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Neural Integration in the First Optic Neuropile of Dragonflies

I. Signal Amplification in Dark-Adapted Second-Order Neurons

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Summary. 1. The responses of retinula cells and large monopolar cells (LMC's) to axial light flashes were recorded intracellularly in dark-adapted dragonflies (Figs. 1 and 4).

2. LMC's respond to retinal illumination with a triphasic graded hyperpolarisation whose amplitude and waveform is intensity dependent. An initial hyperpolarising "on" transient is followed by a smaller amplitude sustained plateau. A rapid positive going "off" transient follows the cessation of the stimulus. Intensity is encoded as hyperpolarisation amplitude for action potentials are not recorded in these cells.

3. Measurements of the difference between LMC and retinula response latency (2 msec, Fig. 6) and the LMC angular sensitivity (Fig. 7) confirm the previous anatomical studies suggesting that the LMC's are post-synaptic to retinula axons and receive their major input from axons with the same fields of view.

4. Comparison of retinula and LMC response/intensity functions (Fig. 2) suggests that the visual signal is amplified when it is transferred from the retinula cell soma to a LMC.

5. The derivation of average normalised response/intensity functions (Fig. 3) leads to an estimation of gain during the transfer of the LMC "on" transient and plateau amplitudes (Fig. 8). Their maximum values are times 14 and times 12, respectively.

6. The possible mechanisms for producing amplification at this level in the visual system are discussed together with the significance of amplification in terms of the performance of the visual system.

7. The synaptic noise level in the LMC's is high, from 4.2% to 15.6% of the maximum response amplitude with an average value of 8.6%. It is shown that this is equivalent to a receptor signal of 400 μV at threshold. It is proposed that the high noise level is the result of multiple synapses. It is shown that multiple synapses increase the visual signal: synaptic noise ratio in proportion to the square root of the number of synapses, in a manner analagous to a signal averager.

8. It is concluded that the retinula-LMC pathway acts, in the *dark-adapted* state as a high sensitivity detection system, and shows several adaptations to maximise the signal:noise ratio.

Introduction

One of the major problems in sensory neurophysiology is that of establishing the functional relationships between neurons that under-

lie the integration of visual information derived from an array of photoreceptors. The ultimate aim should be a description of the system in terms of the responses of the component neuron types and their connectivity. The analysis of a single neuron's integrative functions is only possible if its inputs from lower levels of the system are already understood. The logical place to initiate such a study in the arthropod visual system is at the level of the second order neurons in the first optic neuropile.

In insects the first optic neuropile is the lamina and it is here that retinula axons synapse with the principal second order neurons, the monopolar cells. The anatomy and the physiology of the lamina have been intensively studied recently and these results have been reviewed elsewhere (Laughlin, 1973). In this series of papers on the dragonfly lamina the integrative functions of the neural components of the lamina are analysed by comparing their responses with their retinal inputs under different stimulus conditions. Dragonflies have highly developed visual systems and the retinal input is readily derived by recording intracellularly from the retinula cells (Autrum and Kolb, 1968, 1972; Horridge, 1969; Eguchi, 1971).

This paper analyses the responses of a single class of second order neurons, the large monopolar cells (LMC's), to axial light flashes. These are compared with the responses of retinula cells to the same stimuli, thus allowing an analysis of the transfer of information on intensity under *dark adapted* conditions. The results obtained are similar in many respects to those obtained by Zettler and Järvilehto (Järvilehto and Zettler, 1971; Zettler and Järvilehto, 1972a and b) in *Calliphora* and demonstrate that the signal is greatly amplified at the level of the first synapse.

Methods

Animals. Most of the experiments were carried out in Canberra using the local Corduliid, *Hemicordulia tau* (Odonata, Anisoptera). The experimental determination of latency was made using the giant tropical Aeschnid, *Anax gibbulosa*, while on field work in Darwin, Northern Australia. In both cases the adult animals were caught locally and dark-adapted for at least four hours before use.

Recording Techniques. The dark adapted animals were immobilized with their ventral sides uppermost in such a way that only respiratory movements were left unhindered, and the indifferent electrode was inserted in the abdomen. Less than a quarter of the ventral-most part of the retina was removed to allow insertion of the recording microelectrode into the lamina or the retina.

The electrodes used for recording from the lamina and the retina were filled with 2.5 molar potassium chloride or 6% Procion yellow and had resistances of 150 and 600 megohms respectively. All recordings from LMC's or retinula cells used for the measurements of their response/intensity functions were made with potassium chloride filled electrodes. These were connected to the input stage of a Grass P16 preamplifier which was fully capacity compensated for most recordings

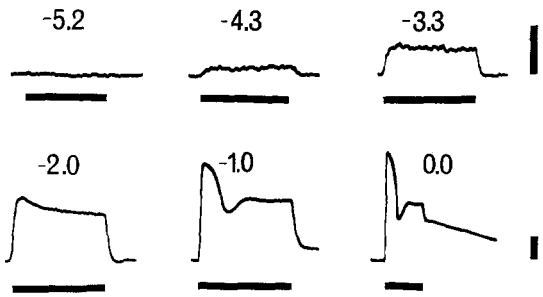

Fig. 1. The response of a single retinula cell to light flashes of increasing intensity. The horizontal bars show the stimulus duration of 0.5 sec and the vertical bars represent 10 mV. The response/intensity function of this cell is illustrated in Fig. 2

and had a frequency response of 1 kHz. For the recordings of retinula cells responses at low intensities (—5.5 to —3.5 log units) a low pass filter was used to cut the frequency response to 50 Hz and reduce the electrode noise to a level that allowed the accurate measurement of response amplitude.

