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The Operation of Connexions Between Photoreceptors and Large Second-Order Neurones in Dragonfly Ocelli

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Summary. Photoreceptors and large second-order neurones (L-neurones) of dragonfly ocelli have been penetrated simultaneously with microelectrodes to study the operation of the synapse between them. The responses of L-neurones to changes in illumination are of opposite polarity and are more phasic than those of photoreceptors.

1. When pulses of light are superimposed on a constant background illumination, the phasic nature of L-neurone responses is often enhanced. Sometimes, during a light stimulus, an L-neurone is depolarised relative to its resting potential. With rapidly repeated short pulses of light, responses of L-neurones decrement markedly, although the full response of photoreceptors is maintained.

2. Passive membrane properties of L-neurones cannot contribute significantly to the cutback in the hyperpolarising response of these neurones to light on.

3. When depolarising or hyperpolarising currents are injected into a photoreceptor, responses in an L-neurone it synapses with are of opposite polarity and markedly phasic. The voltage gain of the connexion between one photoreceptor and an L-neurone can be as great as nine.

4. No evidence for feedback connexions from L-neurones to photoreceptors has been found.

5. Conductance measurements on some L-neurones indicate that synaptically-induced currents may make a small contribution to the cutback in the hyperpolarising response to light on.

6. Some L-neurones make rapidly decrementing inhibitory connexions with other L-neurones.

7. A variety of neurones which respond to changes in illumination by alterations in spike rate have been found. Some of these connect with L-neurones.

8. Possible mechanisms for the cutback in the hyperpolarising response of an L-neurone at light on include intrinsic properties of the pre- or postsynaptic terminals, and excitatory synapses made by small second-order neurones on L-neurones.

Introduction

Large second-order neurones (L-neurones) of insect ocelli can detect rapidly occurring, slight changes in light intensity. Chappell and Dowling (1972), who first made intracellular recordings from ocellar neurones, found that, compared with the responses of photoreceptors, the responses of L-neurones of dragonflies showed: 1. a longer latency; 2. opposite polarity; 3. enhanced photosensitivity; and 4. a more transient character. Receptors and L-neurones of locusts respond in a similar manner (Patterson and L.J. Goodman 1974; Wilson 1978a), as do the photoreceptors and second-order neurones of arthropod compound eyes (review, Laughlin 1981).

Dragonfly ocelli are well suited to study the transmission of signals across the first synapse in a visual pathway because both the photoreceptors and the L-neurones are relatively large. The median ocellus of a dragonfly contains about 1,500 photoreceptors (Cajal 1918; Ruck and Edwards 1964), and each lateral ocellus about 680 (Ruck and Edwards 1964). A photoreceptor is about 230 μm in length, with a cell body 7–8 μm wide and axon 1–2 μm wide (Patterson and Chappell 1980). For the median ocellus, the structures of nine types of L-neurone, with axons at least 10 μm in diameter, have been described (Patterson and Chappell 1980), and for a lateral ocellus four types of L-neurone have been described (Chappell et al. 1978). There are also

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many smaller second-order neurones and some efferent neurones of unknown function (Kondo 1978; Rosser 1974). Dowling and Chappell (1972) have made an ultrastructural study of the synaptic plexus of the median ocellus. Most synaptic contacts made by the receptor terminals are diadic, and the receptor axons are often reciprocally as well as serially synaptic with each other. In addition, Dowling and Chappell (1972) tentatively reported a few reciprocal synapses, made by L-neurones back onto photoreceptors.

In this study, the operation of connexions made between photoreceptors and L-neurones of dragonfly ocelli has been investigated by recording simultaneously from photoreceptors and L-neurones. This method allows both comparison of the responses of the two types of neurone to the same light stimuli, and investigation of the transmission of signals between the neurones by injecting currents into one of them. Of particular interest is the mechanism which enhances the phasic nature of the responses of L-neurones to changes in illumination. Dowling and Chappell (1972) suggested that feedback synapses from L-neurones to photoreceptors could contribute to this, and since then pharmacological (Klingman and Chappell 1978) and physiological (Stone and Chappell 1981) evidence in support of this mechanism has been produced. For locust ocelli, however, Wilson (1978b) has provided evidence that the feedback to receptors comes from neurones other than the L-neurones. The direct test of injecting current into single L-neurones and searching for postsynaptic responses in photoreceptors has not been performed before.

Materials and Methods

Adult dragonflies were caught locally. Experiments were performed on 17 *Hemianax papuensis* (Burmeister), 15 *Aeshna brevistyla* (Rambur) and 17 *Hemicordulia tau* (Selys), all males. In preparation for an experiment, a dragonfly was secured upside down on a plasticence block, and the head embedded securely with the frons uppermost. The mandibles were removed and the wounds sealed with wax. The median ocellus was exposed by removing most of the frons and then two small jaw muscles. To record from the lateral ocelli, the median ocellus and its thick nerve were removed. Throughout the experiment the brain was bathed in saline (Eibl 1978), and the brain and ocelli were ventilated through the tracheae by the animal's own pumping movements. The temperature in the laboratory was 25–27 °C.

Recordings from L-neurones of median and lateral ocelli remained stable for periods in excess of 4 h and penetrations of photoreceptors were held for up to 30 min. Close attention was paid to the condition of neurones from which recordings were made by continually monitoring their responses to pulses of light. This was particularly important when current was injected into a photoreceptor.

