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THE NEURONAL BASIS OF A SENSORY ANALYSER, THE ACRIDID MOVEMENT DETECTOR SYSTEM

II. RESPONSE DECREMENT, CONVERGENCE, AND THE NATURE OF THE EXCITATORY AFFERENTS TO THE FAN-LIKE DENDRITES OF THE LGMD

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SUMMARY

No dendritic spikes occur in the input fan of the lobular giant movement detector (LGMD) neurone. The action potentials are initiated at the point of thickening of the axon, which therefore represents the site of convergence of the retinotopic projection in the MD system. Previous work has shown that the site of decrement in response to repetitive visual stimulation is distal to this point. No change in spiking threshold in the LGMD could be demonstrated, and decrement in the number of LGMD action potentials is completely explained by the observed decrement of EPSPs recorded in the LGMD input dendritic fan. Possible postsynaptic mechanisms which might affect EPSP amplitude are excluded experimentally or shown to be improbable. Latency measurements during electrical stimulation in the second chiasma (which produces a decrementing EPSP in the fan) indicate that the pathway from the chiasma afferents to the LGMD fan is probably monosynaptic. By exclusion, the site of decrement appears to be located at the presynaptic terminal of that synapse. Generalization of habituation of the response to ON and OFF stimuli is demonstrated, showing that the presynaptic neurone at the labile synapse is an ON/OFF unit. The greater part of the previously described sensitivity gradient on the retina, relative to the MD response, appears to be explicable by the geometry of the LGMD fan and of the retinotopic projection. We conclude that the LGMD is fed by a homogeneous population of ON/OFF units running in the second optic chiasma, which form labile synapses on the input fan.

INTRODUCTION

The response of the orthopteran descending movement detector (DMD) neurones are labile, both for movement of contrasting objects in the visual field and to small static stimuli (i.e. changes in the illumination of small areas of the retina) (Rowell, 1971*a*). Some of the variation in response is correlated with change in the arousal state of the animal, as judged by observing motor behaviour, and is relatively unpredictable (Rowell, 1971*b*, *c*); some aspects of this will be considered in the final paper of the present series (O'Shea and Rowell, in preparation). Here we consider the neural

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basis of response decrement, which is a more predictable form of lability that occurs when the same area of the retina is stimulated repetitively.

Response decrement in the DMD neurones has been extensively described (Palka, 1967*a*; Rowell & Horn, 1967; Horn & Rowell, 1968), largely because of the ease with which these neurones can be recorded using extracellular electrodes. Decrement is site-specific, for when the stimulus is applied to an unstimulated area of the retina the response is fully restored. In the absence of dishabituation or strong arousal, habituation is detectable with inter-stimulus intervals as long as 10 min and recovery may take hours. It is clearly the basis of the preference of the DMDs for stimuli which affect new areas of the retina and is therefore the basis of their property of detecting novelty.

Intracellular recordings (O'Shea & Rowell, 1975, and our unpublished observations) from the descending contralateral movement detector (DCMD) neurone, lead us to believe that all its action potentials are derived from input from the lobular giant movement detector (LGMD) neurone (O'Shea & Williams, 1974). The synapse connecting the two is electrical and transmits action potentials reliably even at 300 Hz (O'Shea & Rowell, 1975). It therefore cannot contribute to decrement in the DCMD. This suggests that decrement in the DCMD is the consequence of a process occurring either in the LGMD, or more distally in the optic lobe. Furthermore, the site-specific feature of decrement in the DMDs shows that the locus of decrement is prior to the site of convergence of the retinotopic projection.

The purpose of this paper is twofold. First, we establish the site of convergence in the movement detector (MD) system and show that decrement in the number of DCMD spikes is due to a diminution of the compound EPSP. This diminution is produced by a retinotopic projection of excitatory afferents to the LGMD, which synapse on the dendritic subfield (hereafter referred to as the LGMD fan) as described by O'Shea & Williams, (1974). Evidence will be presented which suggests that this decrement results from presynaptic processes occurring in the excitatory terminals. Secondly, we will describe the nature of these excitatory afferents on the LGMD fan. In the preceding paper (Rowell & O'Shea, 1976), we confirmed the findings of Palka (1967*a*) and Horn & Rowell (1968) that the DCMD is an ON/OFF unit, and showed that under some extreme circumstances the ON and OFF responses varied independently. Since the retinal receptors in the locust, as in other insects, are ON units, and all second order visual cells so far established in insects are OFF units (for further details see the preceding paper), it seems clear that the ON/OFF properties of the MD neurones must derive from convergence of separate ON and OFF afferent channels. The results described here will show that this convergence does not take place at the LGMD fan, but at an earlier stage, and indicate that the LGMD fan receives only a single ON/OFF excitatory retinotopic projection. Other excitatory afferents with different properties also synapse with the LGMD, as we will show in the final paper of this series (O'Shea & Rowell, in preparation), but these play no part in response decrement.

