

## CHARACTERISATION OF LARGE SECOND-ORDER OCELLAR NEURONES OF THE BLOWFLY *CALLIPHORA ERYTHROCEPHALA*

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### Summary

1. Blowflies have twelve large, second-order ocellar neurones (L-neurones) with axons in the single ocellar nerve. These neurones have fairly restricted arborizations in the posterior slope neuropile of the protocerebrum and cell bodies in the nerve, near to the fused ocellar retinae.

2. Like ocellar L-neurones of other insects, or large second-order neurones of the fly compound eye, blowfly L-neurones hyperpolarise in response to increases in light intensity and depolarise in response to decreases in light intensity. Both polarities of response have a strong phasic component. Adaptation to sustained illumination shifts the intensity–response curve, with little change in its gradient.

3. The maximum responses of blowfly L-neurones to sinusoidal changes in light intensity occur at stimulus frequencies of 5–10 Hz.

4. Hyperpolarising an L-neurone with small currents causes an increase in input resistance. Larger hyperpolarising currents cause oscillations in the membrane potential. The amplitude of the oscillations increases with current strength. Repolarisation generates brief rebound spikes of variable amplitude.

5. Injection of small hyperpolarising currents increases the amplitude of a response to a subsaturating pulse of light. This effect is not seen for saturating responses to light and is likely to be due to the increase in membrane resistance caused by hyperpolarisation.

### Introduction

In blowflies, as in many other adult insects, some of the widest axons in the central nervous system are those of the large, second-order neurones of the ocelli (L-neurones). Blowflies have twelve L-neurones with axons in the single nerve that serves the fused neuropile of the three ocelli (Strausfeld, 1976; Nässel and Hagberg, 1985). The ocelli of blowflies are situated at the vertex of the head, and their fields of view are directed upwards (Cornwell, 1955; Schuppe and Hengstenberg, 1993). In locusts and dragonflies, where the ocelli are directed more towards the visual horizon, there is clear evidence that they have a

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role in controlling flight attitude (Taylor, 1981; Stange and Howard, 1979). There has been no direct demonstration of any role for the ocelli of blowflies in stabilising flight. Although anatomical evidence points to a role in controlling head attitude and, indirectly, flight attitude (Strausfeld and Bassemir, 1985), the contribution of the lateral ocelli to head rolling in response to a change in incident light direction during flight is much smaller than that of the compound eyes (Schuppe and Hengstenberg, 1993). In some species of fly, there is evidence that the ocelli can influence the direction of walking, either by sensitivity to the plane of polarisation of light (in *Sarcophaga aldrichi*, Wellington, 1953) or by detection of vertical dark–light boundaries (in *Musca domestica*, Wehrhahn, 1984).

Extracellular recordings have shown that the ocellar nerve of blowflies contains axons that signal changes in illumination by variation in spike rate (Metschl, 1963), and intracellular recordings have shown that ocellar photoreceptors in *Drosophila melanogaster* depolarise in response to light (Labhart, 1977). Electroretinogram measurements from ocelli of *Calliphora erythrocephala* (Kirschfeld and Lutz, 1977; Kirschfeld *et al.* 1988) and *Drosophila* (Hu *et al.* 1978) indicate sensitivity to ultraviolet and blue light, perhaps correlated with their upwardly directed field of view. However, nothing is known of the physiology of fly L-neurones, in contrast to the situation for the equivalent neurones of the compound eyes or for L-neurones of some hemimetabolous insects. Like the second-order large monopolar cells (LMCs) of the fly compound eye (Autrum *et al.* 1970), ocellar L-neurones of all hemimetabolous insect species so far examined hyperpolarise in response to increased illumination of their photoreceptors (e.g. dragonflies, Chappell and Dowling, 1972; locusts, Patterson and Goodman, 1974; Wilson, 1978a; cockroaches, Mizunami *et al.* 1982). In LMCs of flies and L-neurones of cockroaches and locusts, the amplitudes of responses to changes in light are proportional to contrast (regardless of mean light intensity) over a range of mean light intensities spanning several orders of magnitude (fly and dragonfly LMCs, Laughlin and Hardie, 1978; L-neurones in cockroaches, Mizunami *et al.* 1986; and in locusts, Simmons, 1993). L-neurones generate rebound spikes of graded amplitude, which boost the responses to decreases in light intensity (locusts: Wilson, 1978b; Simmons, 1985; Ammermüller and Zettler, 1986; cockroaches: Mizunami *et al.* 1987). Bees are the only holometabolous insects in which intracellular recordings have been made from L-neurones (Guy *et al.* 1979). Some L-neurones in bees are reported to switch between a spiking and a non-spiking state (Milde, 1981), and the distinction between those bee L-neurones that employ graded potentials and those that employ spikes is not always clear (Milde, 1984; Milde and Homberg, 1984).

We have used intracellular methods to investigate the morphology and physiology of large, second-order ocellar neurones (L-neurones) of blowflies. These neurones respond to changes in light intensity in a similar way to L-neurones of other insects. However, injection of hyperpolarising current into blowfly L-neurones reveals some unusual properties, which may boost transient hyperpolarising responses to increases in light intensity.