Glass capillary microelectrodes were filled with 2 mol/l potassium acetate and had dc resistances of 70–120 Mohm. They were attached to amplifiers incorporating bridge circuits. Current passed through an electrode was measured using a virtual ground amplifier, with a silver wire placed in the head capsule acting as an indifferent electrode. Results were filmed directly from the oscilloscope screen. Recordings were made from L-neurones and spiking neurones in the ocellar nerve between the neuropile of the ocellus and the brain. Photoreceptors were penetrated both in the cell body layer and in the neuropile region, often proximal to the pigmented ocellar sheath (see Chappell and Dowling 1972). Neurones were identified by the waveform of their responses to changes in illuminations since other studies involving intracellular recording followed by staining of individual neurones have shown that this method of identifying ocellar photoreceptors and L-neurones is reliable (Patterson and Chappell 1980; Mobbs et al. 1981; Simmons 1982).

As a light source, a Siemens Type LD-57C high intensity light emitting diode, placed 6 cm in front of the median ocellus and approximately aligned with its optical axis was used. This diode emits light with a peak wavelength of 560 nm. The intensity of light emitted by this device was measured using an International Light ISL 700 Research Radiometer, with an SEE 400D Vacuum photodiode, calibrated with a correction factor for wavelength 560 nm. Intensity of light was altered both by varying the current which passed through the light emitting diode and by means of neutral density filters fitted over the aluminium tube which contained the diode. The maximum light intensity emitted by the diode in these experiments was 2.85×10^{12} photons/cm^2/s. Sometimes pulses of light were delivered in darkness (when the room was darkened as much as practicable, the intensity of light falling on the ocellus was less than 10^6 photons/cm^2/s), and at other times, pulses of increased illumination were superimposed on a constant background illumination.

Results

Responses of L-Neurones and of Photoreceptors to Light Stimuli

Figure 1 illustrates the responses of photoreceptors and L-neurones to light stimuli, and confirms and extends the observations of Chappell and Dowling (1972). In each recording, a photoreceptor and an L-neurone were penetrated simultaneously. Subsequently current was injected into the photoreceptor in order to demonstrate that a functional connexion was made between the two neurones. When light intensity increases, a photoreceptor depolarises and, following an initial peak, a plateau level of depolarisation is sustained until light intensity decreases (Fig. 1A). Responses of L-neurones are of opposite polarity to those of the photoreceptors, and more markedly phasic, especially when bright pulses are superimposed on constant background illumination (Fig. 1B).

About 30% of photoreceptors, when initially penetrated, either in the cell body or the axon, gave very bumpy responses to dim illumination (Fig. 1C;