MATERIALS AND METHODS

Experiments were performed on adult males and females of *Schistocerca nitens* (Thunberg, 1815) from laboratory culture. *S. vaga* (Scudder, 1876), the name used

for this animal in our previous publications, has been recently synonomized with *S. nitens nitens* by Dirsh (1974). The thoracic nerve cord was exposed by dorsal dissection and extracellular records from the DCMD axons obtained by placing silver hook electrodes around one prothoracic–mesothoracic connective. The posterior face of the optic lobe was exposed by dissection and the lobula region of the optic lobe supported on a glass 'spoon' placed under the anterior surface. The spoon was raised to apply sufficient tension to the sheath to allow penetration with the microelectrode. Intracellular recordings were made from the LGMD fan and axon by passing capillary electrodes (filled with 2 M potassium acetate) through the sheath overlying the posterior face of the optic lobe. Electrodes were initially of 50–80 mΩ resistance. When penetrated, the LGMD was identified by its response characteristics and the 1:1 synchrony between its spikes and those of the DCMD. On some occasions we used electrodes filled with cobaltous chloride, and the cell was visualized after penetration by precipitation as the sulphide of iontophoretically injected cobalt ions (Pitman, Tweedle and Cohen, 1972).

One eye and the ocelli were covered with an opaque black wax. Both static and moving visual stimuli were presented to the other eye. The apparatus used for the former was described in the preceding paper (Rowell & O'Shea, 1976); it allowed us to generate both positive and negative changes of known amplitude in the luminance of a 5° or 10° area, situated in a much larger 'surround' area which occupied nearly half of the visual field. This surround was painted either white or black, depending on the experiment. Moving stimuli were provided by a circular black target, subtending 9°, mounted on a pen-recorder mechanism driven by a triangular waveform. Movement of the target was monitored by recording the feedback voltage of the servo motor. The target was moved to and fro against a white background of $\sim$ 10 cd/m^2 brightness through an angular distance of 33° at a velocity of 66°/s. When repetitive stimuli were given, the interstimulus interval varied according to the requirements of the experiment, typically between 5 and 30 s.

Extra- and intracellular electrical stimuli were used in some experiments. The LGMD was stimulated intracellularly while recording by means of an active bridge circuit, and the current passed was monitored by a current-to-voltage circuit (Brown, Maxfield & Moraff, 1973) between the insect and ground. Fine bipolar tungsten electrodes (about 5 μm tips), insulated except at their tips, were used to stimulate focally in the optic lobe while recording intracellularly from the dendrites of the LGMD. The stimulating electrodes were connected to the stimulators via an isolated, constant-current circuit with up to 90 V EMF available to avoid variation in stimulus strength due to polarization at the metal/liquid interface. Stimulating electrodes were introduced into the optic lobe through a hole in the retina and advanced axially towards the LGMD with the fine drive of a micromanipulator. The location of the stimulating tips was determined during an experiment by measuring the distance advanced into the optic lobe and comparing this with a large calibrated montage of the optic lobe prepared from photographs of histological sections. This determination was later checked by producing a coagulating lesion at the electrode tips, by passing approximately 20 μA for 20 s and examining histological preparations of the tissue.

RESULTS

(A) *The loci of convergence and of decrement in the MD system*

Previous work has shown that no decrement takes place at the LGMD/DCMD synapse, while the site-specific nature of the decrement in the MD system implies that it occurs before the convergence of the retinotopic projection (see Introduction). The identification of the site of convergence, is therefore, a necessary condition for identifying the site of decrement.