Dye Injection Technique. The method of injection described by Kaneko (1970) was used. After marking a cell the dye was allowed to diffuse for one hour at room temperature before fixing the retina and optic lobe in 70% alcoholic Bouin's for four hours. The specimen was dehydrated, embedded in wax, and sections were cut at 15 microns. The retrieval rate for marked cells was poor and marking experiments were discontinued after the hyperpolarising response had been correlated with the monopolar cell type.

Stimulus Delivery. The stimulator used was of the same type as that described by Laughlin and Horridge (1971). A point source of light, subtending 40′ at the eye, was mounted on a perimeter device that allowed it to be rotated at a constant distance from the retina. The light source was a 40 W tungsten filament lamp. Square wave light pulses with a rise time of less than 5 msec were delivered by means of an electrically driven vane shutter and their intensity was controlled by means of calibrated neutral density filters.

Experimental Procedure. Because both retinula cells and LMC's have an extremely narrow angular sensitivity function (see below) great care was taken to position the stimulus exactly at the point of maximal sensitivity in order to ensure that sensitivity differences between the two cell types did not result from the comparison of stimuli delivered in different regions of the visual field. Throughout all recordings the optical axes of the retinula cells remained stable and did not drift. The sensitivity of a retinula cell or a LMC was examined by measuring the response to flashes of increasing intensity. Flashes were of 500 msec duration and the interstimulus interval was 3 sec. As the dragonfly retina is divided into a dorsal and a ventral region with different facet diameters it was necessary to locate the area of the eye containing the facet giving rise to the response by shadowing the eye during stimulation.

Results

The Response of Retinula Cells

Recordings were made from 12 retinula cells, 5 dorsal and 7 ventral. The maximum response amplitudes fall within the range of 40 and 60 mV

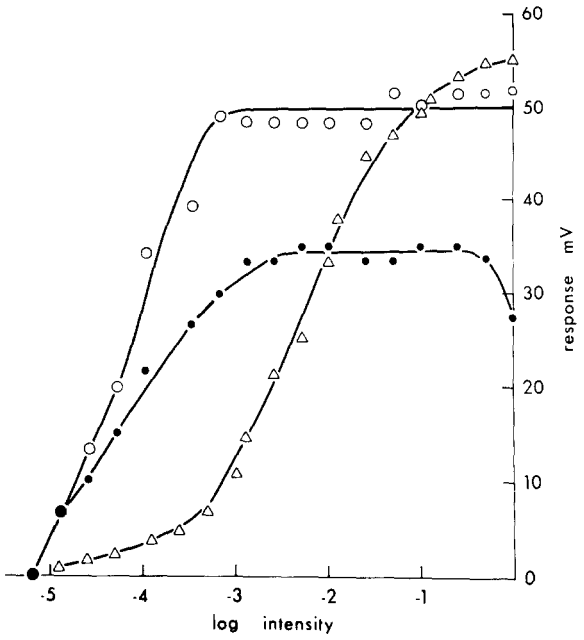

Fig. 2. The response/intensity function of an LMC compared to that of a retinula cell. The response amplitudes of the LMC "on" transient (—o—), and the LMC plateau response (—•—), together with the retinula cell peak response (—▵—) are plotted against stimulus intensity

and their resting potentials vary from —45 to —70 mV. The waveform of the response and the response amplitude are intensity dependent (Fig. 1). At low stimulus intensities, close to threshold, the response is monophasic and the amplitude fluctuates in a way similar to that of the discrete potentials recorded in *Musca* by Kirschfeld (1966). These fluctuations can be attributed to random variations in the frequency of quantal absorption. Unfortunately it has not been possible to record discrete quantal "bumps" of the type described by Scholes (1964) from the locust. As the stimulus intensity increases the response amplitude rises and it becomes biphasic in waveform, with an initial transient peak lasting for approximately 100 msec and a sustained plateau lasting for the duration of the stimulus. At the cessation two types of after potential are seen, a short (25 msec) hyperpolarisation which is only clearly discernable at lower intensities and is not present in the responses of all cells, and a longer lasting positive after potential that decays exponentially following high intensity stimuli. Both these after potentials have been more fully described by Autrum and Kolb (1972) and designated Type II and Type I respectively.

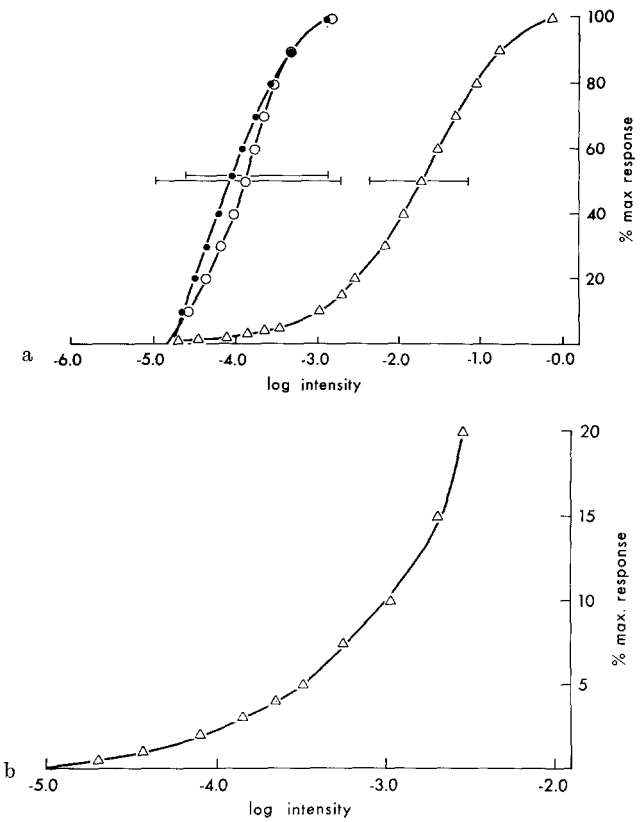

Fig. 3. a The average normalised response/intensity functions for LMC's compared to that for retinula cells. (—o—) LMC "on" transient: (—•—) LMC plateau response: (—▵—) retinula cell peak response: the horizontal bars show the *total* scatter of values for the 50% response intensities. b The average normalised response/intensity function for retinula cells at the low intensities corresponding to the dynamic range of LMC's