### Materials and methods

*Calliphora erythrocephala* were either caught as adults in local gardens, or were purchased as maggots and reared to adulthood. Intracellular recordings were made from

31 blowflies, mostly females because access to the ocellar nerve is more difficult in males. The fly was fastened to a Plasticine block, its head was rotated to direct the ocelli forwards, and insect wax was applied to immobilise the head. Cuticle overlying the ocellar nerve and anterior part of the brain was removed, and the extra-ocular muscles were cut, taking care not to damage the tracheae of the brain. A small platform was manipulated to lie beneath the ocellar nerve. In order to aid penetration by microelectrodes, 1% protease (Sigma type XIV) was applied for 2 min to the ocellar nerve and brain. Microelectrodes, filled with  $2 \text{ mol l}^{-1}$  potassium acetate, had d.c. resistances of 80–100 M $\Omega$  and were connected to a conventional microelectrode amplifier that incorporated a bridge balance circuit.

The light source was usually a bright tungsten–halogen lamp (15 V, 150 W). Light was directed onto the end of a flexible, glass light guide, the other end of which was positioned 100 mm from the head. Either an electronic camera shutter or a vane moved by a servomotor was used as a shutter. The speed with which the vane passed over the light guide could be varied, with the most rapid switching lasting 5 ms and eliciting responses identical to those produced by the camera shutter. Where required, a second, independent tungsten–halogen light and light guide, positioned alongside the first, provided constant background illumination. The intensities of the light sources were altered independently by placing Kodak Wratten gel filters over the ends of the light guides. In addition, the intensity of light pulses was sometimes controlled by an iris diaphragm in the lamp housing. Light pulses were monitored by a photocell placed next to the end of the light guide nearest the fly. This was calibrated with an Ealing Radiometer with its sensor placed at the position of the ocelli. The maximum intensity of light delivered from either light source, measured at the ocelli, was  $28 \text{ mW cm}^{-2}$ . Less than 5% of the light energy was in the range of wavelengths (420–480 nm) that has been shown by electroretinogram recordings to excite fly ocellar photoreceptors (Kirschfeld *et al.* 1988). To determine the frequency responses of L-neurones, light was delivered from a silicon carbide, blue light-emitting diode (LED; Radio Spares no. 577-617; peak wavelength, 470 nm; maximum intensity at the eye,  $7 \mu\text{W cm}^{-2}$ ), placed 10 mm in front of the eye. The current passing through the LED was monitored and related to light output by calibration with the radiometer.

In 12 blowfly brains, L-neurones of either a lateral or the median ocellus were backfilled by application of 5% cobalt chloride to a small hole pierced through the lens of one ocellus. After 24 h at room temperature, the brain was dissected out, and cobalt was precipitated with dilute ammonium polysulphide. For intracellular staining in a further 15 brains, microelectrodes were filled with saturated hexammincobaltic chloride, and 500 ms pulses of 5–15 nA current were applied at 1 Hz for 10–20 min. Some cobalt-stained neurones were silver-intensified (Bacon and Altman, 1977).

## Results

### *Morphology of L-neurones*

Sections through the ocellar nerve revealed 12 clearly defined axons with diameters of 6–12  $\mu\text{m}$ , as well as a number of considerably narrower axons (Fig. 1A). In sections taken near to the ocelli, there are also cell bodies, 10–15  $\mu\text{m}$  across (Fig. 1A).

Intracellular injection of cobalt into L-neurone axons showed that these cell bodies belonged to L-neurones (Fig. 1B). Backfilling neurones of a single ocellus always revealed a bilaterally symmetrical distribution of neuronal processes which, in general appearance, was similar whether a lateral ocellus (Fig. 1C) or the median ocellus (Fig. 1D) had been injected. Usually, backfills of a lateral ocellus revealed seven L-neurones, with one more axon on the side ipsilateral to the injected ocellus than on the contralateral side. Backfills of a median ocellus usually revealed eight L-neurones, including a single pair that cross each other in the brain (long arrow in Fig. 1D). Because the total number of L-neurones revealed by backfilling a median and a lateral ocellus in

