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MORE THAN ONE TYPE OF CONDUCTANCE IS ACTIVATED DURING RESPONSES OF BLOWFLY MONOPOLAR NEURONES

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Summary

The principal second-order neurones in the blowfly compound eye, the large monopolar neurones (LMCs), were studied using intracellular recording and discontinuous current-clamp techniques, in combination with measurement of dynamic input resistance. The LMCs had resting potentials of −35 to −45 mV and showed a linear current–voltage relationship in the lamina in the physiological voltage range. The hyperpolarizing light-on transient was associated with a drop in input resistance from 17 ± 5 to 3 ± 1 MΩ, and had a reversal potential between −60 and −90 mV. The dynamic input resistance of saturated responses and the properties of reversed responses suggested that more than one conductance was activated during the response of the LMCs. In lamina recordings, the input resistance increased beyond the resting level during repolarization, which can be interpreted in terms of a continuous release of transmitter by the photoreceptor terminals, even in darkness. The input resistance of LMCs in axon recordings in darkness and during the light-on response was generally higher than in the lamina recordings. The responses to light in axons also differed from those recorded in lamina by showing regenerative properties.

Introduction

Large monopolar neurones (LMCs) in the first neuropile of the insect compound eye are the principal second-order neurones receiving input from photoreceptors and conducting signals to the second neuropile. The neurones produce a hyperpolarization of the membrane in response to presynaptic depolarization, which is the photoreceptor cells' response to light-on stimuli. These hyperpolarizations are graded, i.e. the amplitude of the hyperpolarization

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depends on the presynaptic photoreceptor voltage and, primarily, on the intensity of the light stimulus. No spikes are normally generated in these neurones in the first neuropile (Autrum *et al.* 1970; Zettler & Järvilehto, 1971, 1973; Laughlin, 1973). The resting potential of LMCs is relatively low, −10 to −40 mV in various studies (Laughlin, 1974; Zettler & Straka, 1987; Laughlin, 1981). The intracellularly recorded hyperpolarizations reach −40 to −50 mV below resting potential in the dark-adapted compound eye.

Relatively little is known about the mechanisms in the monopolar neurone that generate its hyperpolarizing voltage response. The LMC responses show temporal and spatial antagonism, i.e. the responses to sustained stimuli are transient and they are inhibited by spatially displaced stimuli (Zettler & Järvilehto, 1972; for a review, see Laughlin, 1987). These response properties might be the result of the integrative properties of the LMCs or, alternatively, the LMCs could be passive relays of preprocessed information. The hyperpolarizing response of the LMCs to light increase has been shown to be associated with a decrease in input resistance (Shaw, 1968; Laughlin, 1974; Guy & Srinivasan, 1988). The photoreceptor transmitter, which is probably histamine (Hardie, 1987, 1988), activates conductances in the postsynaptic LMC membrane. Recently it has been shown that it is mainly a chloride conductance that is activated by the transmitter (Zettler & Straka, 1987).

The LMCs have three different anatomical regions: the postsynaptic membrane in the lamina, the axonal membrane and the presynaptic terminal membrane in the medulla. It is conceivable that these anatomical regions might also have different electrical properties. The postsynaptic region in the lamina contains the photoreceptor transmitter-activated conductance. The terminal membrane in the medulla probably has special properties associated with transmitter release. Little is known about the properties of the axon. To determine (1) the actual conduction changes during the hyperpolarizing voltage responses and (2) whether there are any voltage-dependent conductances in LMCs, we have applied the discontinuous (switched) current-clamp technique to blowfly monopolar neurones. We have studied the current–voltage relationship in darkness, i.e. without any light stimulus, and the same relationship during the light response.

Materials and methods

In all experiments adult blowflies (*Calliphora erythrocephala*) were used. In addition to conventional intracellular recording, discontinuous (switched) current-clamp techniques were used, which enabled us to study the electrical properties of the penetrated neurones fairly reliably.

In the discontinuous current-clamp technique the voltage responses are recorded with an intracellular electrode, while the current is passed through the same electrode as pulses with a high repetition rate (for further details of the technique see Wilson & Goldner, 1975; Finkel & Redman, 1984). Briefly, the error voltage generated in the electrode is avoided by allowing sufficient time (b

adjusting the switching frequency, in this case 0·6–3·0 kHz) for the electrode voltage to level off before sampling the voltage signal of the cell. For this reason the headstage output of the amplifier (Dagan 8100) was continuously monitored and the electrode capacitance was critically compensated.

