

## EVIDENCE THAT HISTAMINE IS A NEUROTRANSMITTER OF PHOTORECEPTORS IN THE LOCUST OCELLUS

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*Accepted 7 March 1988*

### Summary

The results presented here are consistent with the hypothesis that histamine is the major neurotransmitter released by photoreceptors of locust ocelli.

1. When histamine is injected by iontophoresis into the locust ocellar neuropile, large second-order neurones (L-neurones) hyperpolarize in a dose-dependent manner, and responses to light in these neurones are diminished in amplitude. Both histamine and the illumination of ocellar photoreceptors caused an outward current across the membrane.

2. Hyperpolarizing potentials in L-neurones evoked by histamine had the same reversal potential as hyperpolarizing potentials evoked by photoreceptor illumination.

3. When applied iontophoretically in the ocellus, other biogenic amines, including octopamine, dopamine and noradrenaline, had no effect on the L-neurones. Both gamma-aminobutyric acid and acetylcholine, however, depolarized L-neurones and diminished responses to light.

4. Curare blocked the L-neurone's responses to histamine and light. The histamine response recovered fully. The initial peak hyperpolarizing response to increased light recovered, but the more sustained plateau hyperpolarizing potential did not.

5. Hexamethonium bromide prolonged the response of an L-neurone to histamine, and increased the tonic component of the response to light.

### Introduction

Most insects are endowed with two types of visual organ: the compound eyes and the usually much smaller ocelli or simple eyes. Despite the completely different optical arrangements, early visual processing in these two systems shows interesting parallels. In both cases photoreceptors respond to increases in illumination with a graded depolarization that is converted by the second-order cells into a phasic hyperpolarizing signal (for reviews see Goodman, 1981;

Key words: ocellus, locust, photoreceptor, neurotransmitter, histamine.

Laughlin, 1981). Whereas the compound eye is characterized by the retinotopic projection of photoreceptors to second-order cells (large monopolar cells or LMCs), in the ocelli the photoreceptors converge onto a small number of large second-order neurones known as L-neurones. Each of the three ocelli of a locust is connected with the brain by six or seven L-neurones, each of which is sufficiently large to be reliably impaled with at least two microelectrodes. Both the compound eye and the ocellar system have been extensively used in studies of visual processing (for reviews see Laughlin, 1987; Simmons, 1988).

The identities of the neurotransmitters which insect photoreceptors release has been a matter of conjecture for several years (for reviews see Hall, 1982; Hardie, 1987). However, in the compound eyes of flies and locusts, evidence has recently been obtained which implicates histamine as the most likely candidate. In particular, unusually large quantities of histamine are synthesized in the locust compound eye (Elias & Evans, 1983), and histamine is the only neurotransmitter candidate which mimics the natural transmitter when applied focally to the sites of synapses between photoreceptors and LMCs in the fly (Hardie, 1987). However, in the dragonfly ocellus, Klingman & Chappell (1978) postulated that acetylcholine (ACh) was released by the photoreceptors because curare, which is a cholinergic antagonist, blocked the response to light.

In view of the electrophysiological parallels between the compound eyes and the ocelli, it is of interest to discover whether the photoreceptors use different neurotransmitters. Further, the ocellar preparation offers certain experimental advantages for studies of the actions of neurotransmitters. In particular, the L-neurones are accessible to study under two-electrode voltage-clamp, and the preparation is readily accessible to both bath and ionophoretic application of drugs. In the present work, electrophysiological evidence is presented which supports the hypothesis that, in the ocelli and the compound eyes, histamine is the major neurotransmitter which photoreceptors release.

### Materials and methods

Experiments were performed on two species of locust, *Schistocerca gregaria* and *Locusta migratoria*, in approximately equal numbers. Locusts were prepared for experiments as described previously (Simmons, 1985). Prior to recording, the sheath of the ocellar nerve was softened by a 2-min application of a 1% solution of protease (Sigma Type XIV) in saline. When experiments were performed on the median ocellus, the lateral ocellar nerves were cut. In most experiments where the brain and ocelli were perfused with saline containing drugs, a small tear was made in the sheath of the ocellar cup to facilitate entry of the drug into the ocellar neuropile. This treatment did not noticeably affect the responses of L-neurones to visual stimuli.

Intracellular recordings were made from the axons of L-neurones using microelectrodes filled with  $2 \text{ mol l}^{-1}$  potassium acetate, and having d.c. resistances of about  $80 \text{ M}\Omega$ . For current- and voltage-clamp experiments, two electrodes,

connected to an Axoclamp-2A amplifier, were inserted into the same L-neurone axon. An indifferent electrode, of chloridized silver wire, was placed in the thorax. To apply drugs ionophoretically into the ocellar neuropile, conventional glass microelectrodes with one or three barrels (d.c. resistances 10–20 M $\Omega$  when filled with 0.5 mol l<sup>-1</sup> histamine) were used. The ionophoretic electrodes were attached to a d.c. amplifier which incorporated a high-quality current pump and monitor. When triple-barrelled electrodes were used, one electrode at a time was connected to the amplifier through a remotely controlled miniature relay. With single-barrelled ionophoretic electrodes, a holding current of -1 nA was routinely applied. Injection of current through the ionophoretic electrode often caused a coupling artefact in the recording from an L-neurone, which is evident in some of the records. Experiments were usually conducted in dim red illumination, and the light stimulus was from a high-intensity green light-emitting diode. All L-neurones for observations reported here gave peak responses to increases in illumination in excess of 22 mV.

