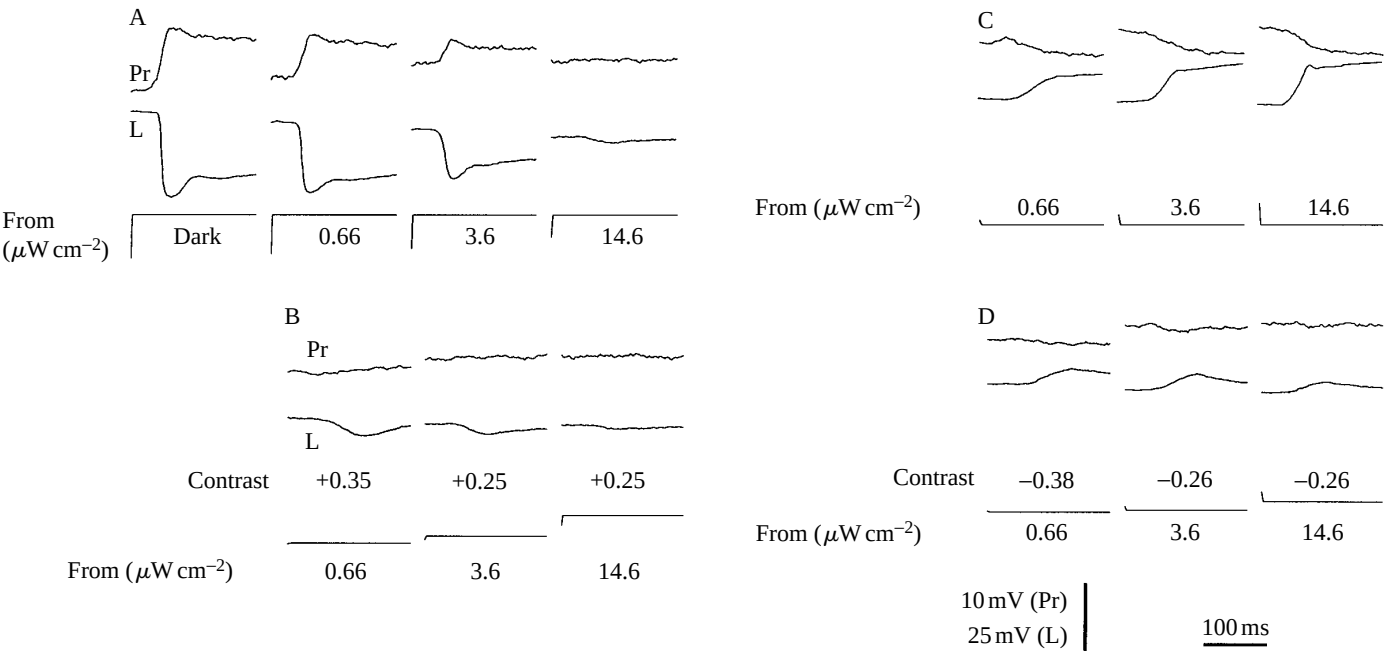
Fig. 3. Responses by a photoreceptor (Pr) and an L-neurone (L) to long-lasting light stimuli. Stimuli were 5 s long, with 7 s between the end of one stimulus and start of the next. (A) Recordings of the responses by the two cells to steps of light at intensities 0.15 μW cm^{-2} (upper record), 1.6 μW cm^{-2} (middle record) and 12.5 μW cm^{-2} (lower record). Arrows indicate possible small rebound spikes during the initial repolarisation. (B) Plots of the amplitudes of the peak responses against the amplitudes of the sustained responses for each cell (open circles, photoreceptor, ± S.D. shown; filled circles, L-neurone, further details in text). (C) Amplitudes of the sustained responses in the two cells are plotted against each other. Each ellipse delimits the range of potential recorded from the two cells during one stimulus. The filled circles with dotted line show a plot of the amplitudes of peak responses in the two cells. (D) Membrane potential fluctuations in the two cells during sustained light stimuli, presented at the same gain. Intensities of the light stimuli are given to the left of the figure in μW cm^{-2}; above and to the right of each recording is the standard deviation for the sustained membrane potential (in mV), measured over 2.5 s.