The responsiveness of a cell to an axial light source is expressed in terms of its response/intensity function which is a plot of the peak response amplitude against stimulus intensity. One such curve, plotted from the neutral density series illustrated in Fig. 1, is shown in Fig. 2. As is typical for insect retinula cells the curve is sigmoidal with a linear central region where the cell is maximally sensitive to changes of light intensity. This is bounded at the base by a toe where the cell is less sensitive to intensity changes but becomes increasingly more sensitive with respect to absolute intensity. At the region close to maximum response amplitude the absolute sensitivity and the sensitivity to change in intensity falls and the response gradually saturates. The detectable thresholds

of retinula cells vary over a range of 1.3 log units. Because the response intensity curves of different cells are approximately parallel this means that the retinula cells sampled are not equally sensitive to the stimulus employed but there is no correlation between sensitivity and the position of a cell within either the dorsal or the ventral region of the retina. The apparent sensitivity differences probably result from the distal or proximal position of a retinula cell within the ommatidium, for the dragonfly retina is tiered (Horridge, 1969; Eguchi, 1972) and/or the interaction of the non-uniform spectral output of the light source with the different spectral types of retinula cells known to exist in dragonfly (Autrum and Kolb, 1968; Horridge, 1969; Eguchi, 1971).

In order to estimate the average amplitude of the retinula input to the lamina at any given light intensity it is necessary to average the response/intensity functions. To achieve this each individual cell's response/intensity function is normalised with respect to amplitude by expressing the response as a percentage of the maximum saturated response amplitude. All twelve normalised response/intensity functions are then averaged and the resulting average normalised response/ intensity function is shown in (Fig. 3) together with the range limits of the 50% sensitivity points.

The Response of Large Monopolar Cells (LMC's)

Intracellular penetration of LMC's is signalled by the appearance of a distinctive low frequency noise and a resting potential of between —15 and —25 mV. The noise has a peak amplitude of up to 5 mV and can be seen superimposed upon the response of the cells. As the response amplitude rises the frequency of this noise increases and its amplitude decreases. This suggests that the noise is synaptic in origin. LMC's respond to stimulation by illumination of the retina with a graded hyperpolarisation of up to 50 mV, whose amplitude and waveform depend upon stimulus intensity (Fig. 4). At low intensities, just above threshold, the response is monophasic and uneven in amplitude. As the intensity of the stimulus is increased the response becomes triphasic, with a rapid "on" transient, a sustained plateau, and a small "off" transient depolarisation following the cessation of the stimulus. The amplitude of both the "on" transient and the plateau reaches a maximum saturated value at a stimulus intensity that is from 1.5 to 2.2 log units above threshold. However, an increase in stimulus intensity above the saturation value leads to further changes in the response waveform. In particular the plateau amplitude tends to decrease relative to the "on" transient amplitude and the "off" transient becomes more pronounced although the size of both these changes varies from cell to cell. In addition, at

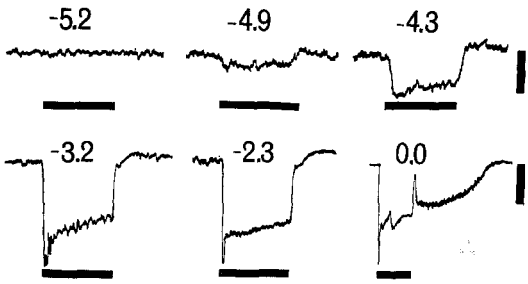

Fig. 4. The response of an LMC to light flashes of increasing intensities, shown in log units above each response. The horizontal bars show the stimulus duration of 0.5 msec and the vertical bar represents 20 mV. The response/intensity function of this cell is shown in Fig. 2

intensities close to the saturation point of retinula cells a slowly decaying hyperpolarisation follows the cessation of the stimulus. This appears to correspond to a sustained input from the retinula positive after potential. In all respects these responses resemble those recorded from the large monopolar cells of Muscoid flies by Zettler and Järvilehto (1971), Arnett (1972) and Ioannides (personal communication). They also resemble the hyperpolarising potentials recorded intracellularly in the lamina of locust by Shaw (1968) and in worker bee by Menzel (personal communication).

The cell type giving rise to the hyperpolarising response has been identified by use of the Procion yellow dye injection technique and a marked neuron is shown in Fig. 5. It is identifiable as a large monopolar cell of the type described by Cajal and Sánchez (1915) from Golgi studies of the dragonfly lamina on the following criteria. The cell body is comparatively large and lies in the monopolar cell soma layer, it has a single axon running radially through the entire depth of the lamina and into the first chiasma, and this axon sends out a dense brush of short dendrites throughout the whole depth of the outer plexiform layer.