Details of most of the drugs used are given by Hardie (1987). *d*-Tubocurarine was dissolved at 10 mg ml<sup>-1</sup>, and hexamethonium bromide at 0.004–0.04 mg ml<sup>-1</sup> in saline. Both were obtained from Sigma. For perfusion, single 0.1-ml drops were delivered by syringe into the rear of the head capsule, which contained 0.8 ml of saline, giving a dilution of about one-tenth. Final concentration at the site of action in the neuropile was presumably much lower. Drugs were washed out by repeated delivery of saline (0.1–0.2 ml min<sup>-1</sup>) from a syringe into the head capsule.

Data were collected on magnetic tape, and later filmed from an oscilloscope. Unless otherwise stated, each observation was made in at least four preparations.

## Results

### *Responses of L-neurones to ionophoretically applied histamine*

Pulses of histamine, ejected by the application of depolarizing current to a micropipette placed in the ocellar neuropile, evoked hyperpolarizing potentials in L-neurones (Fig. 1). Both the amplitude and the speed of these responses varied from experiment to experiment. The largest and fastest responses were recorded when the ionophoretic pipette recorded depolarizing potentials in response to flashes of light. Often, advancing the pipette slightly from this position moved its tip into an L-neurone. During a histamine-evoked hyperpolarization, responses to ocellar illumination were diminished in amplitude (Fig. 1A), and occasionally abolished. After the end of a pulse of histamine, the L-neurone repolarized over a period of some seconds, and the amplitude of its responses to light recovered. Neither control pulses of hyperpolarizing current passed through the ionophoretic pipette, nor current of either polarity passed through pipettes containing saline or potassium acetate evoked responses in L-neurones. Intracellular injection of histamine into an L-neurone did not evoke a hyperpolarizing response.

Most experiments were performed on the median ocellus, because L-neurones arborize over the whole area of the retina in this ocellus (Simmons, 1986), giving a

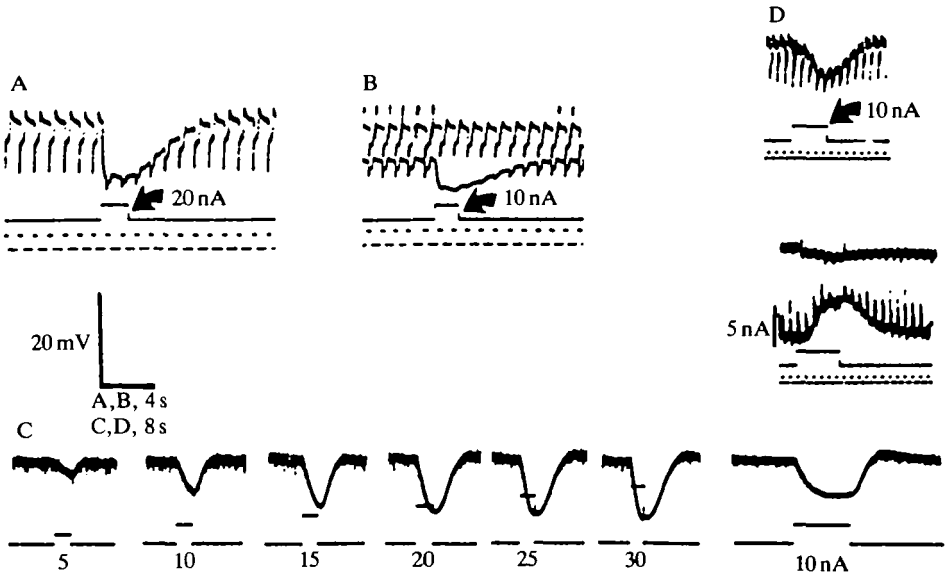


Fig. 1. Responses of an L-neurone to ionophoretically applied histamine. (A) The ocellus was repeatedly illuminated with identical pulses of light (monitor on bottom trace), and a 20 nA pulse of histamine was delivered (middle trace). The histamine hyperpolarized the L-neurone (top trace), and reduced the amplitude of its response to light. (B) Two L-neurons were recorded simultaneously. Histamine hyperpolarized one (second trace), but not the other (top trace). (C) A series of responses of an L-neurone to pulses of histamine of increasing size. The amplitude of the current used to eject histamine is given beneath each record. The record on the right is the response to a longer, 10 nA pulse of histamine. The amplitudes of the responses to a series of histamine pulses of decreasing size were the same as those for the increasing series. (D) Potential changes and currents in an L-neurone evoked by pulses of light and by a 10 nA pulse of histamine. The upper recording is of potential changes in the neurone and, in the lower recording, the potential has been clamped (upper trace) to reveal transmembrane currents (second trace). *Locusta*, lateral ocelli.