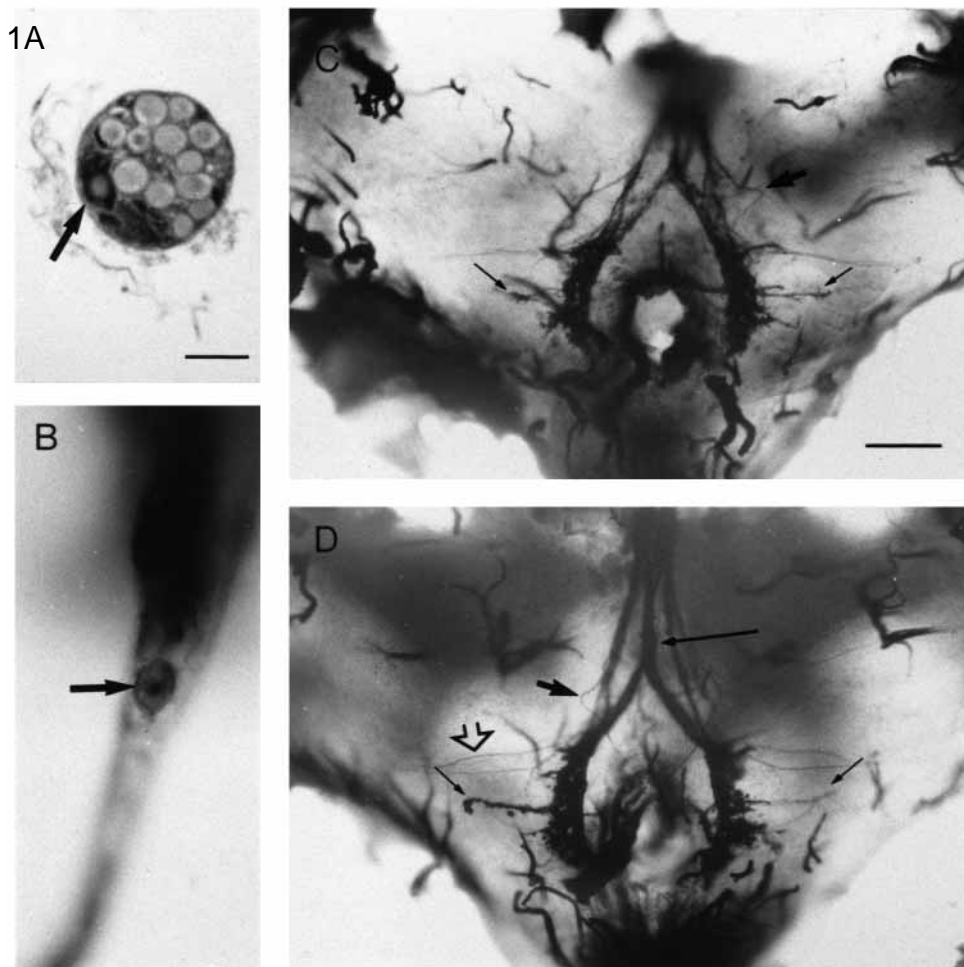


Fig. 1. Photomicrographs showing features of blowfly L-neurones. (A) Toluidene-blue-stained,  $2\ \mu\text{m}$  section through the ocellar nerve, just below the ocellar neuropile. Twelve clearly defined L-neurone axons are seen, as well as some cell bodies (arrow). (B) The cell body of one L-neurone (arrow), stained by intracellular injection of cobalt into the axon. (C,D) The posterior aspect of the brain, in which L-neurones were stained by backfilling from the left (C) or median (D) ocellus. Preparations were silver-intensified. Arrows indicate neurones referred to in the text. Scale bars, A,B,  $20\ \mu\text{m}$ ; C,D,  $100\ \mu\text{m}$ .

different preparations exceeds the number of axons revealed in sections of the ocellar nerve, some L-neurones probably innervate more than one ocellus. No single L-neurone projects to both left and right sides of the brain. All of the L-neurones have arborizations close to the oesophageal foramen, and almost all processes are directed laterally.

Using intracellular injection, L-neurones of four different morphologies within the brain have been stained, each on at least two occasions (Fig. 2). In general, the extent of arborization of the neurones that we have stained by intracellular injection is more restricted than those of the neurones traced from backfills by Nässel and Hagberg (1985). For example, the neurones drawn in Fig. 2A,B have arborizations restricted to the ventromedial area of neuropile, whereas types OL1 and OL2 of Nässel and Hagberg, which have the simplest morphology of the types they describe, resemble more closely the neurone drawn in Fig. 2C (which was stained four times), and have branches in the dorsolateral as well as ventromedial neuropiles. The neurone drawn in Fig. 2D was stained three times by intracellular injection and resembles type OL4 of Nässel and Hagberg (1985). Its distinctive feature is a branch directed laterally into the ventrolateral neuropile (Fig. 2D). Two neurones of this type, one on each side of the brain, were consistently stained in backfills of either one lateral or the median ocellus (Fig. 1C,D; small arrows), indicating that these neurones innervate all three ocelli. Using intracellular injection, we have stained neither a neurone with its arborization restricted to the dorsolateral neuropile (like type OL3 of Nässel and Hagberg, 1985) nor neurones that cross the brain (like types OL2 and OL5 of Nässel and Hagberg, 1985).

Backfills consistently revealed a number of fine processes that could be traced to fine axons in the ocellar nerve, but which could not be associated with any L-neurone. One pair of fine, looping axons (stout solid arrow, Fig. 1C,D) crosses the brain, just anterior to the oesophageal foramen, and is similar to neurones OMDN of Nässel and Hagberg