The responsiveness of the "on" transient and the plateau of the LMC response to square wave stimuli is quantified using exactly the same procedures as those adopted for retinula cells. The responses of 13 LMC's which all gave peak responses of more than 15 mV are analysed. A typical response/intensity curve, measured from the neutral density run illustrated in Fig. 4 is shown in Fig. 2. Note that the dynamic range of response amplitude is less than 2.0 log units and the gradient of the response/intensity function is steep and the LMC's are more sensitive to changes in light intensity than the retinula cells. The scatter of threshold

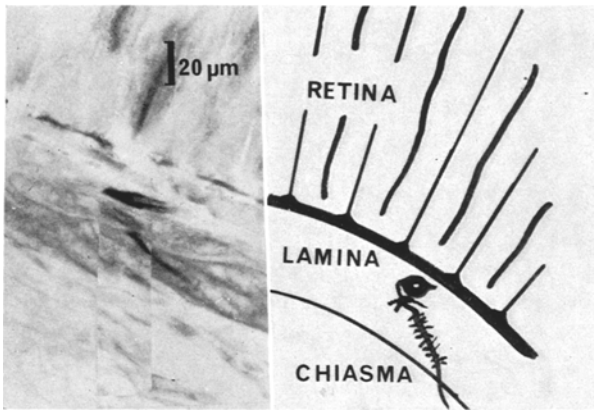

Fig. 5. A montage of horizontal sections through the lamina of *Hemicordulia tau* showing a cell that has been injected with Procion yellow together with a diagrammatic representation of a large monopolar cell in the corresponding position. The montage is printed from a colour positive photomicrograph so that fluorescent objects appear darker than the surround. The lateral dendritic spines shown in the diagrammatic cell are clearly visible in the injected cell but cannot be reproduced photographically

intensities is greater for LMC's than for retinula cells, ranging over 2.0 log units from —5.5 to —3.5 log units. As in retinula cells the response/intensity functions of different cells are parallel and the differences in threshold reflect a difference in sensitivity rather than a recording artefact caused by damage to the cell. In addition to the possible dissimilarities in spectral sensitivity these sensitivity differences could result from changes in the state of neural dark adaptation of second order neurones. In order to obtain a good estimate of the output of LMC's to the medulla, average normalised response/intensity curves are derived using the procedure described above for retinula cells (Fig. 3). The plateau response amplitude is expressed as a percentage of the maximum plateau amplitude.

Both the peak and the plateau of the dark-adapted LMC response have a narrow dynamic range (between threshold and saturation) from —4.8 to —2.9 log units. The response/log intensity curve is linear throughout most of the region and has a steep gradient which is greatest between 50% and 75% of the maximum response amplitude. Here the sensitivity to change of light intensity has a value of 80% of maximum response amplitude per log unit. In contrast the retinula cells have a wider dynamic range from —4.9 to —0.1 log units. They are maximally sensitive to changes in light intensity within the range of 40% to 80%

of maximum response amplitude, with a sensitivity value of 46% of maximum response amplitude per log unit. However this region of optimal sensitivity starts at an intensity which is 0.8 log units greater than the average saturation intensity for LMC's. Within the dynamic range of the LMC's the retinula cell sensitivity is less than 20% of maximum response amplitude per log unit and at threshold this value is 2.0%. The high sensitivity of dark-adapted LMC's, as compared to retinula cells over the same intensity range, is discussed below.

LMC Latency

The latency and rise time of retinula cell and LMC responses are intensity dependent (Järvilehto and Zettler, 1971), so that in order to estimate the latency difference between these two cell types the same stimulus intensities must be used. Experiments were performed on the Aeschnid, *Anax gibbulosa*, to determine the latency/intensity curves for the receptors and the second order neurons over a range of three log units of intensity. As it is apparent that equal amplitude signals in the retinula cells and in the LMC's are not functionally equivalent, because of amplification at the first synapse (see below), the latency is defined in a manner that attempts to be independent of response amplitude and rise time, i.e. latency is taken to be the delay between stimulus onset and the first detectable component of the response. To minimise errors resulting from the amplification of the LMC signal retinula cell responses were recorded with five times the vertical gain of that used for LMC's. The two average curves of latency versus stimulus intensity are shown in Fig. 6 and a latency difference of 2 msec is apparent. This can be attributed to synaptic delay and agrees well with the value of 1 msec in *Calliphora* (Järvilehto and Zettler, 1971).

Angular Sensitivity

The angular sensitivity of LMC's and retinula cells were determined using the methods described by Laughlin and Horridge (1971). In dragonflies the fields of view of retinula cells are extremely narrow (1.0–2.0°) and angular sensitivity can be badly underestimated if a single traverse of the light source, which fails to pass through the optical axis, is used (Horridge, 1969). For this reason the point source was scanned across the visual field in 0.5° steps along a series of horizontal traverses which were separated vertically by 0.5°. In this way the visual field was sampled at a series of points on a 0.5° grid. Only a small number of LMC's have been examined so far by this method but the preliminary result reported here and illustrated in Fig. 7, together with other recordings, show that

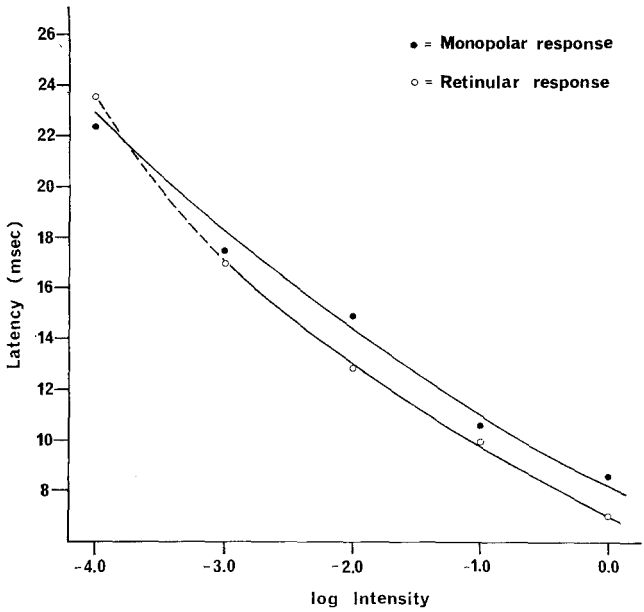

Fig. 6. Response latency plotted as a function of stimulus intensity, for LMC's (—•—), and for retinula cells (—○—), recorded in the Aeschnid, *Anax gibbulosa*. Each point represents the average latency at that intensity derived from measurements from 13 LMC's and 9 retinula cells