good chance of pairing an intracellular recording with ionophoretic injection onto the recorded neurone. In the lateral ocelli, the arborizations of L-neurons are more restricted (Simmons, 1982a, 1986). In a lateral ocellus, when a pair of L-neurons was penetrated simultaneously, focal ionophoresis of histamine often evoked responses in only one L-neurone (Fig. 1B), showing that the action of histamine was local. Two features of Fig. 1B require further explanation. First, the response to light of the L-neurone in the top trace was larger than that of the neurone in the second trace. This is because the light stimulus was directed from the front of the animal, and lateral L-neurons differ in their fields of view (Wilson, 1978a; Simmons, 1986). Second, during the pulse of histamine, the neurone in the top trace did not spike following each pulse of light, although it did before and after. This is probably because this L-neurone received excitatory connections

from other L-neurones (Simmons, 1982a), which were directly hyperpolarized by the histamine.

The amplitude and speed of response of an L-neurone to ionophoretic application of histamine were dependent on the dose of histamine (Fig. 1C). In Fig. 1C, a reduction in background noise in L-neurones is clearly seen during the histamine pulses. The response to histamine for this neurone saturated at about 27 nA. Longer pulses, such as the 10-nA pulse on the right of Fig. 1C, induced maintained hyperpolarizations with only slight repolarization compared with the cut-back in the response to light. Following larger doses, L-neurones typically repolarized initially to a potential slightly positive to the resting potential.

Both illumination of the retina and ionophoretic application of histamine raised the conductance of L-neurones (Fig. 1D). This was shown by employing a two-electrode voltage-clamp to demonstrate that both light and histamine evoke outward currents across the membrane. The time courses of the histamine-evoked potential changes and currents were similar. Because the resting conductance of an L-neurone is high compared with many other neurones (Wilson, 1978b), the magnitude of the conductance change caused by light or histamine is very small.

#### *Reversal potential of the histamine-evoked response*

A sensitive test of whether an exogenously applied drug is activating the same conductance as the natural neurotransmitter is to compare the respective reversal potentials. Unfortunately, it was not possible to hyperpolarize the membrane sufficiently to reverse the response to histamine, because L-neurones have the property of delayed rectification (Wilson, 1978b; Ammermüller & Zettler, 1986) so that, after an initial peak hyperpolarization, the membrane potential repolarizes, or sags, to a less hyperpolarized level. Nevertheless, on two occasions we were able to hyperpolarize the L-neurone sufficiently to eliminate the change in membrane potential evoked by histamine (Fig. 2). This occurred when the L-neurone was hyperpolarized by between 45 and 50 mV from its normal resting potential. By extrapolating the plots of response *versus* holding potential (Fig. 2B) it is apparent that both the light and the histamine response reverse at approximately the same potential. Our measurement of the reversal potential for the response to light corresponds with previously published measurements (Wilson, 1978b; Ammermüller & Weiler, 1985) and is consistent with a conductance increase in response to either potassium or chloride ions. We attempted to obtain further evidence for the nature of the ionic species involved by intracellular ionophoresis of chloride ions from KCl-filled microelectrodes, but it proved impossible to modify the response to light despite injection of 20 nA for 20 min. We conclude that the L-neurones are too large, or the ionic homeostatic mechanisms too powerful, for significant alteration of the intracellular ion concentrations by this method.

#### *Responses to histamine when synaptic transmission was blocked*

When an ocellus is bathed in saline containing cobalt chloride, the hyperpolariz-