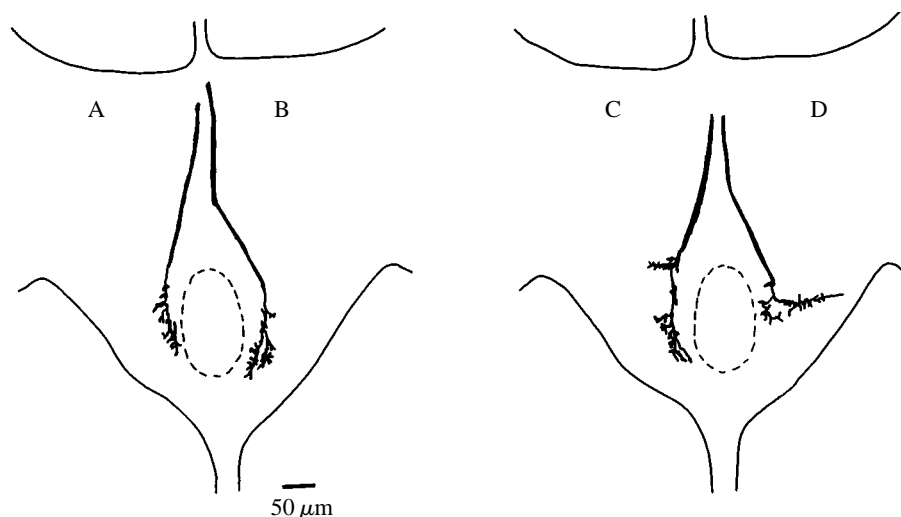


Fig. 2. Drawings of single L-neurones stained by intracellular injection of cobalt followed by silver-intensification. Each of the four neurones, A–D, was stained in a different brain. Brains are viewed posteriorly. The oesophageal foramen is drawn as a dashed line. Scale bar, 50  $\mu$ m.

(1985). A group of three straight fine processes (Fig. 1D, open arrow) travels towards an optic lobe and can be traced as far as the medulla. Nässel and Hagberg (1985) describe a single neurone, OMMe, like this on either side of the brain. Other small neurones have widespread projections within the protocerebrum.

#### *General responses to changes in light intensity*

Stable intracellular recordings were obtained in the ocellar nerve and ocellar tract in the brain from neurones that responded with hyperpolarising potentials of graded amplitude to increases in light. When injected with cobalt to reveal their morphology, these neurones were always revealed to be L-neurones. Occasionally, brief recordings were made from neurones that generated trains of spikes, the frequency of which altered when ocellar illumination changed.

The waveform of responses of L-neurones to pulses of light delivered in darkness (Fig. 3A) or against constant background illumination (Fig. 3B) consisted of an initial peak hyperpolarising potential, which declined in approximately 50 ms to a more sustained level. Rapid decreases in light intensity often produced sharply rising depolarising potentials, which sometimes included a spike. The initial peak hyperpolarising response saturated, in different flies, at 25–28 mV from the dark resting potential (Fig. 3A). At saturation, the repolarising phase had a more complex and variable waveform than that reported for L-neurones from other species, with a brief, rapid phase followed by an irregular repolarisation towards the sustained level (Fig. 3C).

After 2 min of dark adaptation, the intensity–response functions for peak responses typically had maximum slopes of  $-75$  mV per tenfold increase in light intensity (Fig. 3D). For sustained responses, the maximum slope was  $-6$  mV per tenfold increase in intensity. In the presence of sustained background illumination, adaptation maintained the slope of the intensity–response function but shifted it along the light intensity axis (Fig. 3D, two right-hand curves). When a long-lasting step increase in light was delivered, the membrane continued to repolarise slowly towards the dark resting potential following the initial fast repolarisation (Fig. 3E). For the range of light intensities we employed, increasing light intensity was associated with an increase in membrane potential noise (Fig. 3F), which declined only a little as membrane potential repolarised to its dark resting potential.

The effects of the rate of change of light intensity on L-neurone responses were investigated by delivering both ramp and sinusoidal changes in light intensity (Fig. 4). Ramp changes in intensity were produced by moving a vane across the light guide at different velocities (Fig. 4A). The most rapidly rising ramp saturated the response in this neurone, and the amplitude of the response decreased with decreases in the speeds of the ramps. The frequency response was measured by delivering light with sinusoidally modulated intensity from the blue LED (Fig. 4B,C). Although this light was insufficiently intense to saturate the responses (even after 15 min of dark adaptation), responses were largest at stimulus frequencies of 5–10 Hz, a similar figure to that obtained for L-neurones of bees (Baader, 1989) and locusts (Simmons, 1993).

*Electrical properties*

The input resistance of blowfly L-neurones changed when current was injected to alter membrane potential. Resistance was calculated from measurements of changes in potential caused by trains of small hyperpolarising current pulses, which were superimposed on longer pulses of different strengths (Fig. 5A,B). A single microelectrode was used both to inject current and to record membrane potential. Recordings were made in the dark. At resting potential, the input resistance of the neurone was  $6\text{ M}\Omega$  (range from four experiments,  $4\text{--}6\text{ M}\Omega$ ). Depolarising the neurone