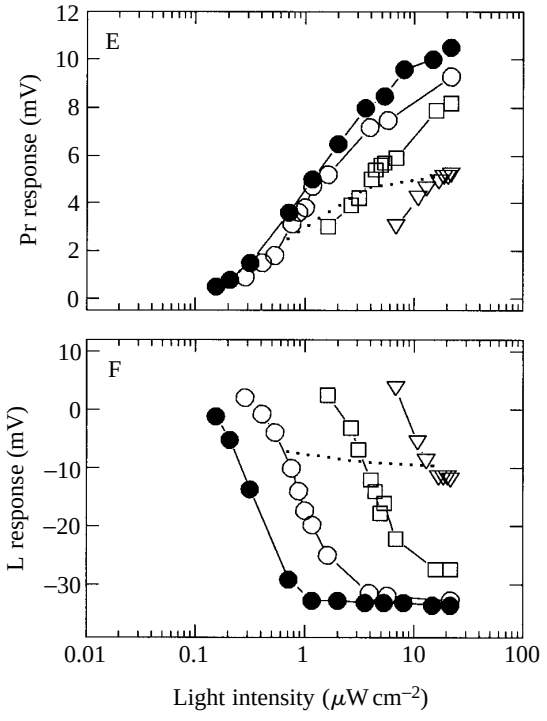
saturating response by an L-neurone decreased as background light intensity increased (Fig. 4A,F). Because background illumination generated a sustained response in both cells (dotted lines, Figs 4E,F), responses to decreases in light in the presence of continuing background illumination were less marked than the responses produced when light was switched off completely. For three different background light intensities, the responses to switching off light are shown in Fig. 4C, and the responses to relatively small reductions in intensity are shown in Fig. 4D. For both cells, the maximum slopes of the intensity–response functions remained unchanged as background intensity altered (Fig. 4E,F).

The maximum slope of the transfer curve relating membrane potentials in the two cells was also maintained as the intensity of background light increased (Fig. 4G). However, the transfer curve shifted towards more depolarised photoreceptor membrane potentials as background light intensity increased, so that a particular response in the L-neurone required the photoreceptor to depolarise by an increased potential from its dark resting level. The transfer curves also shifted towards more depolarised L-neurone potentials, as indicated by a decrease in the amplitude of the saturating response in the L-neurone.

Responses to ramp increases in light

The size of a response depends on the speed as well as the amplitude of an increase in light. In response to sinusoidal changes in light intensity, the transfer of signals across the





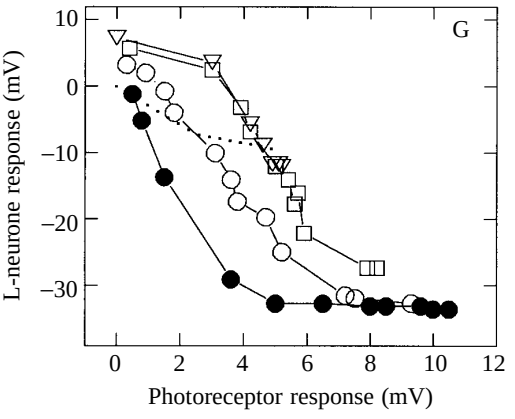


Fig. 4. The effects of mean background illumination on responses by a photoreceptor (Pr) and an L-neurone (L) to increases and decreases in light intensity. (A) Light stepped to 21.7 μW cm^{-2} from dark (left-hand record) and from three different background intensities. (B) Light increased by a contrast of 0.35 (left-hand record) or 0.25 (middle and right-hand records). (C) Background light switched off from the three background levels. (D) Decreases in light intensity, contrasts −0.38 (left-hand record) and −0.26 (middle and right-hand records) from the same backgrounds. Background light intensities for A–D in μW cm^{-2} were: left-hand record, 0.66; middle record, 3.6; right-hand record, 14.6. Contrast is intensity change during stimulus divided by background intensity. (E,F) Plots of the amplitudes of peak responses by the photoreceptor and L-neurone to light stimuli from darkness (filled circles) and to increases and decreases in light intensity from the three backgrounds (open symbols; background intensities were: circles, 0.66 μW cm^{-2}; squares, 3.6 μW cm^{-2}; and triangles, 14.6 μW cm^{-2}). (G) Transfer curves for the connection between the photoreceptor and the L-neurone for stimuli in darkness (filled circles) and stimuli against the three levels of background illumination (open symbols, as in E and F). The dotted lines in E and F track the sustained potentials during illumination by each background light intensity.