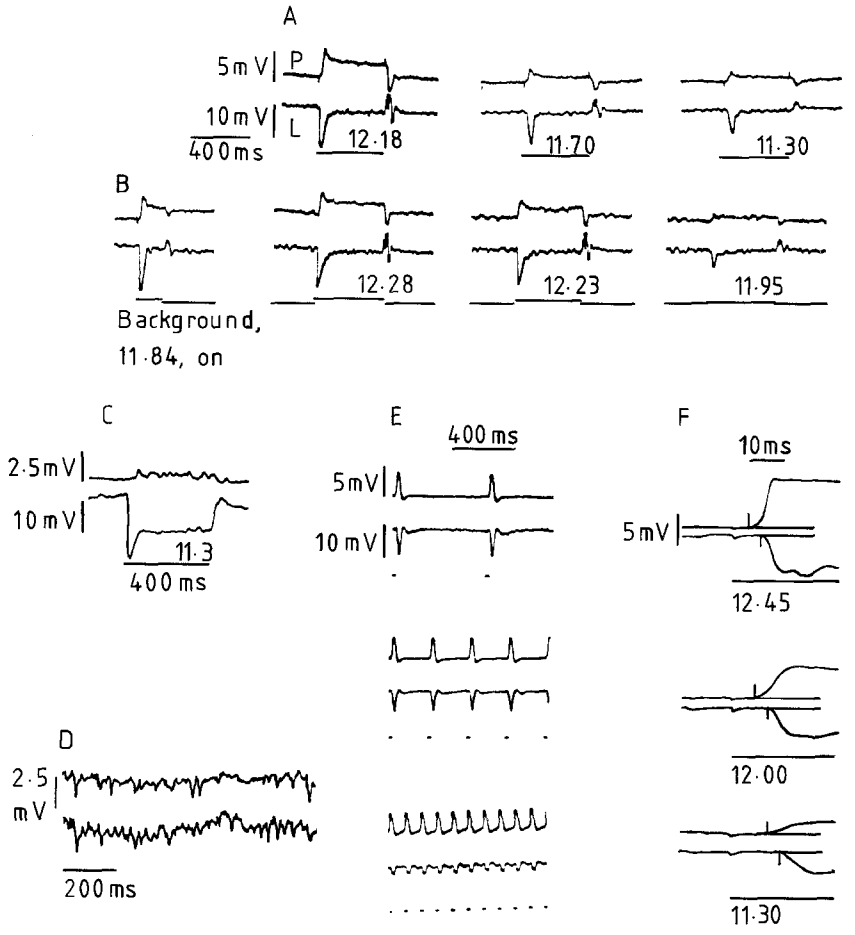

Fig. 1A–F. Responses of photoreceptors and of L-neurones they connect with to light stimuli. In all records in this paper where pulses of light were delivered, the bottom trace indicates the timing of the stimulus, and the intensity of the stimulus is expressed as log photons/cm^2/s incident at the ocellus lens.
A Pulses of light, 400 ms long, delivered in darkness. The animal was dark adapted for 5 min and stimuli were delivered at 5 s intervals.
B Light stimuli delivered when the ocellus was illuminated with a continual background (the background was switched on during a stimulus pulse).
C Response of a newly penetrated photoreceptor and an L-neurone to a dim light stimulus.
D In the darkened laboratory, two L-neurones, recorded simultaneously, show a constant barrage of hyperpolarising potentials.
E Responses of a photoreceptor and of an L-neurone to 20 ms-long pulses of light, delivered at various frequencies.
F Latency of responses of a photoreceptor and of an L-neurone it synapsed with to the onset of 3 pulses of light of different intensities

Stone and Chappell 1981, also show a similar figure). Some discrete hyperpolarising potentials in locust L-neurones correspond with the absorption of single photons by photoreceptors (Wilson 1978c), and similar discrete hyperpolarising potentials are also found in dragonfly L-neurones (Fig. 1D). It should be possible to relate discrete depolarising potentials in receptors with corresponding hyperpolarising potentials in L-neurones, but photoreceptor responses always became smooth too rapidly to allow this.

Figure 1E shows recordings from a photoreceptor and an L-neurone when 20 ms-long pulses of light, repeated at different frequencies, were delivered to the ocellus. At frequencies of repetition up to 10 Hz, each photoreceptor response was the same amplitude as to pulses repeated at lower frequencies. In contrast, responses of the L-neurone declined markedly as the frequency of repetition increased. This experiment indicates that one feature that contributes to the enhancement of phasic responses in L-neurones compared with those of photoreceptors is that transmission at the connexion between photoreceptors and L-neurones cannot be sustained for long. An alternative mechanism, involving a filter which allows rapid changes in potential to be transmitted more effectively than slower changes, would give different results.

Recording simultaneously from a photoreceptor and an L-neurone allows a direct measure of the latency in transmission between them (Fig. 1F). This ranged between 3 and 4 ms in different preparations, which is rather shorter than the measurements given by Chappell and Dowling (1972).

Responses of L-neurones to changes in illumination are examined in more detail in Fig. 2. Figure 2A shows responses to light stimuli delivered in darkness (plotted as crosses in Fig. 2D). When stimuli are superimposed on background illumination (Fig. 2B and 2C; dots in Fig. 2D), the response to each total level of illumination decreases, the intensity/response functions are steeper, and the responses become more transient. Sometimes, when a stimulus is superimposed on constant background illumination, the cutback in response of an L-neurone that follows the initial