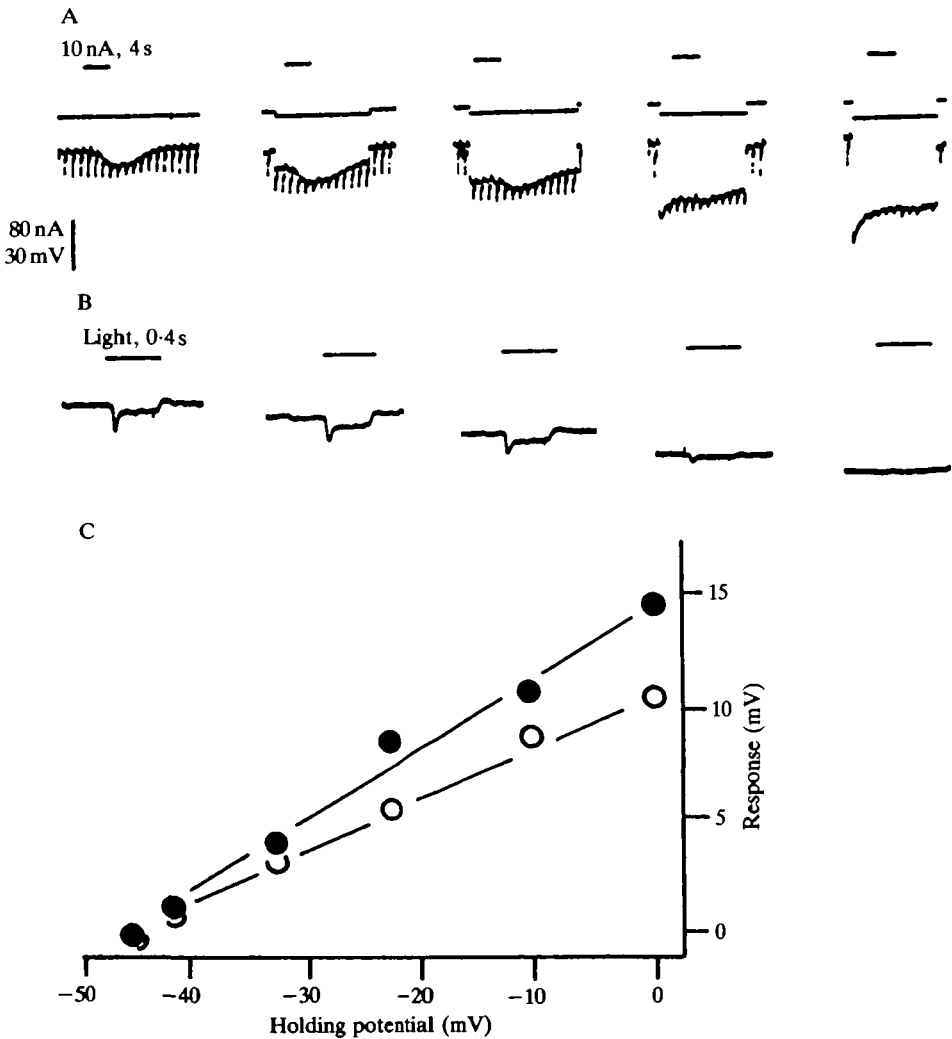


Fig. 2. Hyperpolarization of membrane potential reduces the amplitudes of the responses of an L-neurone to light and to histamine. The neurone was illuminated by a regularly repeated 0.4 s pulse of light, and 4 s pulses of histamine were injected into the ocellar neuropile. One electrode injected hyperpolarizing pulses of current into the neurone (8 s long, monitored on the second trace in A) while a second recorded membrane potential. (A) The responses to histamine. (B) Responses to pulses of light, taken just before application of histamine for each recording. (C) Plot of the amplitudes of responses to light (●) and to histamine (○) against the potential at which the neurone was held, relative to resting. *Locusta*, lateral ocellus.

ing response of L-neurones to light is blocked (Wilson, 1978c), presumably because the cobalt blocks presynaptic calcium channels which regulate the release of transmitter. In our study, the response to light was almost eliminated by  $4 \text{ mmol l}^{-1}$  cobalt within 5–10 min, but large hyperpolarizing responses to iono-

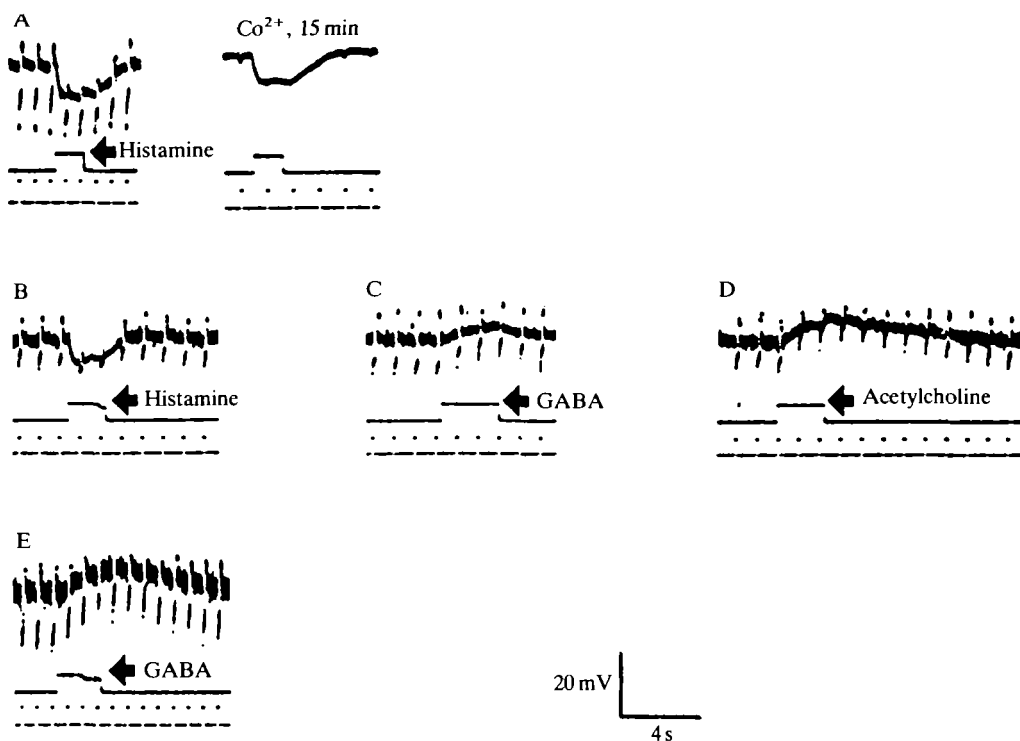


Fig. 3. Response to histamine when synaptic transmission was blocked and ionophoresis of histamine, gamma-aminobutyric acid (GABA) and acetylcholine in the ocellar retina. (A) Responses of an L-neurone to pulses of light and to 15 nA histamine before and after adding cobalt to block synaptic transmission. (B–D) A triple-barrelled electrode was used to compare the responses of one L-neurone to (B) histamine, (C) GABA and (D) acetylcholine, applied at the same location. Each drug was applied by 15 nA current. (E) Response of an L-neurone in another locust to GABA. *Schistocerca*, median ocellus.