the angular sensitivity of LMC's is as narrow or narrower than that of retinula cells. Because of the narrow acceptance angles of retinula cells it is difficult to demonstrate lateral inhibition by measurement of the LMC receptive field. The narrowing of the acceptance angle in LMC's to one half the value of that found in retinula cells described by Zettler and Järvilehto (1972) in *Calliphora* would cause an equivalent change of acceptance angle in dragonfly of 0.5° to 1.0° and this falls within the scatter of experimental values. It is, therefore, impossible to decide at the present time as to whether or not lateral inhibition occurs between adjacent lamina cartridges in the dragonfly and more data are necessary to elucidate this point. However the result presented here together with the results from *Calliphora* (Zettler and Järvilehto, 1972a) and from locust (Shaw, 1968) lead to the conclusion that the major excitatory input to LMC's must be derived from retinula cells with the same field of view. This verifies the anatomical finding that axons from retinula cells with the same field of view project to a single lamina cartridge (Horridge and Meinertzhagen, 1970).

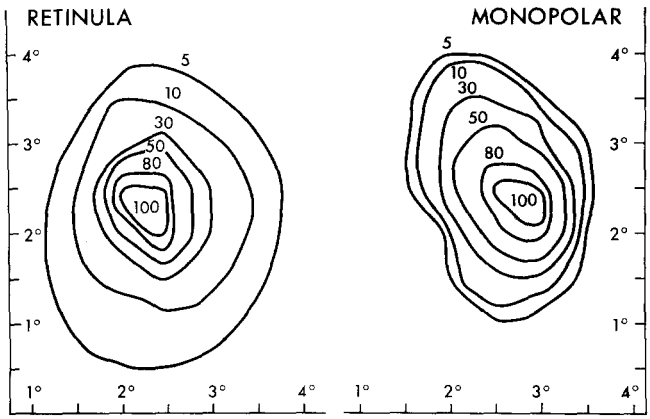

Fig. 7. The field of view of a dorsal retinula cell compared with that of a LMC receiving its input from the same region of the dark-adapted eye of *Hemicordulia tau*. Contours of iso-percent sensitivity are plotted against the two sets of axes which show the 0.5° grid on which measurements were taken

Retinula → LMC Transfer

The short delay between retinula cell excitation and LMC excitation, together with the similarities in their fields of view support the anatomical evidence that retinula axons with the same field of view are presynaptic to a single LMC. Because the signal is inverted the synapse must be chemical and furthermore, as the retinula waveform is monophasic at the low intensities corresponding to the dynamic range of the LMC's, the LMC triphasic waveform must be a property of the LMC's themselves. These same conclusions have been reached by Järvilehto and Zettler (1971) as a result of their studies on *Calliphora*.

It is readily apparent from the data presented above that, within the range of intensities corresponding to the dynamic range of the LMC's, the absolute size of the signal in these second order lamina neurons is greater than the corresponding signal in any single retinula cell. It is not possible, yet to record intracellularly from pre- and post-synaptic terminals simultaneously. Therefore in order to estimate the amplification of the visual signal resulting from its transfer from retina to lamina the averaged normalised response/intensity functions of retinula cells and LMC's are compared.

If the gain, G_I, at any intensity, I, is given by

$$G_I = \frac{V_I^{\mathrm{LMC}}}{V_I^{\mathrm{ret}}} \tag{1}$$

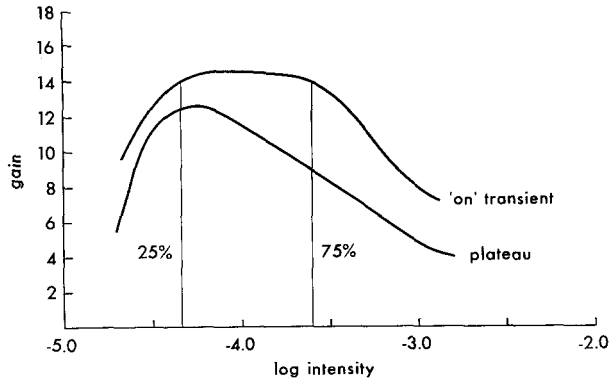

Fig. 8. The amplification of the average retinula cell signal as seen in the dark-adapted LMC's plotted against stimulus intensity. The gain for transfer of the "on" transient and the plateau are plotted separately over a range of intensities corresponding to the dynamic range of the LMC response. The vertical lines show the intensities giving 25% and 75% of maximum response amplitude. Note that between these limits the gain for the "on" transient is constant at times 14

where V_I^{LMC} and V_I^{ret} are the amplitudes of the signal in a LMC and in a single retinula cell soma respectively, the magnitude of G_I throughout the dynamic range of the LMC can be estimated as follows. The maximum LMC "on" transient recorded is 50 mV while the maximum retinula cell amplitude is 60 mV. If it is assumed that these amplitudes are the most representative of signals actually transmitted by these cells in the intact animal then G_I for the "on" transient is given by

$$ {}_{\mathrm{on}}G_I = \frac{{}^{\%}_{\mathrm{on}}R_I^{\mathrm{LMC}}}{{}^{\%}R_I^{\mathrm{ret}}} \cdot \frac{5}{6} \tag{2} $$

where ${}^{\%}_{\mathrm{on}}R_I^{\mathrm{LMC}}$ and ${}^{\%}R_I^{\mathrm{ret}}$ are the normalised average responses at intensity I for LMC's and retinula cells respectively, expressed as a percentage of the maximum response amplitude and given in Fig. 3.