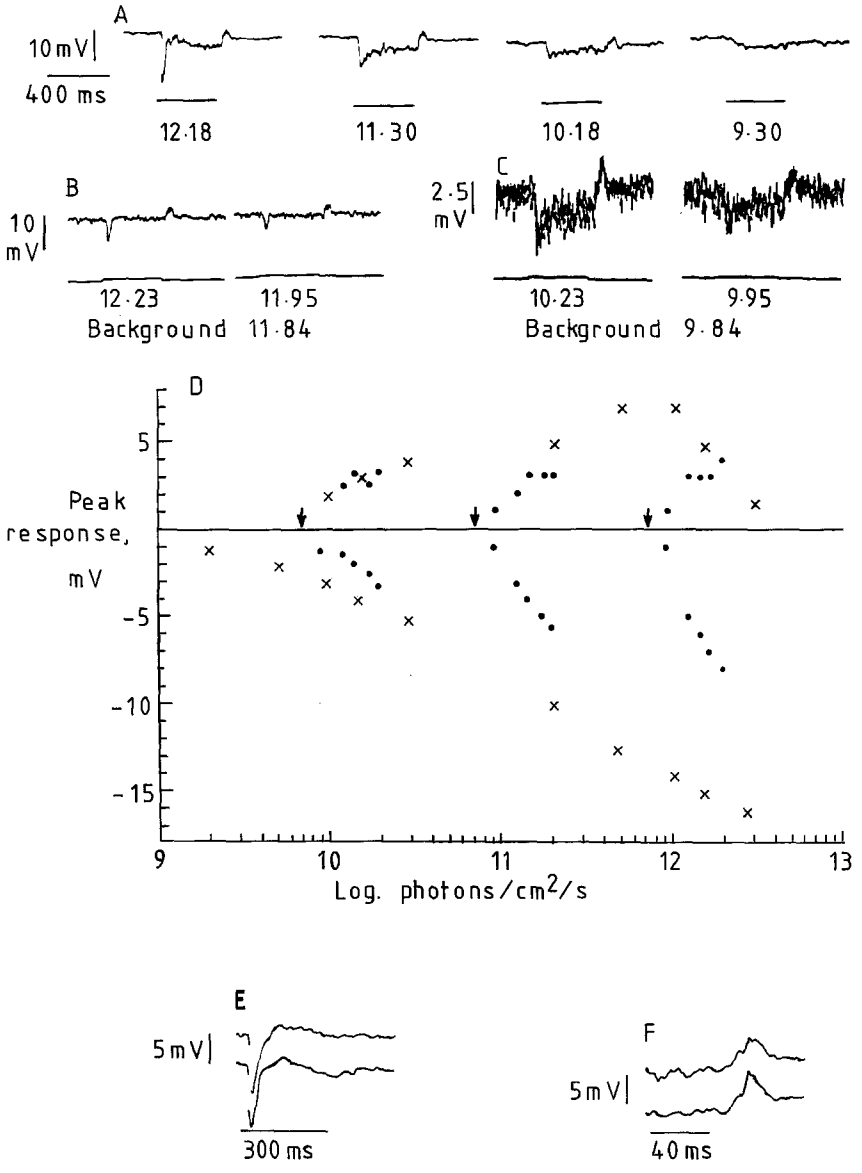

Fig. 2A–F. Responses of L-neurones to light stimuli.
A Light stimuli delivered in darkness, to an ocellus dark-adapted for 2 min.
B, C Light stimuli delivered against constant background illumination. For each record in **D**, 5 repetitions are superimposed.
D Plots of the responses recorded in the experiment of **A–C** to the onset and offset of stimuli. Responses to stimuli delivered in darkness plotted as crosses, and responses to stimuli delivered against background illumination plotted as dots. The three arrows indicate the intensities of the three backgrounds.
E Responses of two L-neurones of a median ocellus to a pulse of bright light delivered in darkness.
F Responses of two median L-neurones to light offset

hyperpolarising transient results in a depolarisation, relative to the neurone's potential before stimulation (Fig. 2E).

Figure 2F shows the responses of two L-neurones of the same ocellus to the offset of a light stimulus. They produced depolarising potentials, about 20 ms in duration. Each depolarising potential consists of a series of components, resembling excitatory postsynaptic potentials (EPSPs). Unlike locust L-neurones (Patterson and Goodman 1974; Wilson 1978a, b) dragonfly L-neurones rarely produce spikes in response to decrease in illumination.

Effects on L-Neurones of Injecting Current into Photoreceptors

Injecting pulses of hyperpolarising current into a photoreceptor causes transient depolarisations in an L-neurone it synapses with, and injection of depolarising current into the photoreceptor causes transient hyperpolarisations (Fig. 3A, B). In Fig. 3A i, 10 nA of hyperpolarising current was injected into the photoreceptor, and this caused a depolarising potential of 1.7 mV in the L-neurone. Probably injection of −10 nA into the photoreceptor hyperpolarised it below the threshold for transmitter release, because injection of any greater currents did not produce larger depolarisations in the L-neurone. Figure 3B shows that each 1 mV postsynaptic hyperpolarisation is caused by 2.6 nA depolarising current injected into the photoreceptor, when the receptor is depolarised above its dark resting potential. One other experiment where the penetration of the photoreceptor remained stable when current was injected into it gave similar values to these. In another five experiments, which

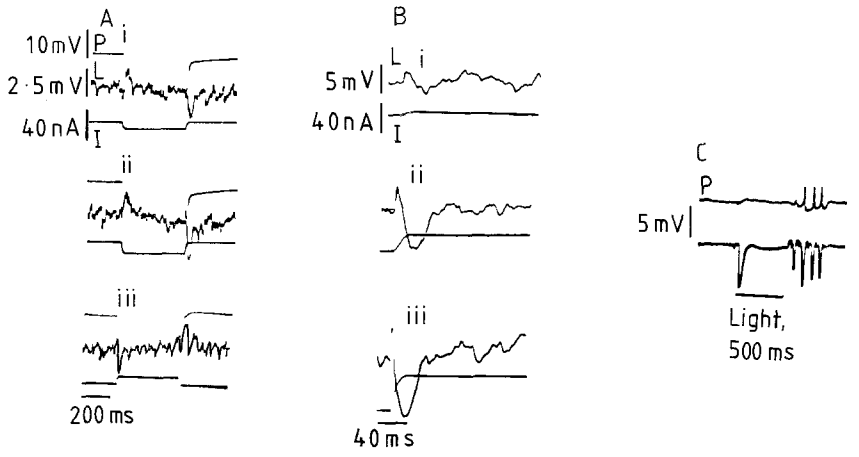

Fig. 3A–C. Operation of connexions between single photoreceptors and L-neurones.
A, B Positive and negative current injected into a photoreceptor causes postsynaptic potentials of opposite polarity in an L-neurone. In **A**, the bridge circuit of the amplifier recording the photoreceptor response was unbalanced, and in **B**, this trace is omitted.
C A photoreceptor spikes at the end of a light stimulus, and each spike is accompanied by an IPSP in the L-neurone

gave similar waveforms in L-neurones to those shown in Fig. 3A and B, much higher currents were required.