Using this technique, 13 LMCs with stable and large (maximum 35–45 mV) voltage responses were successfully impaled. Recordings were made in two different, easily identifiable locations: in the lamina, i.e. near the synaptic contacts between the photoreceptors and the LMCs, and in the axons of the neurones near the chiasma. The recording site in the lamina can readily be identified because of the large extracellular field potential. The axonal recordings, made using a different electrode penetration angle, could be recognized by the absence of an extracellular field potential, by large shifts of the receptive fields from neurone to neurone and by the absence of photoreceptor axon penetrations. The electrode resistance was 80–150 MΩ when filled with 0·6 mol l^{-1} K_2SO_4. The identification of the neurones as large monopolar cells was based solely on their voltage response properties, because filling the electrodes with Lucifer Yellow increases the electrode impedances so as to be incompatible with the switched-clamp method. Ten to twenty records of each response to a brief (30 μs) light flash were averaged, stored and plotted with a micro-PDP-11/23+ laboratory computer.

The dynamic input resistance of the neurones was determined by subtracting from an averaged record of the voltage response a record with a small (0·2–0·5 nA) current injection (van Hateren, 1986). Special care was taken to avoid voltage artefacts caused by the drop in input resistance during the voltage response of the LMCs, as the membrane time constant of LMCs decreases (along with the input resistance, see Table 1) in the peak response to about one-fifth of its value in the resting state.

Results and discussion

The resting potential of the LMCs with reference to a distant indifferent electrode in extracellular fluid varied between −35 and −45 mV, which is similar to that found by Zettler & Straka (1987). The recorded membrane voltage of the neurones was linearly related to current injected in the lamina in the physiological range (Fig. 1), which supports earlier findings in dragonfly (Laughlin, 1974) and dronefly (Guy & Srinivasan, 1988) LMCs. This seems to indicate that there is no significant activation of voltage-sensitive conductances in the resting state. With hyperpolarizations larger than 40 mV the cells' input resistance increased, but this occurred outside the physiological voltages. The input resistance was higher in the recordings from LMC axons than in those from the lamina (see Table 1), as also found in the dronefly by Guy & Srinivasan (1988).

Applying a constant hyperpolarizing current through the recording electrode reduced the hyperpolarizing response of the LMCs, whereas depolarizing currents increased the response. Increasing the hyperpolarizing current eventually led to reversal of the hyperpolarizing transient between membrane potentials of −60 and

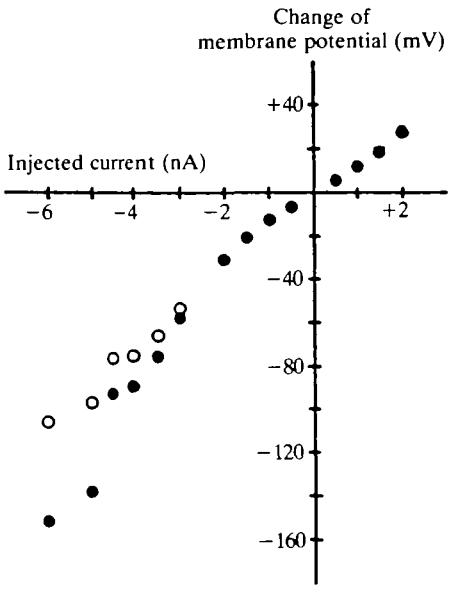

Fig. 1. Current–voltage relationship of a monopolar neurone measured by injecting current into the neurone using the switched current-clamp method described in the text. The input resistance near resting potential is equal to the slope of the curve (in this case about 15 MΩ). With large hyperpolarizing currents, causing a change in the membrane potential greater than 40 mV, the input resistance, measured from the peak voltage change, rises (filled circles). The steady-state relationship (after 100 ms) remains almost constant (open circles). Voltage is scaled to be zero at the resting potential.