Estimation of G_I for the LMC plateau response is complicated by the fact that the relative sizes of the "on" transient and the plateau vary from cell to cell. The average maximum amplitude of the plateau is 55% of the "on" transient maximum response amplitude so that G_I for the plateau is given by

$$ {}_{\mathrm{plat}}G_I = \frac{{}^{\%}_{\mathrm{plat}}R_I^{\mathrm{LMC}}}{{}^{\%}R_I^{\mathrm{ret}}} \cdot \frac{55}{100} \cdot \frac{5}{6} \tag{3} $$

The curves for ${}_{\mathrm{on}}G_I$ and ${}_{\mathrm{plat}}G_I$ are shown in Fig. 8 as a function of stimulus intensity over the average dynamic range of the LMC's. Note that the

gain for the "on" transient is virtually constant over the median half of the dynamic range while the gain for the plateau decreases constantly after reaching an initial maximum.

It must be emphasised that the gain G_I does not represent the difference in voltages across the retinula—monopolar synaptic terminals but quantifies the amplification resulting from signal transfer from the retina to the LMC's. It will become apparent in the discussion that G_I gives an approximate estimate of the differences between pre- and post-synaptic voltages. Again this cannot be directly related to the transfer properties of a *single* synapse because the post-synaptic voltage is a product of a large number of synapses.

The Noise Level in LMC's

The LMC's always show a high intracellular noise level of constant peak to peak amplitude. In order to estimate its effect on the detection of small retinula cell signals the noise is expressed in terms of an equivalent retinula cell voltage in the following manner. The measured peak to peak noise in 9 LMC's giving maximum response of more than 20 mV varies from 1.0 mV to 4.9 mV, after subtracting 0.5 mV which represents the *minimum* level of electrode noise. Because the LMC noise level varies with recording conditions in the same manner as the maximum response amplitude the noise level in each cell is expressed as a percentage of the saturated response amplitude and varies from 4.2% to 15.6% with an average value of 8.6%. This average value is then expressed as an equivalent threshold intensity difference by use of the average normalized response/intensity curve shown in Fig. 3. In order to derive the equivalent noise level in the retinula cell this intensity fluctuation, 0.26 log units, is converted to percent retinula cell response by use of the retinula average normalised response/intensity function. This is then converted to a maximum receptor voltage fluctuation by assuming that the optimal maximum response amplitude is 60 mV. Using this method the equivalent receptor noise level is approximately 400 μV. A value for equivalent receptor noise for the range of maximal sensitivity to intensity change of the LMC's derived by the same method and is 300 μV. Thus the LMC noise level is equivalent to a signal in the retinula cell soma that is 0.005 of the maximum receptor response amplitude.

Discussion

Intensity Coding by LMC Graded Potentials

The results presented above add further weight to the proposal by Zettler and Järvilehto (1971) that visual information is transmitted to the medulla by LMC's encoded in graded hyperpolarisations. The wave-

form of the response is similar in all respects to that found in *Calliphora* and action potentials are never recorded in LMC's. However, in a recent paper, Arnett (1972) proposes that the spiking "on-off" and "sustaining" units that he recorded extracellularly from unmarked loci in the first chiasma of the fly *Phaenicia* are the two large monopolar cells of Muscoid flies, L1 and L2. This data is incompatible with the known intracellular responses of the LMC's, recorded from neurons that have been positively identified by intracellular dye injection, not only in this study but in four previous studies including his own (Autrum, Zettler, and Järvilehto, 1970; Zettler and Järvilehto, 1971; Arnett, 1972; Ioannides, personal communication). These LMC graded potenials resemble those recorded intracellularly from second order neurons in the dragonfly ocellus (Chappel and Dowling, 1972). In this system the hyperpolarisations generated in the second order neurons inhibit a tonic discharge of action potentials and this appears to be the only way that such a hyperpolarisation could act to convert synaptic input to spike frequency. However light inhibited units with a pronounced dark discharge have never been recorded extracellularly from the first chiasma even though LMC's represent the largest diameter fibres present. It is clear that Arnett's units are not LMC's, for LMC's do not support action potentials and they must represent another class of lamina neuron, possibly centrifugal fibres although in the absence of clear evidence as to their latency even this point is difficult to decide.

In addition there is little reason to doubt that, on the basis of available evidence (briefly reviewed, Laughlin, 1973), graded potentials can transmit visual information over considerable distances (e.g. Shaw, 1972). The widespread occurrence of graded potentials encoding visual information in receptors and second order visual neurons, such as insect retinula and monopolar cells, vertebrate cones and horizontal cells (Werblin and Dowling, 1969) points to a common functional role. It is possible that with the high density of receptors required for optimum acuity, space is at a premium in the lower order ganglia of visual systems, and that graded potentials allow a higher rate of information transfer per axon because the signal need not be integrated with respect to time at the next synapse. In addition graded potentials allow the transmission of high frequency but low amplitude signals with an accuracy that is impossible in a digitalised system with an upper carrier frequency of 1 kHz.