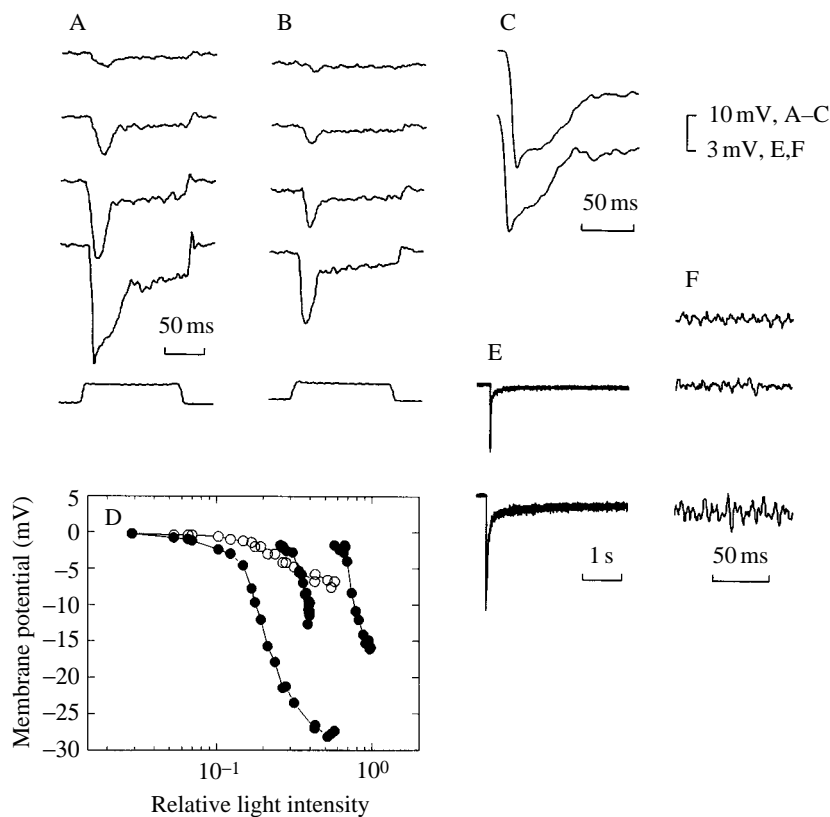


Fig. 3. Intracellular records of responses to light stimuli. (A) Responses to 120 ms pulses of light, delivered in darkness. Responses (top to bottom) to light pulses attenuated from maximum by 0.85, 0.67, 0.5 and 0 log units. (B) Responses to pulses of light of the same intensities as in A, but superimposed on a constant background ( $24\text{ mW cm}^{-2}$ ) to which the eye had been adapted for 2 min. (C) Saturating responses to the onset of two 120 ms light pulses, delivered in darkness with no attenuation. (D) Intensity-response curves for an L-neurone stimulated with 120 ms pulses of increased light intensity. The curves for peak responses (filled circles) are with no background illumination (left-hand curve) or two different intensities of background ( $7.3$  and  $15.7\text{ mW cm}^{-2}$ , right-hand curves). The curve for responses at 100 ms (open circles) is with no background illumination. (E) Responses to 10 s steps of light from darkness to half-maximal intensity (upper record) and maximal intensity (lower record). (F) Fluctuations in membrane potential recorded in darkness (upper record) and 4 s after each of the two steps in E (lower records).

with 1 nA reduced the resistance to its minimum value, 4.5 M $\Omega$ . With steady hyperpolarising currents, the membrane resistance increased, reaching a maximum of 16 M $\Omega$  at -3 nA.

When stronger hyperpolarising currents were injected, the membrane potential oscillated (Fig. 5C). This behaviour was observed in 20 out of 21 L-neurons tested. The strength of current at which oscillations first appeared differed between -1.5 and -4 nA in different neurones. As the strength of hyperpolarising current increased, there was an increase in the amplitude and latency of the oscillations, but not in their frequency (Fig. 5C). Oscillations were not elicited by depolarising currents (Fig. 5D). With strong hyperpolarising pulses, the membrane potential oscillations were irregular at first, but continued indefinitely once initiated (Fig. 5E). The largest oscillations included a

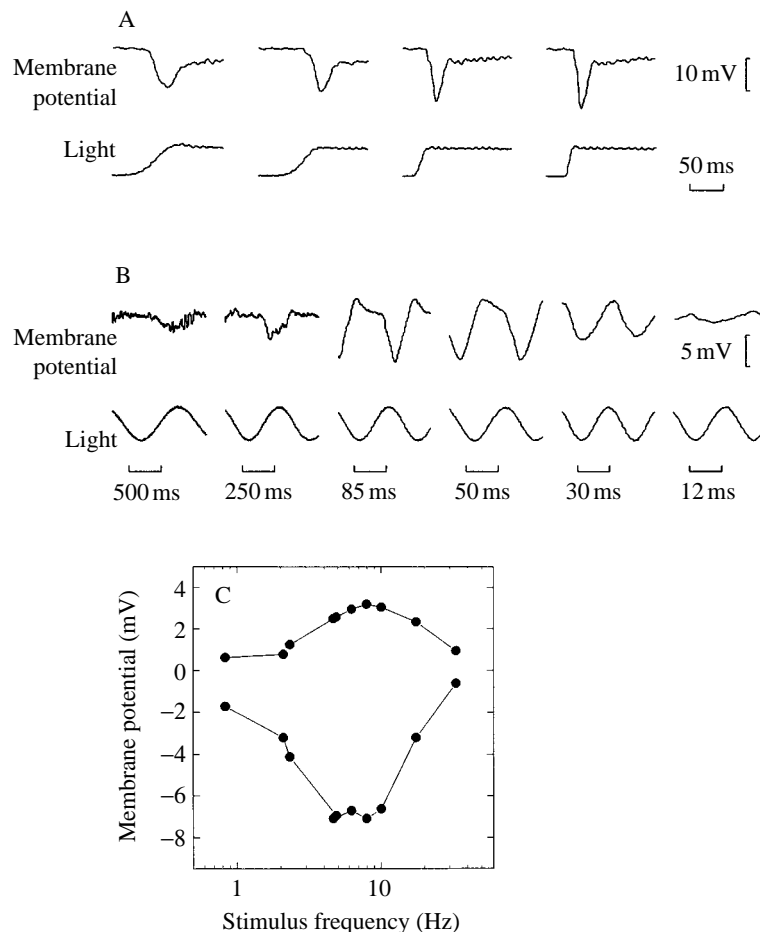


Fig. 4. Responses to different rates of change of light intensity. (A) Ramp increases in light intensity from dark to half-maximal intensity. (B) Responses to sinusoidally modulated light intensity, delivered from a blue LED. Maximum intensity was 7  $\mu\text{W cm}^{-2}$ ; minimum was darkness. (C) Frequency-response curves derived from the experiment in B. The two curves show peak levels of depolarisation and hyperpolarisation elicited by each frequency.



