Resistance Changes Associated with the Response of Insect Monopolar Neurons

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Intracellular recordings from the dragonfly lamina show that the light initiated hyperpolarising response of large monopolar cells (LMC's) is associated with a resistance decrease. The response reversal potential is at least 40 mV negative to the dark resting potential. The adaptation of the response to a maintained stimulus is associated with a decrease in hyperpolarising input.

The large monopolar cells (LMC's) of the dragonfly lamina are second order interneurons that respond to input from photoreceptors with a triphasic graded hyperpolarisation, consisting of an initial 'on' transient, a sustained plateau and a depolarising 'off' transient [1]. One of two general types of membrane process could generate the LMC hyperpolarisation which can attain amplitudes of up to 50 mV [1]. As in vertebrate cones [2], and probably horizontal cells and bipolar cells [3], the hyperpolarisation could be associated with a cell input resistance increase that is indicative of a decreased sodium ion membrane conductance. Alternatively the response could be linked with a resistance decrease, the response being generated by increased potassium and/or chloride ion conductances, as in the hyperpolarising photoreceptors of scallop [4]. Although the propagation of the LMC signal has been studied in fly [5] the resistance change associated with lamina hyperpolarisations has only been examined in locust [6] where they are associated with a resistance decrease and a reversal potential compatible with a process dependant upon potassium and chloride ions. The resistance changes associated with the dragonfly LMC intracellular response were measured to show that they are probably generated by a conductance increase and to confirm that locust lamina hyperpolarisations resemble the responses of LMC's in yet another detail of their response.

The preparation of the lamina of the dragonfly *Hemicordulia tau* has already been described, together with the intracellular recording techniques [1]. However, instead of using 150 MΩ fibre-filled microelectrodes, the electrodes used here had resistances of 80 – 100 MΩ and were filled by the ethanol substitution method. Although these electrodes were less effective for intracellular penetrations they had the stable current/voltage characteristics that are essential for any estimate of input resistance. With these electrodes stable intracellular recordings are possible and maximum LMC response amplitudes of between 10 and 40 mV were obtained.

Input resistance was measured using two different methods, both of which utilise a bridge circuit [7] for current injection. In the first method the change in input resistance during the response is measured from the change in bridge imbalance. Positive current pulses (70 ms, 5 Hz) are applied across the bridge and the bridge is balanced with the electrode within the cell. The LMC is then stimulated by a 1.0 s square wave light pulse of saturating intensity, delivered at the centre of the LMC visual field to minimise lateral lamina inputs, and the bridge imbalance observed (Fig. 1). Over

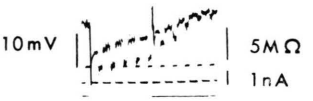

Fig. 1. An intracellular recording using the bridge imbalance technique during determination of the input resistance change from an LMC. Upper trace — intracellular recording *via* Wheatstone bridge; middle trace — current pulses, upwards is positive; lower trace — photocell monitoring axial stimulus.

the range of resistance changes measured, the voltage imbalance is approximately proportional to the change in resistance in the electrode and preparation. Each run is then calibrated by switching a 5 MΩ resistor in series with the preparation. A total of 35 separate determinations carried out upon 13 cells showed that associated with the response there is decrease of input resistance of between 1.2 and 3.8 MΩ (average = 2.4 MΩ). This recorded resistance change is assumed to arise from a change of LMC input resistance during stimulation.

The bridge imbalance technique only allows for the measurement of resistance changes occuring during the response plateau because the 'on' transient is too brief to be accurately monitored. A second series of experiments determines the resistance changes associated with the 'on' transient and the plateau responses by applying steady currents to the cell and observing the change in response amplitude. Under current clamp conditions the response amplitude is reduced by hyperpolarising currents and increased by depolarising currents and the relationship between amplitude and current is approximately linear (Fig. 2). This

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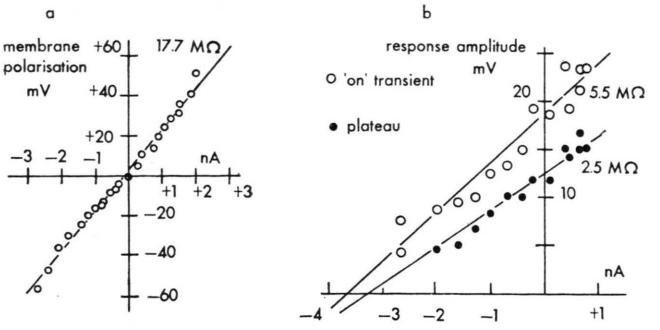

Fig. 2. Plots of changes in LMC membrane potential against applied current. a. LMC membrane polarisation *vs* applied current for the unstimulated membrane. b. Response amplitude *vs* applied current. Values for input resistance (a) and resistance decrease associated with the response (b) are derived from the slopes of the straight lines fitted to the points by linear regression.

result agrees with the lamina recordings of fly LMC responses [5]. The slope of the curve of response amplitude plotted against applied current gives the change in input resistance associated with the response [2]. Measurements were made upon 7 cells and the resistance change estimated by carrying out a linear regression upon the data points. The average resistance decrease associated with the 'on' transient is 5.0 MΩ (total range = 2.5 – 7.9 MΩ) while the average decrease associated with the plateau response is 2.7 MΩ (total scatter = 1.2 – 3.4 MΩ). Note that both methods of measurement give approximately the same result for the plateau resistance decrease. Moreover the larger resistance decrease associated with the 'on' transient response suggests that the decline of response amplitude causing the plateau is accompanied by an increase in input resistance. This in turn suggests that a principal component of the plateau decrement results from a decrease in retinula input at the first synapse rather than synaptically driven depolarisation or shortcircuiting of the LMC membrane, or the passive spread of extracellular lamina positive potentials [6, 8]. Because the LMC triphasic waveform is a property of the lamina rather than the retina [1] it is proposed that inhibition acts upon the retinula axon terminals or is intrinsic to the retinula-LMC synapses themselves.

In three current clamp experiments the input resistance of LMC's could be measured because the bridge was balanced immediately before penetration of the cell and immediately after withdrawal from the neuron. The current/voltage characteristics of the unstimulated cell are approximately linear (*e. g.* Fig. 2) and linear regression analyses of the data yield values for the input resistance of 18, 22 and 33 MΩ (av. = 27 MΩ). When the input resistance values are combined with the currents required to reverse the LMC response values for reversal hyperpolarisations of 64, 49 and 83 mV respectively (av. = 65 mV) are derived, suggesting that the reversal potential lies close to the K^+ and Cl^- equilibrium potentials. Therefore, in dragonfly, as in locust [6], the LMC response is produced by a typical inhibitory synaptic mechanism rather than by the reduction in sodium ion membrane conductance found in vertebrate retinal neurons.

Finally it must be pointed out that despite the larger diameter of dragonfly LMC's their measured values of input resistance greatly exceed those reported for fly LMC's [5]. This serves to emphasise that all intracellular measurements performed upon such small fibres are subject to gross leakage artefacts. When this type of error is so readily compounded by the temperamental current/voltage characteristics of high resistance micropipettes it is obvious that conclusions based upon measurements of absolute response amplitude and membrane resistance must be treated with extreme caution.

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