phoretically applied histamine were still recorded (Fig. 3A). This experiment, which was performed twice with identical results, indicates that the effect of histamine is directly on the L-neurone and not mediated *via* other interneurons. Unfortunately, on both occasions, the microelectrode was dislodged while washing the preparation in ordinary saline in an attempt to restore the response to light.

#### *Responses of L-neurones to other transmitter candidates*

The specificity of the response to histamine was tested by injecting a number of other aminergic transmitter candidates into the ocellar neuropile. These drugs were injected through a triple-barrelled ionophoretic electrode, one barrel of which also contained histamine. A negative result was only attributed any significance when combined with a positive effect from the histamine barrel. In a series of experiments in three preparations, dopamine (four cells), noradrenaline

(three cells) and octopamine (two cells) were found not to affect the L-neurones under conditions when histamine did evoke a clear hyperpolarization. A negative result was also obtained with glutamate (one cell).

We took particular care to investigate the effects of gamma-aminobutyric acid (GABA) and acetylcholine (ACh) since these might also be considered as candidates for the photoreceptor neurotransmitter. GABA produces hyperpolarizing potentials in some insect muscle fibres (see review by Usherwood, 1978), and there is good evidence that it is the transmitter at some inhibitory connections in the locust central nervous system (Watson & Burrows, 1987). In addition, Klingman & Chappell (1978) postulated that GABA is released by the L-neurones, causing the photoreceptors to depolarize. Acetylcholine has previously been implicated as the neurotransmitter released by ocellar photoreceptors because curare blocks the hyperpolarizing response of L-neurones to light (Klingman & Chappell, 1978). We conducted five experiments with triple-barrelled ionophoretic micropipettes, in which one barrel contained histamine, the second GABA, and the third ACh (Fig. 3B–E). GABA and ACh were ejected after clear hyperpolarizing responses by an L-neurone to histamine had been recorded (Fig. 3B). In four experiments, GABA evoked a clear depolarizing response in the L-neurone (Fig. 3C,E; in the fifth, the pipette containing GABA became blocked). In two experiments, ACh also evoked depolarizing responses (Fig. 3D), and no clear response to ACh was recorded in the other three. The responses to GABA and ACh had slower rise and decay times than those to histamine (Fig. 3B–D). During the depolarizing responses evoked by GABA and ACh, the amplitudes of the responses to light stimuli were reduced. In one experiment employing a two-electrode voltage-clamp, the depolarizing GABA response was shown to be associated with an inward current, and hence a conductance increase. In one further experiment, the response to GABA persisted after the L-neurone's response to light had been abolished by bathing the ocellus in saline containing cobalt ions.

#### *Drugs that alter responses to light and histamine*

In the present work, no attempt was made to characterize the pharmacology of the histamine-sensitive receptors which are presumably sited on the membrane of the L-neurones in the ocellus. However, it was considered to be important to investigate whether drugs which altered the responses of L-neurones to ocellar illumination had the same effects on the responses of L-neurones to ionophoretically applied histamine. Two drugs were tested: first curare, which is the only compound to date which has been reported to block the L-neurone response to light (Klingman & Chappell, 1978); and second hexamethonium, which was the most potent of a range of drugs tested on fly LMCs (Hardie, 1988*a,b*).

#### *Curare*

*d*-Tubocurarine has been reported to block the response of L-neurones to ocellar illumination, but only at concentrations greater than  $10^{-4}$ – $10^{-3}$  mol l<sup>-1</sup> (in