synapse was independent of the stimulus frequency, over the range 0.5–20 Hz. In order to study slower changes in light intensity, different speeds of ramp increases in light, from darkness, were delivered to the ocellus (Fig. 5). In these experiments, a number of features emerged consistently. In many of the records, two phases of response by the two cells to ramp increases in light intensity were apparent: an initial, rapid response, followed by a more sustained potential. For the most rapid stimuli (left-hand recordings, Fig. 5A), the initial peak response was followed by a repolarisation towards a sustained membrane potential. For the slowest stimuli (right-hand recordings, Fig. 5A), membrane potential continued to increase throughout the duration of the ramp increase in light intensity (0.99 s). Note that, in the L-neurone, the peak of the response to the slow increase in light intensity was greater in amplitude than the potential to which the response rapidly decayed following the most rapid increase in light intensity (dotted lines, Fig. 5A).

Clear, initial rapid responses to an increase in light intensity were most apparent for stimuli where changes in intensity were either rapid (left-hand records, Fig. 5A) or large (upper records, Fig. 5A). The slope of the transfer function between the photoreceptor and the L-neurone for these initial, rapid changes in potential depended on the speed of the ramp stimulus (Fig. 5B). For the fastest ramps, the curve almost saturated, at an L-neurone potential about −33 mV from the dark resting potential (circles, Fig. 5B), but saturation was not achieved at slower stimulus speeds (diamonds and triangles, Fig. 5B). As expected, the transfer function for potentials in response to sustained illumination was not affected by the speed of a stimulus.

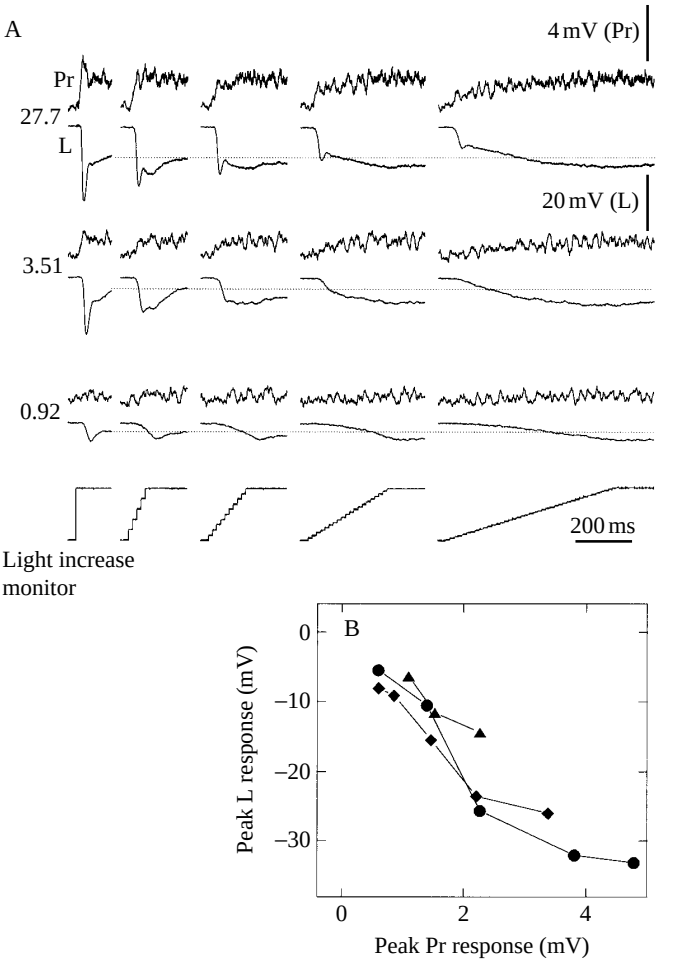


Fig. 5. Responses to ramp increases in light intensity, delivered with no background illumination. (A) Recordings from a photoreceptor (Pr) and an L-neurone (L) in response to ramp stimuli with five different speeds (light monitor at bottom of panel) to the three different light intensities (in $\mu W\,cm^{-2}$) given to the left of each paired recording. The dotted line is the L-neurone potential 200 ms after the fastest stimuli (left-hand recordings). (B) Amplitudes of the initial peak transients in the two cells are plotted against each other for three speeds of stimuli: circles, left-hand records; diamonds, second records; triangles, fourth records in A. Only those potentials where the initial peak transient was distinct from the sustained response are plotted.