regenerative depolarising phase, which was much longer in duration than that of rebound spikes (Fig. 5F). As in other insects, the amplitudes of rebound spikes depended on both the amplitude and duration of the preceding hyperpolarising pulse (Fig. 5G).

*Changes in response to light when current was injected*

Injection of current into a blowfly L-neurone consistently altered the amplitude of the response to a pulse of light. The effect is illustrated for responses to light pulses of two different intensities in Fig. 6A,B. Depolarising the neurone decreased the amplitude of

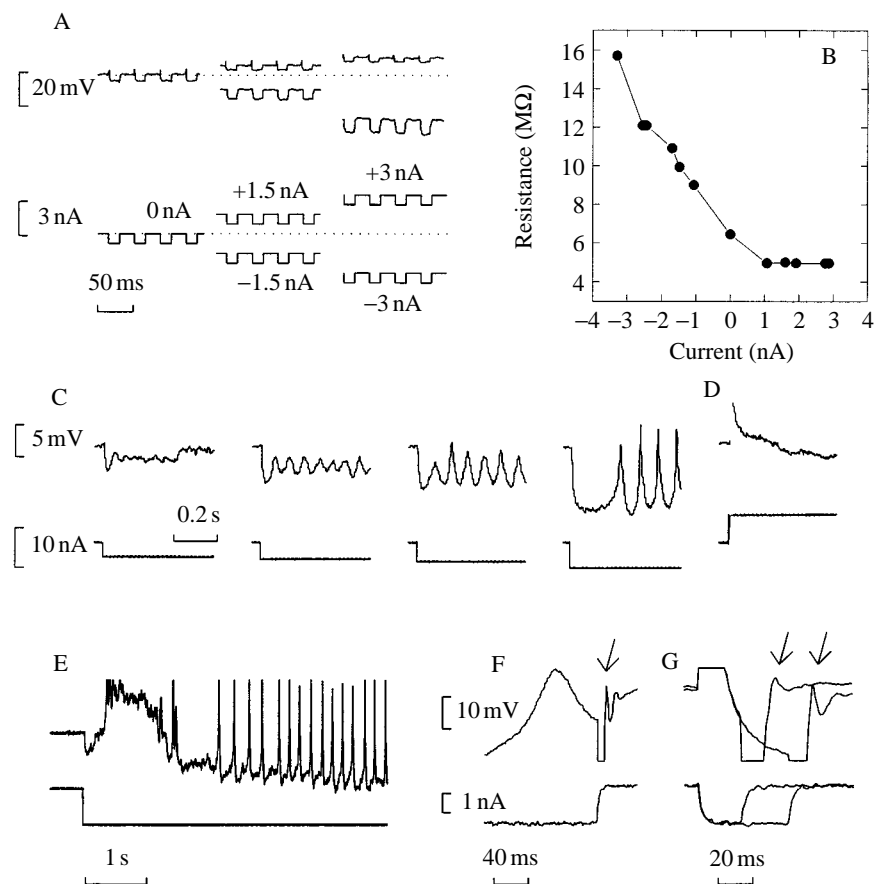


Fig. 5. Membrane characteristics of blowfly L-neurones. (A) The effect of membrane potential on input resistance. Resistance was monitored by measuring voltage deflections caused by a train of  $-0.75$  nA square pulses of current. (B) Plot of input resistance against amplitude of steady hyperpolarising or depolarising injected current. (C) Oscillations in membrane potential caused by hyperpolarising pulses of current. (D) Response to a pulse of depolarising current. (E) Response to injection of sustained hyperpolarising current ( $-10$  nA). (F) Comparison between a large, oscillatory potential and a rebound spike (arrow). (G) Two rebound spikes (arrows) in response to the ends of pulses of hyperpolarising current of different durations. In A and C–G, the injected current is shown in the lower traces and the membrane potential in the upper traces.

the response to increased light intensity, whereas injecting up to  $-4$  nA hyperpolarising current into the neurone increased the response amplitude. The effects of currents of greater strength were obscured by the rhythmic oscillations these currents caused (bottom trace, Fig. 6A). Similarly, when most neurones were hyperpolarised by injection of small currents, intense pulses of light caused the membrane potential to oscillate briefly. In only one neurone out of 21 were the effects of current on responses to saturating pulses of light recorded without being obscured by oscillations (Fig. 6C). Here, the effects of current on the initial peak hyperpolarising response were different from the effects on the more sustained response. Injection of  $-4$  nA decreased the amplitude of the peak response from 28 to 22 mV, whereas it increased the response at the end of the 200 ms light pulse from 5 to 10 mV. In the same neurone, injection of a train of hyperpolarising current pulses showed that, during the sustained part of the response to a pulse of light, membrane resistance was greater than that in the dark (Fig. 6D).