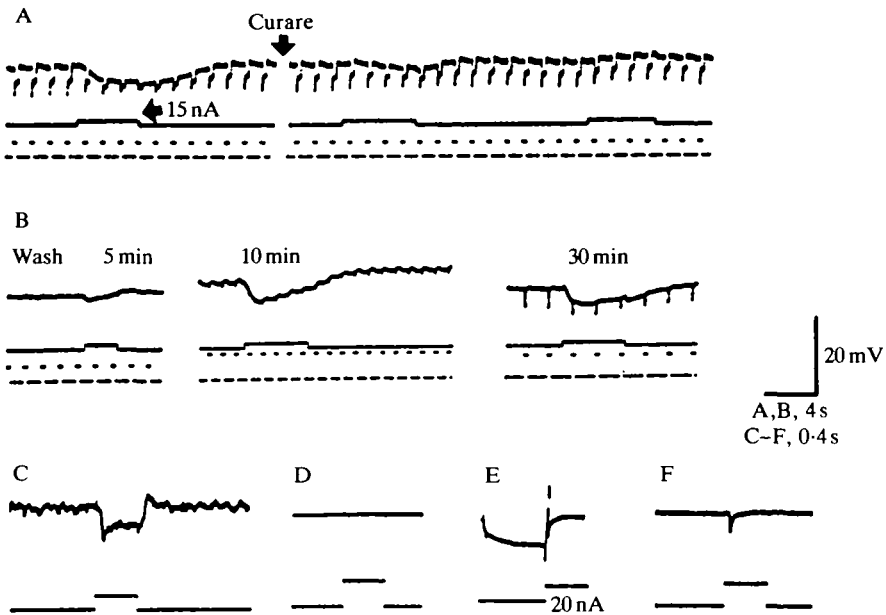


Fig. 4. Effects of curare on the responses of an L-neurone to pulses of light (monitored on the bottom trace) and of histamine (middle trace). (A) Immediately after addition of the curare, the amplitude of the response to histamine declined. (B) The response to histamine recovered more rapidly than the response to light when the curare was washed out. (C) Response to a pulse of light before curare. (D) Response to a pulse of light is abolished 20 min after addition of curare. (E) 20 min after addition of curare, the neurone produced a rebound spike at the end of a pulse of current injected through the recording electrode. (F) The response to a pulse of light 30 min after the curare had been washed out was only phasic. *Schistocerca*, median ocellus.

dragonflies, Klingman & Chappell, 1978; in locusts, Ammermüller & Weiler, 1985). This result has been interpreted as evidence that acetylcholine is the photoreceptor transmitter, and is still the only evidence for this hypothesis. We were able to repeat this result using a bath concentration of  $1.4 \text{ mmol l}^{-1}$  *d*-tubocurarine but showed, in addition, that this concentration was effective in blocking responses to histamine (Fig. 4). In our experiments, the time course of the effect on the light-evoked response was slower than the effect on the histamine-evoked response. The histamine-evoked response was reduced and then blocked within a few seconds, before the light-evoked response showed any reduction (Fig. 4A). After 20 min, the baseline of the recording was smooth, with no deflection when the ocellus was illuminated (Fig. 4D). At this time, the neurone could still be made to spike (Fig. 4E), showing that curare did not have a general poisoning effect on the neurones, and that the recording electrode was still in place. On washing the preparation to remove the curare, the histamine-evoked response started to recover within a few minutes, its initial amplitude being

recovered within 10 min (Fig. 4B). The light-evoked response recovered much more slowly, and recovery was incomplete. After 30 min, the peak initial response was still depressed from its amplitude before the addition of curare, although a response of the same amplitude could be evoked by using the maximum stimulus intensity available (Fig. 4B). A more dramatic effect was that the waveform of the response was altered. Before the application of curare, L-neurones produced a peak hyperpolarization, followed by a more sustained plateau hyperpolarization, with a depolarization following light-off (Fig. 4C). After 30 min washing in saline, only the initial peak hyperpolarization was recorded (Fig. 4F). Soon after this, the recording electrode became dislodged. This incomplete recovery of the light response was recorded in three experiments, and two others showed that curare blocked the hyperpolarizing potentials in L-neurones evoked by both light and histamine. Inspection of previously published records (Klingman & Chappell, 1978; Ammermüller & Weiler, 1985) suggests that, here too, the light response did not recover fully. The difference in time courses of the effects can easily be explained, as histamine was injected locally, close to the edge of the neuropile, and the site of ejection would have been relatively accessible to the saline bathing the ocellus. In contrast, synaptic contacts between photoreceptors and L-neurones are made throughout the depth of the ocellar neuropile. A possible implication of the alteration in waveform of the response to light is that the L-neurone membrane has two different receptors for the transmitter which the photoreceptors release.

### *Hexamethonium*

Low concentrations of the cholinergic antagonist hexamethonium (Gillman *et al.* 1985) have been found to have pronounced effects on the putative histamine receptors of the housefly lamina (Hardie, 1988a). This was also the case in the locust ocellus. Addition of  $10^{-4}$ – $10^{-5}$  mol l $^{-1}$  hexamethonium bromide to the saline (giving a final bath concentration of  $10^{-5}$ – $10^{-6}$  mol l $^{-1}$ ) caused similar changes in the responses of L-neurones to both light and histamine (Fig. 5). The most obvious effect was an increase in the time taken for an L-neurone to repolarize following a pulse of histamine (Fig. 5C,D). After 5 min in hexamethonium saline, the membrane potential hyperpolarized tonically by about 5 mV in the experiment shown in Fig. 5. The response to increases of light became less phasic after addition of hexamethonium (compare Fig. 5B and 5E). Hexamethonium at final bath concentrations greater than  $10^{-5}$  mol l $^{-1}$  caused hyperpolarization of L-neurones and loss of response to both light and histamine; concentrations of less than  $10^{-6}$  mol l $^{-1}$  produced no apparent effect on the response to light. The effects of hexamethonium were not reversed after 20 min of washing in saline.

Attempts to test the effects of various established antihistamine drugs by adding them to the saline in the head capsule were inconclusive. For example, metiamide at 1 mmol l $^{-1}$  produced no obvious effects, whereas at 2 mmol l $^{-1}$  it caused an irreversible blocking of any responses in an L-neurone.