Responses by L-neurones to injection of current pulses into photoreceptors

Depolarising pulses of current injected into photoreceptors elicited hyperpolarising responses in L-neurones, after a delay of about 4 ms, in about 50 % of all paired recordings tested (Fig. 6). All paired recordings of responses to light that are presented in this paper were from neurones shown to be connected. The amplitude of the L-neurone response depended on the strength of the current used to stimulate the photoreceptor (Fig. 6A). After the peak hyperpolarising response, the L-neurone repolarised to its dark resting potential within 25 ms, irrespective of the duration of the stimulus applied to the photoreceptor. Hyperpolarising current injected into photoreceptors did not elicit responses from L-neurones, which is not surprising in view of the number of photoreceptors which probably drive each L-neurone. However, rebound potentials were produced in photoreceptors when pulses of hyperpolarising current ended; they were followed by small, hyperpolarising responses in L-neurones (Fig. 6B). The amplitudes of the response by an L-neurone to injection of depolarising current into a photoreceptor depended on the speed with which the current increased as well as on its peak amplitude (Fig. 6C). Following one pulse of depolarising current injected into a photoreceptor, the size of the response by an L-neurone to subsequent pulses was reduced, recovering its initial amplitude over a period of 200 ms (Fig. 6D).

The amplitudes of the responses in the L-neurone, both to light and to electrical stimulation of a photoreceptor, were increased by sustained negative current injected into the L-neurone and were decreased by sustained positive current (Fig. 6E). Over the range −14 nA to +5 nA injected into the L-neurone (the range over which changes in membrane potential could be recorded), there was a linear relationship between the amplitudes of the responses of the two stimuli. This observation is consistent with a common ionic mechanism for the two types of responses in an L-neurone. Note the brief duration of the response by the L-neurone to injection of

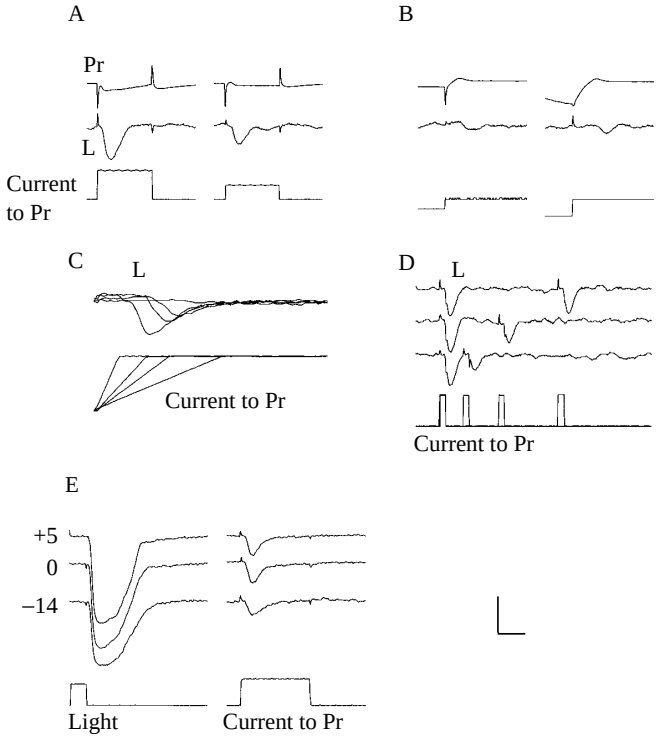


Fig. 6. Responses by an L-neurone to the injection of current stimuli into a photoreceptor. (A) Pulses of depolarising current. (B) Responses following the ending of two pulses of hyperpolarising current. (C) Ramps of depolarising current. (D) Three recordings of responses to pairs of depolarising pulses, separated by different intervals. (E) The effects of direct current injected into the L-neurone on responses to identical light stimuli (left-hand records) and to identical pulses of depolarising current injected into the photoreceptor (right-hand records). The strengths of the currents (in nA) injected into the L-neurone are shown on the left. Photoreceptor potential is not shown in C–E. A and B were from one experiment, and C–E were each from separate experiments. Calibrations: L-neurone, 5 mV (A–D) and 4 mV (E); photoreceptor, 10 mV; current, 1.5 nA; 50 ms (A,B,D,E) and 12 ms (C).

current into a photoreceptor compared with the duration of the response to light in Fig. 6E.