Following the end of a pulse of negative current injected into a photoreceptor, an L-neurone produces a transient hyperpolarising potential (Fig. 3A i, ii). One possible explanation for this is that regenerative depolarising activity is triggered in the photoreceptor when the current injected into it is switched off (better balancing of the bridge circuit in the amplifier might have revealed such activity). Another possible explanation is that transmission across the synapse is facilitated by the hyperpolarisation of the presynaptic terminal. Following the end of a pulse of depolarising current applied to a photoreceptor, an L-neurone produces a transient depolarising potential (Fig. 3A iii). This might be caused by the same mechanism which causes the cutback in the light-induced hyperpolarisation of an L-neurone; or it might be due to a transient hyperpolarisation of the photoreceptor, as is sometimes seen after the end of a pulse of light.

On two occasions, while responses of an L-neurone were being recorded, photoreceptors into which pulses of current had been injected, produced spikes at the end of pulses of illumination (Fig. 3C). In Fig. 3C, the first spike in the photoreceptor has an amplitude of about 0.5 mV, and produces an inhibitory postsynaptic potential (IPSP) of 4.5 mV in the L-neurone. Subsequent spikes are 2.5 mV in amplitude, and produce IPSPS of 7 mV, 6 mV and 5.5 mV. These measurements suggest first, that a synapse between one photoreceptor and one L-neurone can have a gain of at least 9 (first spike and IPSP), and second, that the gain is reduced with usage of the synapse (subsequent spikes and IPSPs).

Chappell and Dowling (1972) also report recording spikes from dragonfly ocellar photoreceptors on two occasions, and spikes are routinely recorded from photoreceptors in the compound eyes of drone bees (Shaw 1969). Here they are undoubtedly an artefact, caused by penetration of the receptor by an electrode, but the recording (Fig. 3C) does indicate the presence of voltage-sensitive conductances in ocellar photoreceptors.

Current/Voltage Relations of L-Neurones

The input resistances of five L-neurones, in different dragonflies, were measured by employing one electrode to pass current into an L-neurone and a second electrode to record voltage changes in the neurone (Fig. 4). One of these electrodes was placed in the ocellar neuropile and the other at the base of the ocellar nerve. When hyperpolarising currents were injected, a peak hyperpolarising potential was attained rapidly, and then decayed to a more sustained plateau level (Fig. 4B). With currents greater than 50 nA, the peak decays by 30% in 60 ms, and for the neurone of Fig. 4, no further significant decline in hyperpolarisation occurred after 500 ms.

In the neurone of Fig. 4, single spikes were produced at the end of hyperpolarising pulses 20 mV or greater in amplitude. These spikes never exceeded 10 mV in amplitude. Two of five L-neurones tested did not produce any. Spikes were never elicited when depolarising current was injected into an L-neurone, although some neurones did show some evidence of regenerative responses. Following the end of a depolarising pulse, L-neurones produced transient hyperpolarising potentials (Fig. 4B, bottom record).

The five neurones gave a mean value for input resistance of 1.2 MOhm, at resting potential (range, 1.0 to 1.5 MOhm). This is slightly lower than 1.74 MOhm measured by Wilson (1978b) for locust L-neurones, but axons of dragonfly L-neurones are generally wider than their counterparts in locusts. Compared with locust L-neurones (Wilson 1978b)

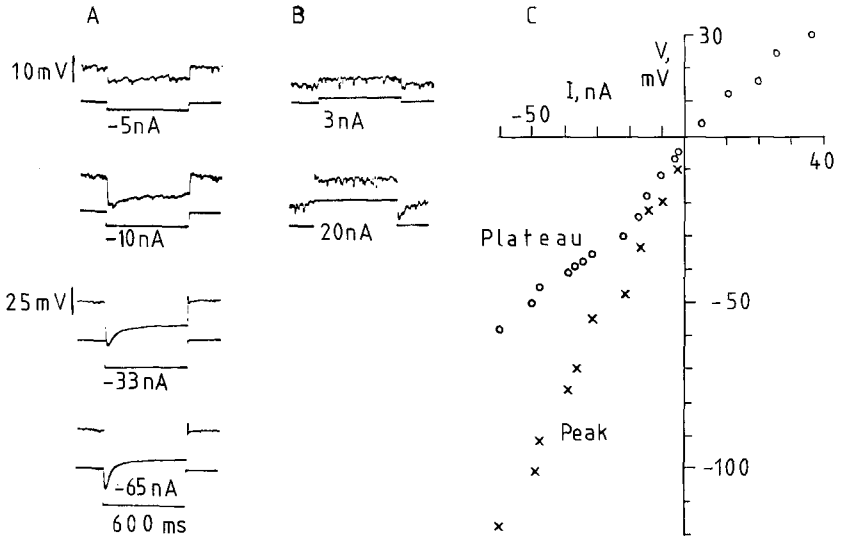

Fig. 4A–C. Current/voltage relations of an L-neurone of a lateral ocellus.
A Hyperpolarising current.
B Depolarising current.
C Plot of the current/voltage relation.