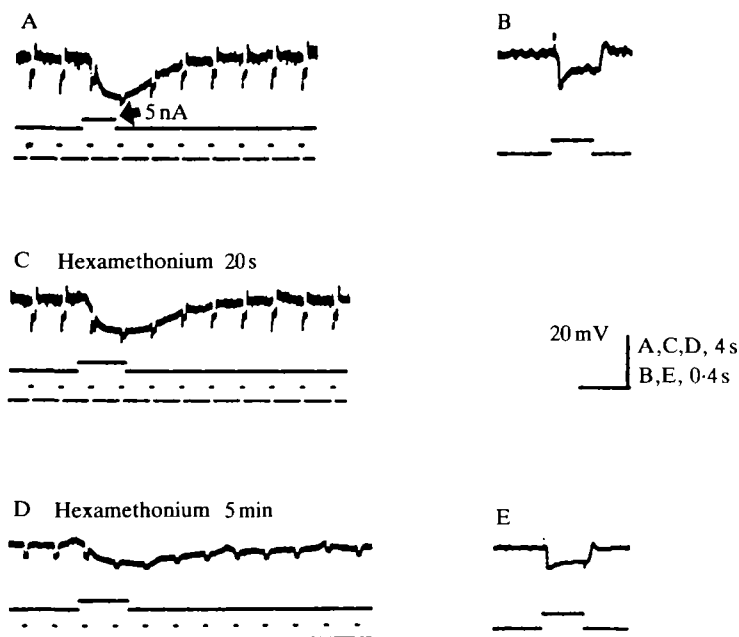


Fig. 5. Effects of hexamethonium on the responses of an L-neurone to pulses of light (bottom traces) and to pulses of histamine (middle traces). (A,B) Before addition of hexamethonium. (C) After 20 s the repolarization of the L-neurone following a pulse of histamine was prolonged. (D) After 5 min, the neurone was continually hyperpolarized and had a quieter baseline. The response to histamine was considerably prolonged, and the amplitude of the response to light reduced. (E) The difference between the initial peak hyperpolarizing response to light and a more sustained plateau is reduced. (Some artefacts due to movement of the animal are apparent in D.) *Schistocerca*, median ocellus.

### Discussion

This study has shown that focal, exogenous application of histamine to L-neurones in the ocellus can mimic the action of an endogenous transmitter which photoreceptors release, and that certain pharmacological agents have similar effects on the response to ionophoretically applied histamine and the response to light. Before histamine can be unequivocally accepted as a transmitter that ocellar photoreceptors release, it is necessary to demonstrate that these cells contain, synthesize and release histamine. However, the similarities with the results from the compound eye (Hardie, 1987) are striking. For the locust compound eye, there is biochemical evidence that the retina contains high endogenous concentrations of histamine and also has the ability to synthesize and metabolize histamine (Elias & Evans, 1983). To date, the retina-lamina projection of the compound eye represents the only putative histaminergic system amongst the insects. In general, histaminergic neurotransmission has been only rarely reported (for a review see Prell & Green, 1986), and it has only been clearly established in the case of identified cells in *Aplysia* (e.g. McCaman & Weinreich,

1985). The potential inclusion of the ocellar preparation in the list of histaminergic systems is hence of some significance.

*The effect of histamine on L-neurones: comparison with the compound eye*

In both ocelli and compound eyes, light depolarizes the photoreceptors, resulting in the release of a neurotransmitter which hyperpolarizes large second-order neurones: L-neurones in ocelli and LMCs in compound eyes. This hyperpolarization is mediated by a conductance increase with a reversal potential about 45 mV negative to the dark resting potential (L-neurones: Wilson, 1978*b*; Ammermüller & Weiler, 1985; LMCs: S. B. Laughlin, in preparation). In the LMCs of the compound eye there is evidence that chloride is the major ion species carrying the light-evoked current (Zettler & Straka, 1987).

In ocelli and compound eyes the action of light on large second-order neurones can be mimicked by the ionophoretic application of histamine, but not by a wide range of other neurotransmitter candidates, including other biogenic amines such as octopamine. In both types of eye, it has been shown that the histamine-evoked responses survive a synaptic blockade induced by cobalt, indicating that histamine affects the postsynaptic membrane directly, rather than through additional synaptic connections. In the present study, we were able to show that potentials evoked by light had similar reversal potentials to those evoked by histamine (45 mV from resting, within an experimental error of about 5 mV). In the LMCs, it was possible to obtain evidence that the histamine-evoked current was carried by chloride ions (Hardie, 1987).