The effects of injecting sustained current into photoreceptors

When sustained direct current was injected into a photoreceptor, L-neurone responses to electrical stimulation of the photoreceptor were affected in a similar way to that in which background illumination affected the responses to light stimuli. The amplitudes of L-neurone responses to electrical stimulation of a photoreceptor were increased when the receptor was hyperpolarised with direct current and were decreased when the photoreceptor was depolarised with direct current (Fig. 7). The recordings in Fig. 7A show the responses of an L-neurone to two amplitudes of depolarising current, superimposed on three strengths of direct current (indicated on the left), injected into a photoreceptor. The amplitudes of the two current pulses were 0.7 nA (left-hand recordings) and 1.25 nA (right-hand recordings). The recordings from the photoreceptor in Fig. 7A show how its response to a pulse of light was increased by hyperpolarising direct currents and was almost eliminated by the depolarising direct current.

The relationship between the strength of a depolarising stimulus pulse of current injected into a photoreceptor and the amplitude of the hyperpolarising response in an L-neurone was smoothly graded (Fig. 7C, filled circles). The response by the L-neurone saturated at −6.5 mV, compared with a potential of −36 mV for saturation by this neurone to pulses of light. When direct current was injected into the photoreceptor, the curve describing the relationship between the peak current injected into the photoreceptor and the response by the L-neurone shifted along the current axis in proportion to the direct current (Fig. 7C, open symbols; note, current here is the sum of the direct current and each stimulating pulse). The slope of the curve was not sensitive to the direct current, although the potential at which the response by the L-neurone saturated was slightly affected. The effects of hyperpolarising direct current injected into a photoreceptor, up to a strength of −0.5 nA, were consistent in seven different experiments. For hyperpolarising direct currents stronger than −1 nA, a decline in the amplitudes of L-neurone responses was sometimes observed, but in other experiments the opposite effect or no effect was observed. The effects of depolarising direct current injected into the photoreceptor varied from experiment to experiment, and this treatment often caused a decline in the quality of recordings from the photoreceptor.

Discussion

Comparison with other synapses

By recording simultaneously from pairs of connected photoreceptors and L-neurones, it has been demonstrated that L-neurones are remarkably sensitive to changes in photoreceptor potential. At the steepest gradient in the transfer function for the connection between the two cells, an e-fold change in L-neurone potential is accompanied by a change in photoreceptor potential of just 0.38–0.7 mV. It is probably safe to accept the higher figure of 0.7 mV, although the lower figure of 0.38 mV must be treated with caution because of the possibility of damage to photoreceptors in some experiments. In comparison, at other output synapses from photoreceptors where measurements have been made, the photoreceptor potential changes by more than 1 mV for each e-fold change in postsynaptic potential: 6 mV for synapses made by rod photoreceptors in the dogfish *Scyliorhinus canicula* (Ashmore and Falk, 1980); 1.5–1.86 mV for the synapse between a photoreceptor and an LMC in the compound eye of the blowfly *Calliphora stygia* (Laughlin *et al.* 1987); and 2.2–4.4 mV at the synapses between a photoreceptor and second-order neurone in the median eye of the barnacle *Balanus nubilus* (Hayashi *et al.* 1985). At one output synapse made by some locust L-neurones, an e-fold change in postsynaptic potential is associated with a change in L-neurone potential of 2.3–4.0 mV (Simmons, 1981, 1993). Synapses involved in the early stages of visual processing