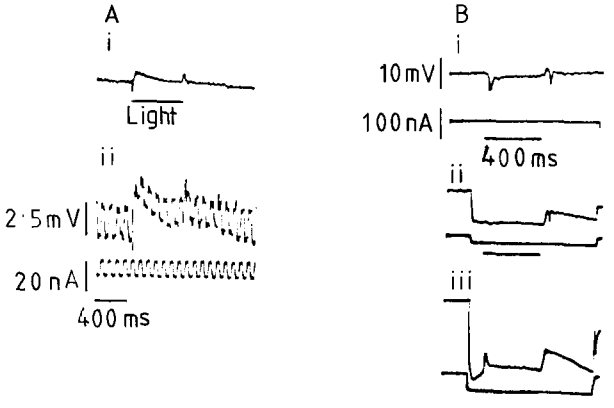

Fig. 5A, B. Conductance changes in L-neurones during increased illumination. **A** (i) when illuminated, following the initial hyperpolarising potential, this L-neurone depolarised relative to the dark potential. (ii) Pulses of hyperpolarising current show that the conductance of the neurone is greater during this depolarisation than in the dark. Pulses of depolarising current gave the same result, reducing the possibility that the conductance change was due to non-linearity in the neurone's resistance. **B** Reversal of the hyperpolarising responses of an L-neurone to increased illumination

those of dragonflies show a decay in hyperpolarisation at much smaller potentials. The hyperpolarising potential following depolarising pulses (Fig. 4B) has not been reported from locust L-neurones.

Conductance Changes in L-Neurones Following Changes in Illumination

If the cutback in hyperpolarisation of an L-neurone following increased illumination is due to synaptic input which depolarises the L-neurone, then the neurone's conductance should increase as its hyperpolarisation decreases. The neurone of Fig. 5A depolarised following the initial hyperpolarising transient, when a bright stimulus was superimposed on constant background illumination. The voltage changes too rapidly during the initial hyperpolarisation to allow measurement of conductance changes. However, during the depolarisation which follows, the conductance of the neurone, measured by injecting short pulses of current of constant amplitude into it, was clearly greater than the conductance preceding the stimulus. Most neurones where the membrane potential cut back as far as the resting level showed only a small increase in conductance, but a few showed a decrease. Thus, although there may be some synaptic input onto an L-neurone which depolarises it following an increase in light intensity, it is unlikely to be important in producing the cutback in hyperpolarisation in the L-neurone.

Results of an experiment to determine the reversal potential of the responses to a light stimulus by an L-neurone are shown in Fig. 5B. Both the initial transient and the plateau reversed at a potential 11 mV hyperpolarised from resting (Fig. 5B ii). Since both reversed at the same potential, they are probably both mediated by the same ionic mechanisms (this has also been reported for locust L-neurones, Wilson 1978c). Five experiments gave reversal potentials ranging from -10 mV to -15 mV. In each experiment, both the initial transient and the plateau reversed at the same potential, which indicates that conductance changes in the L-neurone do not contribute to the cutback in light-induced hyperpolarisations. In locusts, L-neurone responses to increased illumi-

nation reverse at about −40 mV, so different ionic mechanisms must operate in the two types of insect.

Connexions Among L-Neurones

From thirty pairs which were tested, no excitatory connexions between L-neurones were found. Between five pairs of L-neurones, inhibitory connexions were found (Fig. 6A). One of these pairs was in a lateral ocellar nerve, and the four remaining pairs in the median ocellar nerve. When depolarising pulses of current were injected into the presynaptic neurone, an IPSP was produced in the post-synaptic neurone. In three cases the connexions were clearly two-way, and in the remaining two results were not so clear. These inhibitory connexions appear to operate in a similar way to those between locust L-neurones (Simmons 1982) in that the IPSP has a duration of 10 ms, whatever the duration of the current injected presynaptically.

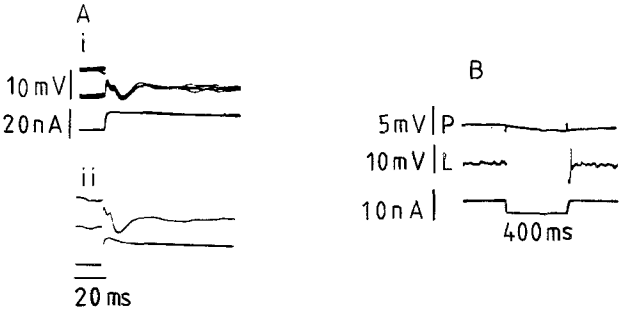

Fig. 6A, B. A connexion between two L-neurones and an experiment to search for feedback from an L-neurone to a photoreceptor. **A** Depolarising current, monitored on the bottom trace, was injected into one L-neurone (top trace), and an IPSP was recorded from a second L-neurone (middle trace). The bridge circuit for the presynaptic neurone could not be balanced, and there is some capacitative coupling between the two electrodes. In (i), five sweeps are superimposed. More current was injected in (ii), which shows a single sweep. **B** Hyperpolarising current was injected into an L-neurone, and it spiked at the end of the pulse. No sign of any postsynaptic activity was recorded from a photoreceptor which was subsequently shown to synapse with this L-neurone

Experiments to Investigate Feedback from L-Neurones to Photoreceptors

Twenty different pairs of photoreceptor and L-neurone were tested to search for evidence for feedback from an L-neurone to photoreceptors. The tests were made by injecting hyperpolarising and depolarising pulses of current into the L-neurones. Most tests were performed in darkness, but in order to search for PSPs that reverse at the dark potential of a photoreceptor some were also performed while the ocellus was illuminated. In the example in Fig. 6B, the L-neurone spiked at the end of a hyperpolarising pulse, and no sign of any synaptic input is seen in the photoreceptor. The tests for feedback were performed soon after penetration of the two neurones, and, in all cases, light stimuli were delivered before and after the tests to ensure that both neurones were operating normally. Subsequent tests were made to examine whether the photoreceptor synapsed with the L-neurone, and in seven cases clear evidence for this was obtained. Equal numbers of recordings were made from cell bodies and from axons of the photoreceptors.