Lamina Amplification as a Property of Dark-Adapted Apposition Compound Eyes

As in dragonfly, all previous studies of the responses of dark-adapted LMC's show that they have a threshold close to that of the receptors

and a relatively short dynamic range (Shaw, 1968; Zettler and Järvilehto, 1971; Arnett, 1972; Ioannides, personal communication). There is also a rapid increase of response amplitude with intensity compared with retinula cells. Both these facts can be explained as the result of an amplification of the visual signal during its transfer from the retina to the lamina. Shaw (1968) was the first to suggest that amplification occurred here, for discrete potentials seen in lamina hyperpolarising units were larger in amplitude than the quantum bumps seen in retinula cells at the same stimulus intensity. In addition Järvilehto and Zettler (1971; Zettler and Järvilehto, 1972b) found an amplification of times eight by comparing the responses of retinula cells and LMC's to sinusoidally modulated stimuli in the frequency range of 10–100 Hz. However they were unable to demonstrate an amplification of response amplitude during square wave stimuli although they observed an amplification of approximately times two by comparing the first derivative with respect to time (dV/dt) of the rising phases of the individual responses. Thus amplification appears to be a general property of all the *dark-adapted* LMC's which have been investigated, but the stimulus conditions by which it can be demonstrated experimentally vary from species to species. It is difficult to account for the apparent absence of signal amplification in response to square wave stimuli in *Calliphora*. Square wave stimuli can be described in terms of high frequency components at "on" and "off" and extreme low frequency components during the sustained duration of the stimulus. Amplification is described in *Calliphora* at high frequency both by comparison of the rates of rise of the square wave response and in response to sinusoidally modulated stimuli. In addition it is seen at low frequency, the gain being times three at 8 Hz (Zettler and Järvilehto, 1972b). The clear cut amplitude gain seen in dragonfly both on the peak and the plateau of the response is not just a result of the larger amplitude responses reported here, for this would account for a factor of two, nor is it solely the result of the higher upper frequency response in signal transfer as the plateau is maintained throughout the stimulus duration. Although the frequency response of retinula-LMC amplification has not been tested in the dragonfly, the result presented here does strongly suggest that it is not obligatory to express retinula-LMC transfer in terms of the first derivatives (dV/dt) of the rising phases (cf. Järvilehto and Zettler, 1971).

Possible Mechanisms for Producing Amplification

It is now established beyond all reasonable doubt that in insects with apposition compound eyes the retinula axons with the same fields of view project onto a single lamina cartridge (for a review of the evidence

see Laughlin, 1973). Anatomical and ultrastructural studies on muscoid flies suggest that six retinula axons are presynaptic to one LMC (Trujillo-Cenóz, 1965), and that each LMC synapses many times with one retinula axon. This would explain the large number of short radial dendrites seen along the length of the lamina axon of the LMC's (Cajal and Sánchez, 1915; Strausfeld, 1971). The visual signal is encoded in the retinula axons, not in action potential frequency, but as a graded receptor potential conducted electrotonically to the axon terminals (Ioannides and Walcott, 1970; Järvilehto and Zettler, 1970). Amplification of this retinula signal could come about by a combination of three mechanisms.

1. An amplification of the signal in the receptor axon terminals as a result of retinula axon interaction.

2. The convergence of inputs from six retinula axons onto a single LMC.

3. Multiple synapses from one retinula axon terminal onto a single LMC.

The evidence for each of these mechanisms and their functional consequences will now be considered in turn.

1. Inter-Axonal Interaction. There is at present no conclusive anatomical evidence for direct synaptic contact between retinula axons and the only evidence for an interaction comes from the recordings of positive potentials, similar in waveform to the receptor potential, in the lamina (Scholes, 1969; Gemperlein and Smola, 1972; Smola and Gemperlein, 1972). Scholes found that the inputs all six retinula axon terminals in the fly lamina neurommatidium contribute to the positive potential. Gemperlein and Smola have continued this study and have shown that at low intensities these potentials are more sensitive than retinula cell soma and conclude that this results from a summation of the signal together with an improvement in the signal to noise ratio. There is however some doubt as to whether all lamina positive can be assumed to be intracellular recordings from retinula axons (Scholes, 1969; Laughlin, 1973). In any case it is difficult to envisage that retinula axon summation at a level distal to the LMC, can amplify the signal fourteen times for in dragonfly the retinula axons are relatively short, 50–100 μ, and attenuated signals from synaptic inputs to the axon should be seen in the cell soma (Autrum and Kolb, 1972). It must be concluded therefore that inter-axonal interaction can only account for a small fraction of the amplification measured in LMC's and that additional mechanisms must be invoked.

2. Axonal Convergence onto LMC's. Convergence is well supported by the anatomical evidence (see above). The summation of the inputs from six retinula cells in a single LMC will not only increase the effective

stimulus strength by a factor of six (assuming linear summation) but will reduce the fluctuation of the retinula signal at low intensities due to the random nature of quantal absorption (the discrete potentials) by a factor of $\sqrt{6}$. This effectively reduces a major source of *receptor* noise at low intensities.

3. Multiple Synapses. Multiple synapses from one retinula axon terminal onto a single LMC are known to occur in muscoid flies and the number of such synapses is estimated to be 40 (Strausfeld, 1971). The similar morphology of dragonfly LMC's suggests that a similar organisation exists in the lamina of Odonata as well. Multiple synapses would undoubtedly amplify the signal and more important still would act in a manner analagous to that of a signal averager, removing synaptic noise (see below).

In summary all three mechanisms could combine to produce an amplification of the visual signal but the mechanisms (2) and (3) would not only be more effective but are extremely advantageous since they decrease the effective level of noise from several sources (see below).