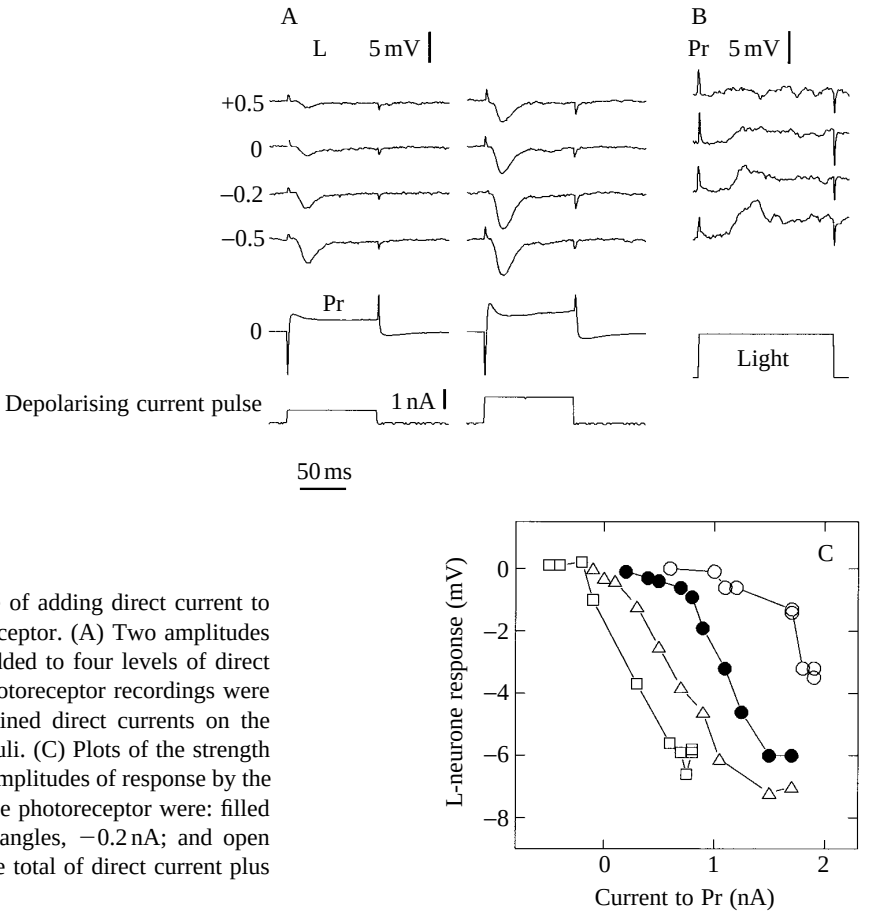


Fig. 7. The effects on the responses by an L-neurone of adding direct current to pulses of depolarising current injected into a photoreceptor. (A) Two amplitudes of depolarising current pulse (bottom traces) were added to four levels of direct current (strengths in nA on the left of the figure). Photoreceptor recordings were with no direct current. (B) The effects of the sustained direct currents on the responses by the photoreceptor to identical light stimuli. (C) Plots of the strength of current injected into the photoreceptor against the amplitudes of response by the L-neurone. Strengths of direct current injected into the photoreceptor were: filled circles, no current; open squares, −0.5 nA; open triangles, −0.2 nA; and open circles, +0.5 nA. For each measurement, current is the total of direct current plus stimulus.

generally have high gains, compared with synapses elsewhere in the nervous system (Siegler, 1985). Clearly, the synapse between a photoreceptor and an L-neurone in the locust ocellus operates with a very high gain, and this is responsible for the high sensitivity that L-neurones show in their responses to changes in light intensity compared with locust compound eye LMCs (Wilson, 1978*a*). Also, the maximum gain for the transfer function for the first synapses in the ocellus is reached for very small depolarisations of the photoreceptor from the dark resting potential, which ensures that an L-neurone is very sensitive to light stimuli delivered in darkness. This is partly because, as indicated by extrapolation of the curves relating responses to light in the two cells, the threshold for transmitter release is more negative than the dark resting potential of the photoreceptor and is partly a consequence of the convergence of a large number of photoreceptors onto an L-neurone, which affects the size of the constant *a* in equation 1 in the results (Laughlin *et al.* 1987).

Each L-neurone arborizes over a large part of the ocellar retina (Goodman *et al.* 1979; Simmons, 1982*a*), enabling the L-neurone to respond to changes in illumination averaged over a wide panorama (Wilson, 1978*a*). The number of photoreceptors that an L-neurone contacts has not been determined, although a lateral ocellus contains about 1000 photoreceptors (Goodman *et al.* 1979) that converge on seven L-neurones (C. S. Goodman, 1974; L. J. Goodman *et al.* 1975), and each photoreceptor probably synapses with more than one L-neurone because, in dim illumination, some discrete hyperpolarising potentials occur synchronously in pairs of L-neurones (Wilson, 1978*b*). Where all photoreceptors view a uniformly lit visual field, convergence should provide a mechanism for reducing the noise due to random arrival of photons at single photoreceptors and to the process of transduction. In blowfly compound eyes, careful analysis has enabled measurement of the relative contributions of this source of noise and of synaptic transmission to the noise in LMCs (Laughlin *et al.* 1987). At low light intensities, there is an improvement in signal-to-noise ratio by the square root of 6 as signals cross the first synapse, which is consistent with synaptic transmission making only a small contribution to the noise, and convergence of six photoreceptors onto an LMC (Laughlin *et al.* 1987; that six photoreceptors converge onto one LMC is also known from an anatomical study, Nicol and Meinertzhagen, 1982). In the locust ocellus, if just 100 photoreceptors converged on a single photoreceptor, the signal-to-noise ratio should be improved by a factor of ten. Measurements made during steady illumination indicate an improvement by between three- and eightfold. However, these measurements only give a rough indication of the relative signal-to-noise ratios in the two cells because, as the experiments with ramp changes in light show, relatively high-