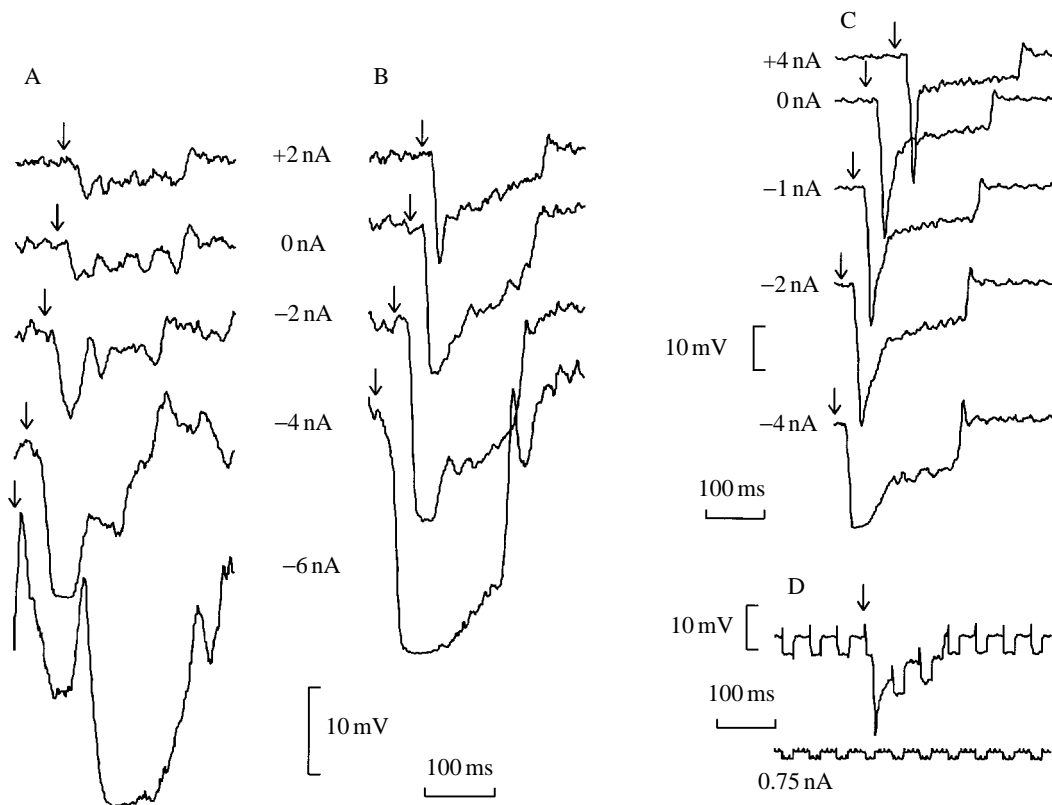


Fig. 6. Effects of steady injection of depolarising or hyperpolarising current on responses to light. (A,B) Light pulses of two different, sub-saturating intensities. The light was stronger in B. (C) Light pulses of saturating intensity. (D) Changes in resistance monitored by voltage deflections caused by a train of square current pulses, during illumination with a pulse of light. A and B were from one fly, and C and D were from two separate flies. Arrows indicate the times at which pulses of light started. These times are offset in successive traces in order to facilitate comparison of the response amplitudes.

## Discussion

### *Morphology*

L-neurones of the blowfly have a number of unusual structural and physiological features, although many of their general characteristics correspond with those of other species of insect. The most unusual morphological feature is the location of blowfly L-neurone cell bodies outside the brain, as described by Strausfeld (1976). As far as we are aware, no other insect interneurone has been reported to have its cell body outside the central nervous system. L-neurone cell bodies are located in the brain for all other orders so far examined, including both holometabolous (Hymenoptera, Pan and Goodman, 1977; Lepidoptera, Pappas and Eaton, 1977; Trichoptera, Hagberg and Nässel, 1986) and hemimetabolous (Orthoptera, e.g. L. J. Goodman *et al.* 1975; C. S. Goodman, 1974; Koontz and Edwards, 1984; Blattoidea, Mizunami *et al.* 1982; Odonata, Chappell *et al.* 1978) insects. During development in *Drosophila*, the ocelli and compound eyes arise from a common imaginal disk, outside the central nervous system (Vogt, 1946). In locusts, the pathway between each ocellus and the brain is pioneered by the axons of photoreceptor cells (Mobbs, 1979) so, clearly, there are differences in the ways in which ocelli develop in flies compared with other insects.