In both LMCs and L-neurones a number of drugs have been found which affect the responses to light in the same way as they affect the responses to ionophoretically applied histamine. Curare is the only substance previously reported to block the L-neurone light responses, and this has been taken as evidence for cholinergic transmission (Klingman & Chappell, 1978). Apart from the fact that rather high concentrations of curare (greater than  $10^{-4}$  mol l<sup>-1</sup> in the bath) are required to block transmission, our finding that responses to ionophoretically applied histamine are also blocked by the same concentration of curare indicates that the actions of curare can no longer be used as evidence for cholinergic transmission in this system. In the only other putative histaminergic system reported in arthropods, involving neurones of the stomatogastric ganglion of the spiny lobster, curare, at similar concentrations ( $2 \times 10^{-4}$  mol l<sup>-1</sup>), is also the only effective antagonist so far reported (Claiborne & Selverston, 1984). Curare also blocks the response to light in fly LMCs (R. C. Hardie, unpublished results). It should be noted that a similarity between the pharmacology of histamine and acetylcholine receptors is not without precedent – H<sub>1</sub> antagonists, for example, have moderate blocking actions on muscarinic receptors (Prell & Green, 1986). For both L-neurones and LMCs (Hardie, 1988*a*), hexamethonium, at relatively low concentrations, is particularly potent in prolonging the response to light and histamine. The simplest explanations for the effect of hexamethonium are that it interfered with inactivation of the transmitter within synaptic clefts, or that it prolongs the

open time of the postsynaptic receptor. At higher concentrations, hexamethonium appears to block all responses.

The most recent evidence for histamine as a neurotransmitter in insect photoreceptors comes from immunocytological studies using an antibody raised against histamine conjugates. In two species of fly (*Musca* and *Calliphora*), photoreceptors of the ocelli and the compound eyes show strong immunoreactivity (D. R. Nässel, M. H. Holmqvist, R. C. Hardie, R. Hakanssin & F. Sundler, in preparation). Despite numerous studies (for a review see Hardie, 1988b), the only other antibody yet found to label insect photoreceptors is one raised against taurine (Schäfer *et al.* 1988). Although taurine was not tested in the present study, it has no effect when applied ionophoretically onto fly LMCs (Hardie, 1987), and its widespread occurrence in nervous systems generally (Oja & Kontro, 1983) may suggest some role other than as a neurotransmitter.

#### *Responses to acetylcholine and gamma-aminobutyric acid*

ACh has previously been postulated to be the neurotransmitter in ocellar photoreceptors (Klingman & Chappell, 1978). However, in the present study, the only action of ACh was, on occasion, to depolarize L-neurones in situations where histamine evoked hyperpolarizing responses when ejected from a neighbouring ionophoretic barrel. The response to ACh indicates that this transmitter was reaching sites of action in the neuropile, without first being inactivated enzymatically when it was released ionophoretically.

GABA has previously been suggested to play a role in the cut-back of the response by L-neurones to increased ocellar illumination (Klingman & Chappell, 1978; Stone & Chappell, 1981; Ammermüller & Weiler, 1985). Originally it was proposed that L-neurones themselves make synapses back onto photoreceptors (Dowling & Chappell, 1972), and that they used GABA as their transmitter (Klingman & Chappell, 1978). However, paired recordings from dragonfly L-neurones and ocellar photoreceptors failed to find direct evidence for such connections (Simmons, 1982b), and immunocytochemical staining indicates that some of the small-axoned ocellar S-neurones, rather than L-neurones, are GABAergic (Ammermüller & Weiler, 1985). S-neurones are strong candidates for playing a role in the mediation of the cut-back in the response by L-neurones to light, operating perhaps in parallel with possible presynaptic mechanisms which reduce the rate of transmitter release from photoreceptors (Simmons, 1982b, 1985). (Another mechanism, postsynaptic desensitization, is unlikely to be significant because, as reported in this paper, histamine induces a maintained hyperpolarization with little cut-back.) The discovery that two different transmitters, ACh and GABA, can depolarize L-neurones when injected into the ocellus suggests strongly that L-neurones receive a variety of synapses in addition to those from photoreceptors.

The results are consistent with the hypothesis that histamine is the major neurotransmitter released by the locust ocellar photoreceptors. There are strong parallels with the compound eye, and further investigations in each preparation

are likely to yield results relevant to both. It would also be of interest to know more of the identities of the transmitters employed by photoreceptors in other arthropods. Stages in the processing of information in the ocellus of the barnacle (Stuart, 1983), for example, are very similar to those which occur in the locust ocellus (Simmons, 1988). Originally it was proposed that barnacle photoreceptors employ GABA as their transmitter (Koike & Tsuda, 1980), but pharmacological experiments have refuted this (Timpe & Stuart, 1984), and recent experiments suggest that, here too, histamine is a likely candidate (A. E. Stuart, personal communication). The insect ocellar preparation has the advantage that it is feasible to employ two or more electrodes simultaneously in the second-order neurone. This allows detailed measurements of ionic currents by employing a voltage-clamp, and also allows elucidation of circuitry, by recording from pairs of neurones. The fly compound eye preparation has the advantage that a wide range of drugs can be applied quickly and locally to the particular cartridge in which an LMC synapses with photoreceptors (Hardie, 1987), in contrast to the ocellar preparation, where L-neurones have diffuse input zones, spread throughout the whole depth of the retina.

This work was partly supported by a grant from the SERC; Dr R. C. Hardie is a Royal Society Research Fellow. We would like to thank Simon Laughlin for helpful comments on the manuscript.

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