Recordings from Spiking Neurones in Ocellar Nerves

Several neurones which responded with alteration in the frequency of spikes to changes in illumination were encountered in the ocellar nerves. Probably most of these were small second-order neurones, but some may have been afferents (Rosser 1974;

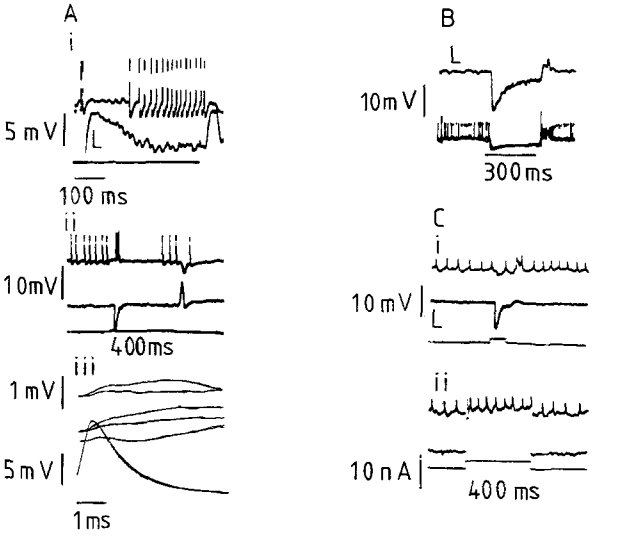

Fig. 7A–C. Simultaneous recordings from L-neurones and presumed second-order neurones which produce trains of spikes. **A** Spike frequency in the neurone of the upper traces increased transiently when the ocellus was illuminated. In (i), stimulus delivered in darkness, in (ii) stimulus superimposed on a constant background illumination. (iii), each spike in the spiking neurone (now on the lower trace) is followed, after 1 ms, by a small EPSP in the L-neurone. These spikes were caused by illumination. Both neurones were in a lateral ocellar nerve. **B** Median ocellar L-neurone, top trace, and a spiking neurone which hyperpolarised upon illumination. **C** Another pair of median ocellar neurones. In this case, the L-neurone is on the middle trace. (i), illumination hyperpolarises both neurones. (ii), depolarising the L-neurone increases the spike frequency in the spiking neurone

Kondo 1978) although this is unlikely as the time course of the responses to altered illumination was generally the same as that of L-neurones (Fig. 7). Some neurones responded to illumination by an increase in spike frequency (Fig. 7A), and others by a decrease (Fig. 7B, C). Similar neurones have been reported previously in extracellular recordings (Chappell and Dowling 1972; Rosser 1974; see also L.J. Goodman 1981, Fig. 20A–D).

In a few cases, evidence for connexions between the spiking neurones and L-neurones was obtained. Twice, small EPSPs in an L-neurone followed spikes in a spiking neurone (Fig. 7A iii). On five occasions, injection of current into an L-neurone altered the frequency of spiking in a spiking neurone (Fig. 7C ii). On all these occasions, the spiking neurone hyperpolarised in response to increased illumination, and depolarising an L-neurone increased the frequency of spikes. Similar connexions from L-neurones to spiking neurones in ocellar nerves have been observed in locusts, and here sometimes depolarising an L-neurone will reduce the frequency of spikes (P.J. Simmons, unpublished observations).

Discussion

Photoreceptors make inhibitory chemical synapses with L-neurones and, in darkness, the photoreceptors are depolarised above the threshold for transmitter release. When a photoreceptor is depolarised a postsynaptic L-neurone produces an initial peak hyperpolarisation, which cuts back to a more sustained potential. Because the time course of this cutback is the same whether a photoreceptor is depolarised by light or by injecting current into it, then it is likely that the mechanisms causing the cutback in the response of an L-neurone to increased illumination can be activated by depolarisation of a single photoreceptor.