(i) *Convergence*

The LGMD fan runs across the receiving face of the lobula (Figs. 1, 8), very close to the point of entry of the afferent cells from the proximal face of the medulla which run in the second optic chiasma (cells of which are hereafter referred to as 'chiasma afferents'). The branches of the fan cover the whole of the retinotopic projection. Visual stimuli of the sort which produce spike activity and decrement in the MD system elicit EPSPs that are at their largest when the electrode is inserted in the fan itself, and may be invisible at other sites in the LGMD neurone. *A priori* the fan is the probable zone of synaptic contact from the excitatory afferents. The ultimate site of convergence in the MD system is, therefore, the point at which spikes are initiated on the single, centripetal, axon of the LGMD. The bimodal nature of the LGMD and the separate, proximal, site of spike initiation for auditory input (O'Shea, 1975) enabled the point of the initiation of the visual spike to be precisely located (Fig. 2). The conduction velocity of the LGMD axon (directly determined by two-point recording) is 3 m s^{-1}, which is effectively the same as that of the DCMD (Burrows & Rowell, 1973). Auditory and visual spikes are recorded with different latencies at electrodes placed in the LGMD fan (x), in the proximal part of the LGMD axon (y), and on the DCMD axon in the thoracic nerve cord (z). This is because the visual spikes are initiated distally, close to electrode x, move past the second recording electrode (y) and then down the cord to the third electrode (z). Auditory spikes, by contrast, are initiated close to electrode y, and propagate in opposite directions to electrodes x and z. Under these circumstances, the difference between the time of arrival of a spike at electrode x and electrode z differs for visual and auditory spikes by a time equal to twice that taken to propagate between the two sites of spike initiation. As the conduction velocity of the LGMD axon is known and, also, the point at which the auditory dendrite joins it (O'Shea & Rowell, in preparation), the position of the initiation zone for visual spikes can be accurately determined by measurement. When this is done, it is found to coincide with the point at which the LGMD axon thickens. This conclusion can be tested by moving electrode a from the fan and placing it instead in the LGMD axon at the calculated distance from the postulated spike initiation zone which would eliminate the conduction difference between auditory and visual spikes. This distance is about 580 μm, and when this is done, the prediction is confirmed (Fig. 2C). We may therefore conclude that visual spikes are initiated in the LGMD axon where it thickens, and that this is the site of convergence of the retinotopic input in the MD system.

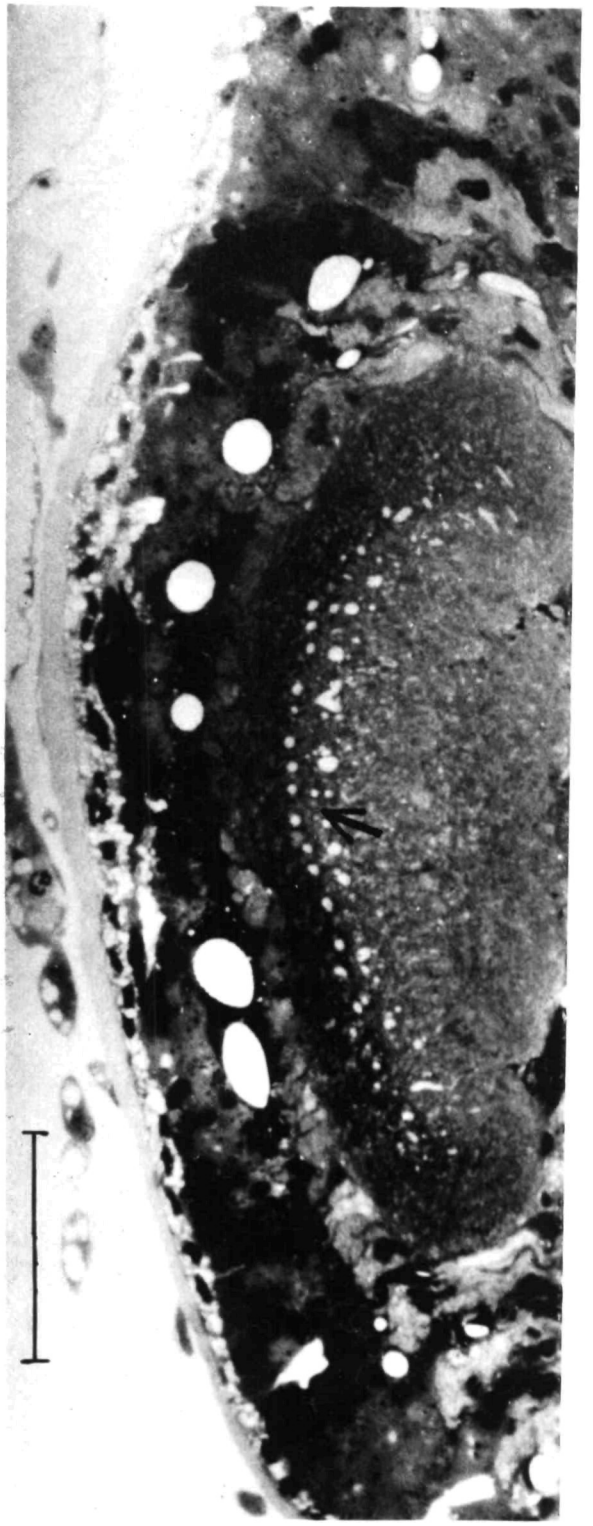


Fig. 1. For legend see opposite.