−90 mV (Fig. 2). The reversal was usually observed only when recording from the lamina, although in some cases it was achieved with larger currents in axon recordings. The reason for this is probably that the voltage change induced by current injection in LMC axons is strongly attenuated before it reaches the site of generation of the postsynaptic response in the lamina, as proposed by Guy & Srinivasan (1988). Thus, it was not usually possible reliably to inject enough current into the axons to lower the membrane potential beyond the reversal potential in the synaptic region. As chloride injection also decreased the hyperpolarizing transient, by depolarizing the chloride equilibrium potential of the LMCs (Zettler & Straka, 1987), it is likely that the light-elicited response transient was caused by inward chloride current. This is consistent with the observation that the reversal potential of the light response is near the presumed chloride equilibrium potential of the LMCs (in insect neurones around −80 mV; Pinnock *et al.* 1988).

With moderate (subsaturating) stimulus intensities, the calculated dynamic resistance was usually a monophasic transient resistance decrease (Fig. 3A). This again indicates that the hyperpolarizing transient was caused by an increase in the transmitter-activated conductance. The repolarization of the hyperpolarizin

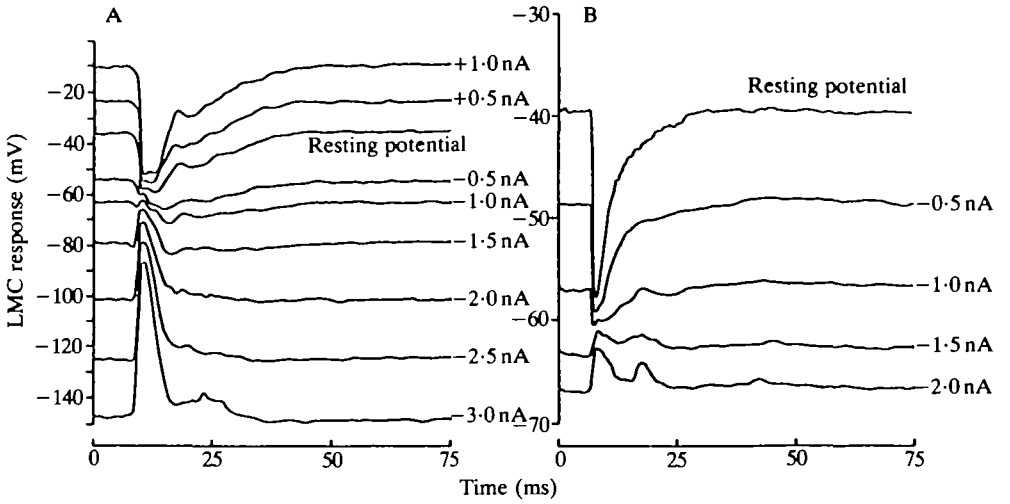

Fig. 2. Two representative sets of LMC responses to near-saturating (30 μs) stimuli recorded in the lamina region with different (steady-state) currents applied through the recording electrode (compare with Fig. 1). The switched current-clamp technique was used at a switching rate of 2 kHz. The responses are averaged 10 times; current levels are marked. Note the changes in the time course of the reversed responses, the different form of the later part of the response at different initial voltage levels and the prominent oscillation in the reversed responses in B.

transient was mainly due to the shutting off of the same conductance, as shown by the increase in input resistance during the repolarizing phase. With near-saturating intensities, the resistance drop in many cases was followed by a resistance increase beyond the resting level, which was followed by a long-lasting resistance decrease (Fig. 3B; Table 1). The increase in the resistance beyond the resting input resistance of the LMCs with saturated responses (in Fig. 3B) suggests that the input resistance in darkness is probably decreased by continuous transmitter release from photoreceptors. After a brief flash this background release is also transiently reduced. In the saturated response the resistance and voltage did not follow each other exactly: in the late hyperpolarization the resistance dropped to the same level as that reached during the initial transient, whereas the voltage was much more depolarized. This is consistent with the delayed activation of a depolarizing conductance.