Three types of mechanism which could contribute to this cutback are discussed here. First, the membrane of an L-neurone shows delayed rectification to hyperpolarising current (Fig. 4). The contribution this makes to a cutback in the response to increased illumination is unlikely to be large since, when even strong currents were injected into an L-neurone, the plateau depolarisation was never more than a third less in amplitude than the peak. Second, properties intrinsic to the pre- or postsynaptic terminals may cause a cutback in transmission. In synapses between giant neurones of the stellate ganglion of the squid, there is an initial peak transient postsynaptic potential, which is followed by a more sustained plateau level when a prolonged pulse of depolarising current is injected into the presynaptic terminal (Katz and Miledi 1973; Kusano and Landau 1975); and at inhibitory connexions between L-neurones of locust ocelli, transmission decrements within 10 ms (Simmons 1982). In neither of these examples is it known whether the mechanisms underlying decrement in transmission are pre- or post-synaptic. It is difficult to determine whether similar mechanisms operate at the connexions between ocellar photoreceptors and L-neurones because of the difficulty of activating a connexion made between a single receptor and an L-neurone. Injection of current into a receptor may achieve this, but photoreceptors may connect with other neurones, such as other photoreceptors (Dowling and Chappell 1972) and small second-order neurones, which could contribute to the cutback in transmission. The third type of mechanism which could contribute to the cutback in the hyperpolarising responses of an L-neurone is one involving additional circuitry, including synapses either on L-neurones or on photoreceptors. Such synapses should be detectable by looking for appropriate conductance changes in the neurones, or by looking for evidence for appropriate connexions. Evidence from conductance measurements and measurements of reversal potentials in L-neurones (Fig. 5) argues against a major role for synaptic input onto L-neurones in causing the cutback in hyperpolarisation. However, some presumed spiking second-order neurones, which are excited by increased illumination, do make connexions with L-neurones (Fig. 7A iii), and they may contribute, especially when an L-neurone depolarises above its dark potential, since a small increase in L-neurone conductance accompanies these depolarisations (Fig. 5A). Connexions made by small second-order neurones with L-neurones may also be important in generating the depolarising transients in L-neurones at light off, which usually appear to consist of several components (Fig. 2F). The connexions L-neurones make among themselves (Fig. 6A) are inappropriate for contributing to the cutback in hyperpolarisation. No evidence was found in the present work for synaptic input onto photoreceptors which would reduce the release of transmitter from them, leading to a cutback in hyperpolarisation of postsynaptic L-neurones. At low intensities of light stimulation, hyperpolarisations in L-neurones can cut back strongly, with no decrease in the depolarisation of photoreceptors which drive them (see Fig. 1B, right record). Despite specific search, no evidence for connexions from L-neurones to photoreceptors was found.

Several lines of evidence for the existence of feedback connexions from L-neurones to photoreceptors have been proposed before. First, Dowling and Chappell (1972) presented ultrastructural evidence for these connexions. However, they report that this type of connexion is rarely found, and only tentatively identified the presynaptic neurones as L-neurones. Second, Klingman and Chappell (1978) showed how feedback from L-neurones to photoreceptors would result in an economical circuit for shaping L-neurone responses to changes in illumination. They found that the acetylcholine antagonist, curare, eliminates responses to light stimuli in L-neurones, and also eliminates the hyperpolarising afterpotential which often occurs in receptors following the end of a light stimulus. Picrotoxin, a GABA agonist, potentiates the hyperpolarising afterpotential in photoreceptors. While these observations provide evidence that acetylcholine is employed by photoreceptors as their transmitter, and that the hyperpolarising afterpotential in photoreceptors is synaptically mediated, they do not specifically show that L-neurones synapse with photoreceptors. Similar results would be obtained if small second-order neurones, for instance, mediate the hyperpolarising afterpotentials in photoreceptors. Third, Stone and Chappell (1981) found that stimulating the ocellar nerve extracellularly caused hyperpolarising potentials in photoreceptors. It is not clear what the effect of extracellular stimulation would be on L-neurones, and whole-nerve stimulation would stimulate many different types of neurone. Dragonfly ocellar photoreceptors may receive synaptic input from small second-order neurones, or from higher order neurones, but connexions from L-neurones are probably absent, or too rare to contribute greatly to the shaping of neuronal signals.

A further mechanism which could reduce the amplitude of light-induced hyperpolarisations in L-neurones is by an electrical field in the extracellular space, set up as a result of photoreceptor depolarisation, which opposes release of transmitter from photoreceptor axons. Such a mechanism has been proposed for insect compound eyes (Shaw 1975). By effectively subtracting the background signal for different conditions of illumination, this mechanism could also be important in allowing L-neurones to signal small increments in illumination over a wide range of backgrounds (Fig. 2A–D), although its time course would be inappropriate for causing the initial cutback in L-neurone hyperpolarisation.

A synapse between a single photoreceptor and an L-neurone can have a gain of up to nine (Fig. 3C). This is higher than the gains of many other types of chemical synapse (e.g. squid giant synapse, Katz and Miledi 1967; crab sensorimotor synapse, Blight and Llinás 1980; output synapse from locust L-neurones, Simmons 1981, 1982). In the fly compound eye, however, synapses between single photoreceptors and second-order neurones may have gains of up to four (Dubs et al. 1981), and so nine is not an unusually large gain for the first synapse in a visual system. The gain of a synapse is greatest when the presynaptic neurone is depolarised well above the threshold for transmitter release (Katz and Miledi 1967) and it would be of interest to know how far depolarised above this threshold a photoreceptor is in darkness.

The high gain of each synapse between a photoreceptor and an L-neurone, the large number of photoreceptors which converge on a single L-neurone and the lack of a sustained hyperpolarising potential to sustained background light all enable L-neurones to be very sensitive to changes in light intensity. The relation between the gain of a single photoreceptor to L-neurone synapse and the summing of signals from several receptors within one L-neurone requires investigation, because the amplitude of the response of an L-neurone to increased illumination is at most four times that of the response of a photoreceptor, and often less (Chappell and Dowling 1972). It is interesting that the responses of L-neurones, in a light-adapted ocellus, can be almost entirely phasic because movements of the head in response to ocellar stimulation under similar illumination are much more tonic (Stange 1981). This implies some other ocellar neurones, which are less phasic in their responses to changes in illumination, must be involved in mediating these movements. The most likely candidates are some of the smaller second-order neurones.

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