frequency fluctuations in potential are transmitted at a higher gain across the synapse than are sustained potentials. Despite their limitations, the results obtained here indicate that the improvement in signal-to-noise ratio is lower than would be expected for the convergence of several hundred photoreceptors onto an L-neurone. A possible reason for a relatively low improvement in signal-to-noise ratio is coupling of photoreceptors, which would reduce their independence and also their effective convergence onto L-neurones, while improving their own signal-to-noise ratio. There is evidence, from ultrastructural studies, for electrical synapses between ocellar photoreceptors in adults of one species of insect, the moth *Trichoplusia ni* (Dow and Eaton, 1976), and ultrastructural studies of the locust ocellus reveal both conventional chemical synapses and a type of contact termed 'capitate' between photoreceptors (Goodman *et al.* 1979). Electrophysiological methods have demonstrated that photoreceptors are coupled in the compound eyes of locusts (Shaw, 1969; Lillywhite, 1978) and flies (van Hateren, 1986), although the anatomical basis for this coupling is not known. Coupling between photoreceptors is found in many visual systems and can serve a number of functions (reviewed by Laughlin, 1994). It is unlikely that synaptic transfer contributes significantly to the noise in L-neurones under dim illumination because of the high gain of the synapse, which is an effective means of reducing synaptic noise (Laughlin *et al.* 1987). However, it should be noted that L-neurones also receive input synapses from cells other than photoreceptors, including those from each other (Simmons, 1982*a*; Littlewood and Simmons, 1992), and these will contribute noise.

When mean light intensity changes, both photoreceptors and L-neurones adapt by shifting their intensity–response curves without a significant change in slope. L-neurones have a steeper intensity–response curve than do photoreceptors, and the curves of both cells shift in register. The same behaviour has been well documented for photoreceptors and LMCs in a dragonfly and a blowfly (Laughlin and Hardie, 1978) and appears to be a general feature of peripheral visual systems, enhancing the responses of second-order neurones to the contrast of a light signal over a wide range of absolute intensities (e.g. Norman and Werblin, 1974; Laughlin, 1989).

A major functional difference between locust ocelli and the compound eyes of blowflies and dragonflies is in the time course and extent of adaptation. The time course of adaptation is slow in locust ocellar neurones, and some third-order ocellar neurones track gradual changes in illumination (Simmons, 1993). Sustained responses may be important for some of the functions that have been suggested for ocelli, such as the control of circadian rhythms (Rence *et al.* 1988), but may not be a general feature in all species because they are more marked in L-neurones of locusts than in those of dragonflies (Simmons, 1982*b*) or blowflies (Simmons *et al.* 1994). As in the compound eyes of a dragonfly and a blowfly (Laughlin and Hardie, 1978), changes in the sensitivity of locust ocellar neurones to light probably follow the time course of repolarisation during a sustained change in background illumination, although the study of this was limited by the difficulty in maintaining stable recordings from photoreceptors. Complete adaptation is unlikely to be required by locust ocellar neurones because, in natural conditions, changes in illumination over the whole visual field of an L-neurone will occur relatively slowly, following changes in the time of day or in meteorological conditions.