Multiple Synapses and the Reduction of the Signal:Noise Ratio

One of the perplexing properties of the LMC's is the apparently high noise level of up to 5 mV which is seen in the unstimulated cell. However it has been demonstrated that such a noise level is equivalent to a threshold receptor signal of 400 μV. It has been suggested (Laughlin, 1973) that this noise is synaptic in origin because its amplitude decreases and its frequency increases with increasing intensities of stimulation. Discrete quantal transmitter release appears to be a common property of all synapses and it is a potential source of random noise in nervous systems. Quantal transmitter noise from a single synapse has a low frequency that follows a Poisson distribution (Hubbard *et al.*, 1969). In LMC's however the noise frequency is abnormally high and at first sight this would appear to introduce unnecessary noise into a system that in all other respects is adapted to maximise the signal:noise ratio. The following analysis shows that multiple synapses from a single retinula axon onto a LMC, although increasing the absolute level of synaptic noise, considerably increases the signal:synaptic noise ratio.

Let a single retinula axon make n synapses onto a LMC. The axonal signal that is transmitted across each synapse is composed of two parts, a signal resulting from light absorption S_q and a signal representing all other sources of noise in the retinula cell itself (excluding transmitter release noise) N_r. At *each* synapse the signal is transferred to the LMC, to give a signal of S_s, given by the equation

$$S_s = x(S_q + N_r) \tag{4}$$

where x is the transfer coefficient for a single synapse. Because the retinula axon terminal and the adjacent LMC axon make synaptic contact within a relatively short length and the conduction of signal in both axons is electrotonic the pre- and post-synaptic signals will appear virtually simultaneously at all synapses. If the post-synaptic signals sum linearly then the total signal set up in the LMC by the retinula signal, S_{LMC}, is given by

$$S_{\mathrm{LMC}} = n\,x\,(S_q + N_r) \tag{5}$$

where n is the number of synapses. Each synapse, however, produces post-synaptic noise with a variance of $\bar{\sigma}_s^2$ and because the frequency distribution of noise events is binomial the variance of the noise generated in the LMC by n synapses, $\bar{\sigma}_n^2$, is given by

$$\bar{\sigma}_n^2 = n\bar{\sigma}_s^2. \tag{6}$$

As the variance is a measure of the square of the deviations of the signal, the peak to peak fluctuation of the synaptic noise (the component that is effective in destroying the signal), N_{LMC}, is given by

$$N_{\mathrm{LMC}} = k\sqrt{n\cdot\bar{\sigma}_s^2} \tag{7}$$

where k is an arbitrary constant.

From Eqs. 5 and 7 the ratio of signal:synaptic noise is given by

$$\frac{S_{\mathrm{LMC}}}{N_{\mathrm{LMC}}} = \frac{x}{k}\cdot\frac{S_q + N_r}{\bar{\sigma}_s}\cdot\sqrt{n}. \tag{8}$$

Thus the ratio of signal:synaptic noise is proportional to the square root of the number of synapses. Note that noise already present in the receptor terminals is not averaged out by this system. At present it is not possible to estimate the number of synapses made by a single retinula axon onto a LMC in the dragonfly lamina. In Muscoid flies the number of synapses has been estimated to be approximately 40 (Strausfeld, 1971) and this would lead to an improvement of the signal:synaptic noise ratio by a factor of approximately 6.

Several cases are known from other animals of neurons receiving multiple parallel inputs, e.g. cerebellar Purkinje cells (Eccles *et al.*, 1967) and it has been suggested that parallel pathways act to improve the signal:noise ratio (Knox, 1970) however in these cases the parallel pathways are derived from a large number of similar neurons. In LMC's multiple synapses act to reduce the synaptic noise in a single pathway, in a manner that is analagous to a signal averager but here, as opposed to the more conventional signal averager, the signal is repeated in space rather than time and temporal correlation is achieved by the simultaneous appearance of input and output in all channels.

The Functional Significance of Amplification

Given that amplification is accompanied by a reduction in the effective noise level, and the considerations outlined above point to this conclusion, amplification has several important consequences for the performance of the visual system.

1. Amplification increases the size of the voltage fluctuations set up by the movement of contrast boundaries across the visual fields of the retinula cells. The importance of this "contrast amplification" in increasing spatial acuity has been emphasised by Gemperlein and Smola (1972) in connection with the amplitude gain shown by retinula axons. The amplification found at the level of the LMC's is far greater and will be more significant. In addition Northrop has postulated that an amplification of retinula input takes place in the locust lamina as a necessary part of a model proposed to account for the anomalously high acuity found in ventral cord movement detectors (R.B. Northrop, personal communication).

2. Amplification increases the reliability of detection of signals of a low absolute level by increasing the size of the available input to higher order neurones. Amplification thus goes some way to explain the mechanism by which the apposition eye can monitor movement at intensities that give receptor potentials of less than 1 mV (Scholes and Reichardt, 1969), despite the occurrence of synaptic noise at the first synapse. The multiple synapse signal averager allows transmitter release noise to be reduced and although quantum bumps have not been observed in dragonfly retinula cells a bump of 0.5 mV (as is commonly found in locust, Scholes, 1964) could be detected above the level of synaptic noise. In addition the convergence of retinula axons onto a single LMC increases the accuracy with which low intensity signals are monitored by reducing the fluctuation in receptor potential produced by the random nature of light quantal absorption by a factor of $\sqrt{6}$.

In summary it is suggested that the high level of amplification found in the dragonfly LMC's reflects the specialisation of the retinula axon—LMC pathway as a high sensitivity detection system which allows the visual system to operate with high acuity at low light intensities. This amplification is achieved by the summation of retinula inputs from several receptors without the loss of spatial acuity and by an amplification of the signal by multiple synapses. Dragonflies have preempted electrical engineers by several million years in the design of a high sensitivity detection system by placing the initial stage of signal amplification as close as possible to the receptors and maximising the signal: noise ratio at every possible opportunity. Whether or not the high sensitivity to change of light intensity shown by dark-adapted LMC's is also found in the light adapted state at which dragonflies are most visually active is an important question that begs further experimentation.

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