Our description of the morphology of blowfly L-neurones is in general agreement with that of Nässel and Hagberg (1985), who used only backfill preparations and restricted their description to projections within the brain. We found more limited arborizations, perhaps because of differences in staining technique, with single L-neurones being difficult to trace in backfill preparations. There may also be real variability between flies (Nässel and Hagberg, 1985). This has been well documented for L-neurones in locusts (Goodman, 1974). The similarities between the projection patterns revealed by backfills from the median and from a lateral ocellus indicate that many neurones innervate more than one ocellus, as also reported by Strausfeld (1976). The smaller ocellar neurones that we have stained are similar to some of those described by Nässel and Hagberg (1985). In general, insect L-neurones are anatomically simpler than the smaller neurones that have axon in the ocellar tract (e.g. locusts, Goodman and Williams, 1976; bees, Heinzeller, 1976; moths, Eaton and Pappas, 1978). Some of the smaller neurones have complex arborizations in the optic lobes, indicating interaction between ocellar and compound eye pathways.

### *Physiology*

L-neurones of blowflies resemble those of other insects, and large monopolar cells of the fly compound eye, in that they generate graded, hyperpolarising potentials in response to increases in light intensity, with response curves that shift with changes in mean illumination (LMCs, Laughlin and Hardie, 1978; cockroach L-neurones, Mizunami *et al.* 1986; locust L-neurones, Simmons, 1993). This adaptation allows visual pathways to operate over a wide range of environmental conditions and occurs at many different levels (Laughlin, 1989). As in L-neurones of other insects, depolarising responses to decreases in light intensity are boosted by regenerative properties, which are activated by rebound from hyperpolarisation and sometimes give rise to small spikes. The strengths of

current required to elicit rebound spikes are smaller in blowflies than in locusts (Wilson, 1978*b*), dragonflies (Simmons, 1982) or cockroaches (Mizunami *et al.* 1987), consistent with a relatively high input impedance for L-neurons (5–6 M $\Omega$  in the dark, compared with 1–2 M $\Omega$  for locusts, Wilson, 1978*b*).

The large oscillations in the membrane potential of blowfly L-neurons, revealed by injection of hyperpolarising current, have not been described for other neurons of insect visual pathways. Faster, spike-like changes in potential can sometimes be unmasked by hyperpolarisation. For example, some neurons in the blowfly optic lobe, which normally operate without spikes, will generate spikes in response to visual stimuli if steady, hyperpolarising current is injected into them (Hengstenberg, 1977). In bees, some ocellar neurons apparently switch between a non-spiking and a spiking state (Milde, 1981, 1984; Milde and Homberg, 1984), although the voltage changes responsible for this switch have not been characterised. No indication of this kind of switch was found in blowfly L-neurons. A possible mechanism for producing these oscillations involves voltage-sensitive channels that are inactivated at the dark resting potential of an L-neurone. Similar channels have been shown to contribute to graded, active responses and to oscillations in membrane potential of neurons in the mammalian brain stem (Jahnsen and Llinás, 1984). However, unlike these responses in mammalian brain-stem neurons, the oscillations in blowfly L-neurone membrane potential occur during hyperpolarisation of the neurone, without the need for a depolarising trigger. The depolarising phase of the oscillations in blowfly L-neurone potential might contribute to the phasic nature of the initial hyperpolarising potential that follows a step increase in light intensity. However, oscillations are not seen during prolonged illumination of the ocelli, probably because the rate of release of transmitter from the photoreceptor cells is reduced.

A second unusual feature of L-neurone physiology is that small, hyperpolarising currents injected into an L-neurone cause an increase in the amplitude of the hyperpolarising response to increased light intensity. There are a number of possible explanations for this effect. First, the photoreceptors might make conductance-increase, excitatory synapses with the L-neurons. This is unlikely, because it requires that the photoreceptors hyperpolarise when illuminated, unlike all arthropod photoreceptors so far investigated, including those of *Drosophila* ocelli (Labhart, 1977). Second, the neurotransmitter released by the photoreceptor cells might mediate a decrease in L-neurone conductance. This would be unlike the action of the probable neurotransmitter histamine on fly LMCs (Hardie, 1987) or locust L-neurons (Simmons and Hardie, 1988). Third, there might be inhibitory feedback synapses from L-neurons onto photoreceptors, so that hyperpolarising an L-neurone would augment the release of transmitter from the photoreceptors. Such feedback was proposed to underlie the shaping of the responses of dragonfly L-neurons to step increases in light intensity (Dowling and Chappell, 1972), and an ultrastructural study of the flesh fly ocellus has shown that photoreceptor cells receive synapses from interneurons (Toh and Kuwabara, 1975). In dragonflies, however, dual recordings from photoreceptors and L-neurons failed to reveal functional feedback connections (Simmons, 1982).

Finally, and most likely, hyperpolarising an L-neurone may increase its input resistance, which would lead to an increase in the amplitudes of hyperpolarising

potentials produced in response to small increases in light intensity. We have provided direct evidence for this explanation by showing that membrane resistance does increase when the membrane is hyperpolarised, either by injection of current through a microelectrode, or during the sustained phase of a response to a pulse of light. Supporting evidence is provided by the observation that hyperpolarising current does not increase the amplitude of the saturating response to light, which would be limited by the reversal potential for the postsynaptic potential and would, therefore, be unaffected by changes in input resistance. In thoracic, non-spiking local interneurons of locusts, the amplitudes and shapes of postsynaptic potentials are significantly affected by membrane resistance (Laurent, 1990), brought about by activating or inactivating a voltage-sensitive potassium current (Laurent, 1991). The results presented in the present paper point to similar roles for voltage-activated currents in shaping responses of blowfly L-neurons to visual stimuli.

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