Mechanisms of signal transformation

There are a number of possible mechanisms for the shift in the transfer curve between a photoreceptor and an L-neurone as background illumination alters. The most likely sites for the shift are within the photoreceptor: desensitization of postsynaptic receptors can be discounted because L-neurones give sustained responses to prolonged ionophoresis of the most likely neurotransmitter, histamine, into the ocellar neuropile (Simmons and Hardie, 1988), and no evidence has been found in the present work for synaptic feedback from L-neurones onto photoreceptors (see also Simmons, 1982*b*). A possible presynaptic mechanism for the shift in the transfer curve is partial inactivation of the voltage-sensitive calcium channels that regulate neurotransmitter release from the photoreceptors. This has been suggested to underlie the decrement in transmission at inhibitory synapses between L-neurones in the locust *Schistocerca gregaria* (Simmons, 1985), where there is a distinct limit of a few milliseconds over which transmission can be sustained. However, in median ocellar photoreceptors of the barnacle, calcium entry does not inactivate during prolonged depolarisations (Callaway *et al.* 1993). It has been proposed that the most probable mechanism for the shift in the transfer function for this synapse is a local interaction between depolarisation, which opens voltage-sensitive calcium channels, and repolarisation mediated by calcium-sensitive potassium channels (Hayashi *et al.* 1985). Another possible means for shifting a transfer curve involves subtraction of an extracellular signal from the intracellular depolarisation of the presynaptic potential, as has been proposed to occur at the first synapse of compound eyes (Shaw, 1975, 1984). This is thought to occur when current, driven by relatively large extracellular potentials, is forced to flow through photoreceptor axons where they pass through a high-resistance barrier, the basement membrane, into the lamina. Large extracellular potential changes are recorded in the ocellus and distal ocellar nerve, but there is no information on the existence of suitable resistance barriers for such a mechanism to operate here.

A full understanding of the mechanisms of adaptation in the ocellus must account for the difference observed in L-neurones between the duration of their responses to sustained increases in illumination and the duration of their responses to the injection of depolarising current into single photoreceptors. Unlike the situation in the locust ocellus, postsynaptic neurones of the barnacle median ocellus sustain hyperpolarising potentials when current is injected into presynaptic photoreceptors, but the experimental design in the work on the barnacle ensured that all four photoreceptors were depolarised in synchrony (Hayashi *et al.* 1985), and in barnacle

ocelli the photoreceptors are coupled electrically (Shaw, 1972). Although locust ocellar L-neurones do not produce a sustained response when a long pulse of depolarising current is injected into a photoreceptor, many properties of the brief, hyperpolarising response which is evoked are similar to the initial, peak response to an increase in light: they are graded in amplitude; their size depends on the speed as well as on the amplitude of the photoreceptor stimulus; and injection of direct current into the L-neurone indicates a common ionic basis. Some of these effects have also been reported for the synapse between an ocellar photoreceptor and an L-neurone in the dragonfly (Simmons, 1982*b*). In addition, it has been shown here that the effects of background illumination on the transfer curve between ocellar photoreceptors and L-neurones are mimicked by injection of sustained current into a photoreceptor. The reasons why no sustained, hyperpolarising response is recorded in an L-neurone when a photoreceptor is depolarised by experimental injection of current are unclear and their elucidation requires more knowledge of the electrical properties of ocellar photoreceptors. The effects of current on the responses by photoreceptors to light show that injected current has a sustained effect on photoreceptor membrane potential, and it is unlikely that voltage-sensitive potassium channels, like those that play a role in light adaptation in some insect photoreceptors (e.g. Weckström *et al.* 1991; Laughlin and Weckström, 1993), would be responsible for completely curtailing the responses by L-neurones to depolarisation of photoreceptors by electrical current but not by light. Two other types of explanation can be proposed. First, the sustained release of transmitter from a photoreceptor could be linked to the absorption of light by a mechanism other than membrane potential, which persists after the voltage-activated release of transmitter has decreased. Although calcium channels controlled by diffusible intracellular messengers are involved in generating maintained potential changes in responses to light in insect photoreceptors (Hardie and Minke, 1992), the anatomical separation of the rhabdom from presynaptic sites makes it unlikely that such a mechanism would control transmitter release. Second, in order for an L-neurone to generate a sustained hyperpolarising response, it might be necessary for several photoreceptors to depolarise synchronously, as they do during ocellar illumination. Reduction in the release of transmitter when a single photoreceptor was depolarised might occur if electrical coupling with its neighbours developed following the onset of depolarisation, providing a sink for the current flowing across the membrane of a single photoreceptor. A number of features of the operation of the first ocellar synapse, including the lower than predicted reduction in noise as signals pass across it, as well as the way it operates when a single photoreceptor is depolarised, point to functional interactions between photoreceptors.

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