This view of more than one conductance being activated during the hyperpolarizing response can be supported by differences in the waveforms of the reversed and unreversed LMC response. In reversed (now depolarizing) responses, the voltage sometimes showed oscillations which were not necessarily present in unreversed responses (Fig. 2, see also Djupsund *et al.* 1987). If there were only one type of transmitter-activated conductance present, the reversed and unreversed responses should be similar in shape; this was clearly not the case. If these response anomalies are caused by voltage-activated conductances, the determined

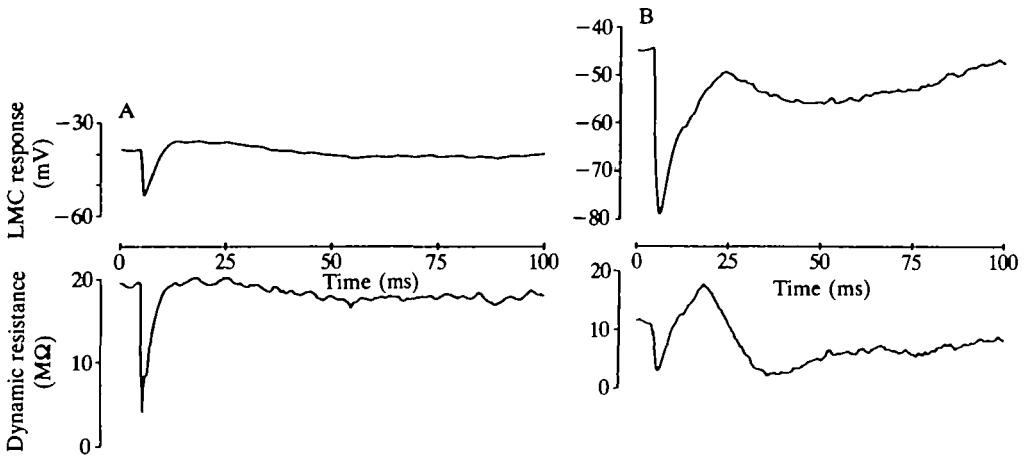

Fig. 3. Dynamic changes of input resistance in LMCs in the lamina when the photoreceptors are stimulated with a brief (30 μs) flash. Upper curves show the voltage response and lower curves the calculated dynamic resistance (applied steady-state current −0·5 nA, switching rate 1·5 kHz). (A) Stimulus intensity in the midrange of the operating function of the LMC (relative intensity 0·14); (B) recording from another cell with stimulus intensity near saturating (relative intensity 1·0). The dynamic resistance is monophasic in A and multiphasic in B. Note that the calculated dynamic resistance is correct only if there is no voltage-activated conductance involved.

Table 1. *Input resistance of blowfly LMCs as recorded in lamina and axon*

	Resistance (MΩ)	
	Lamina ($N = 6$)	Axon ($N = 7$)
Resting input resistance	17 ± 5	27 ± 11
Minimum resistance during light response	3 ± 1	18 ± 10
Resistance increase beyond resting level in saturated responses	4 ± 3	

Values are mean ± S.D.

dynamic resistance may not be quantitatively correct during the off-response, because the method requires that the membrane should be mainly Ohmic. Taken together, the multiphasic resistance change and the oscillating reversed responses seem to indicate conductances (possibly voltage-dependent) other than chloride being activated. This could be the consequence of synaptic input from a source other than photoreceptors.

In the axon recordings, the LMC responses often showed a rapid delayed spike-like depolarization of 10–20 mV, which appeared when the membrane potenti

was lowered by 30 mV or more. This depolarization seemed to have a variable latency of 30–60 ms, and it gradually disappeared when the membrane potential was lowered further. The absence of this type of phenomenon in lamina recordings indicates that the properties of the axonal membrane or the medulla terminal are far from linear, in contrast to the linear properties of the lamina. The spike is reminiscent of axonal membrane phenomena in ocellar interneurones (Ammermüller & Zettler, 1986), and might be caused by a spike generated in the medulla terminal or by a normally inactivated regenerative conductance in the axon (Guy & Srinivasan, 1988).

In conclusion, the light-on transient of the LMCs seems to be caused primarily by the increase of a voltage-insensitive (probably chloride) conductance in the first neuropile. The repolarization is mainly due to shutting-off of the same conductance in the lamina, implying that the transmitter release from photoreceptors is also transient in nature. Other (unknown) conductances seem to be activated during the physiological light-off response, suggesting additional synaptic inputs. The recordings of responses in axons showed regenerative-like properties and suggest that the voltage-insensitivity of the LMC membrane in the lamina cannot be extended to cover the axonal membrane properties.

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