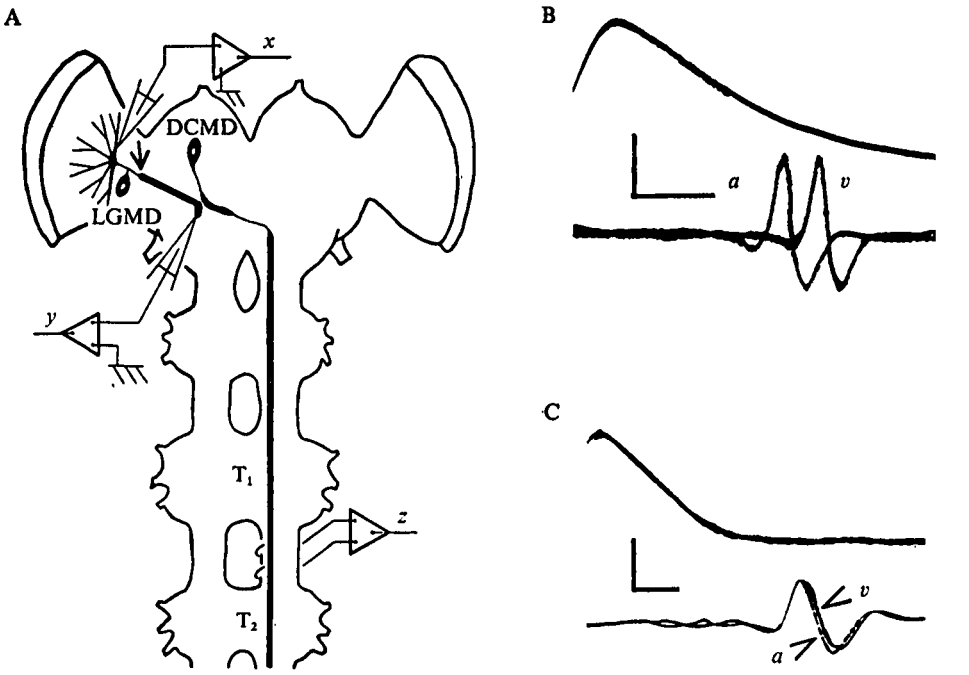


Fig. 2. Determination of the point of initiation of the axonal spikes of the LGMD.

(A) Diagrammatic representation of the LGMD and its postsynaptic neurone, the descending contralateral movement detector (DCMD), to show placement of the electrodes. Visual input initiates spikes in the LGMD via input to the distal fan of dendrites; auditory input initiates spikes near the proximal end of the LGMD, close to its synapse with the DCMD.

(B) Superimposed records of the response to auditory (*a*) and visual (*v*) spikes, recorded in the fan (upper trace) and in the cord (lower trace) respectively. The difference between the apparent latencies of the extracellular spikes relative to the intracellular is double the temporal separation of the two initiation sites. As the conduction velocity is known, the temporal separation of the two sites can be converted into a measure of spatial separation. Calibration, 0·5 ms and (upper trace only) 40 mV.

(C) As in (B) except that the intracellular electrode (upper trace) is inserted proximally in the LGMD. There is now little remaining difference between the apparent latencies of auditory and visual extracellular spikes, relative to the intracellular one, showing that the intracellular electrode is close to the site of origin of the auditory spike. By measuring from this point (on a calibrated photograph of a cobalt-impregnated LGMD neurone) a distance equal to that calculated to separate the two sites of initiation (as derived in B above) the point of initiation of the visual spike is found. It corresponds to the point at which the axon thickens (arrow), some distance proximally from the fan. For further details see text and O'Shea (1975). Calibration, 0·5 ms and (upper trace only) 25 mV.

Legend to Fig. 1

Fig. 1. Portion of a parasagittal section across the optic lobe, at approximately the level indicated on Fig. 8. The posterior face of the lobe is shown. Inside its neural lamella is a layer of perineurial and nerve cell bodies, with numerous large tracheae; then a densely staining layer, composed principally of the axons of chiasma afferents running into the lobula; then a more lightly staining area which represents the neuropil of the lobula. At the margin between the latter two zones, a vertical row of large neuronal profiles can be seen (arrow). These are the larger branches of the dendritic fans of the LGMD and other large lobular fan cells, running around the posterio-distal receiving face of the lobula. See also Fig. 8. Scale, 100 μm. Plastic embedded section, stained in toluidine blue.

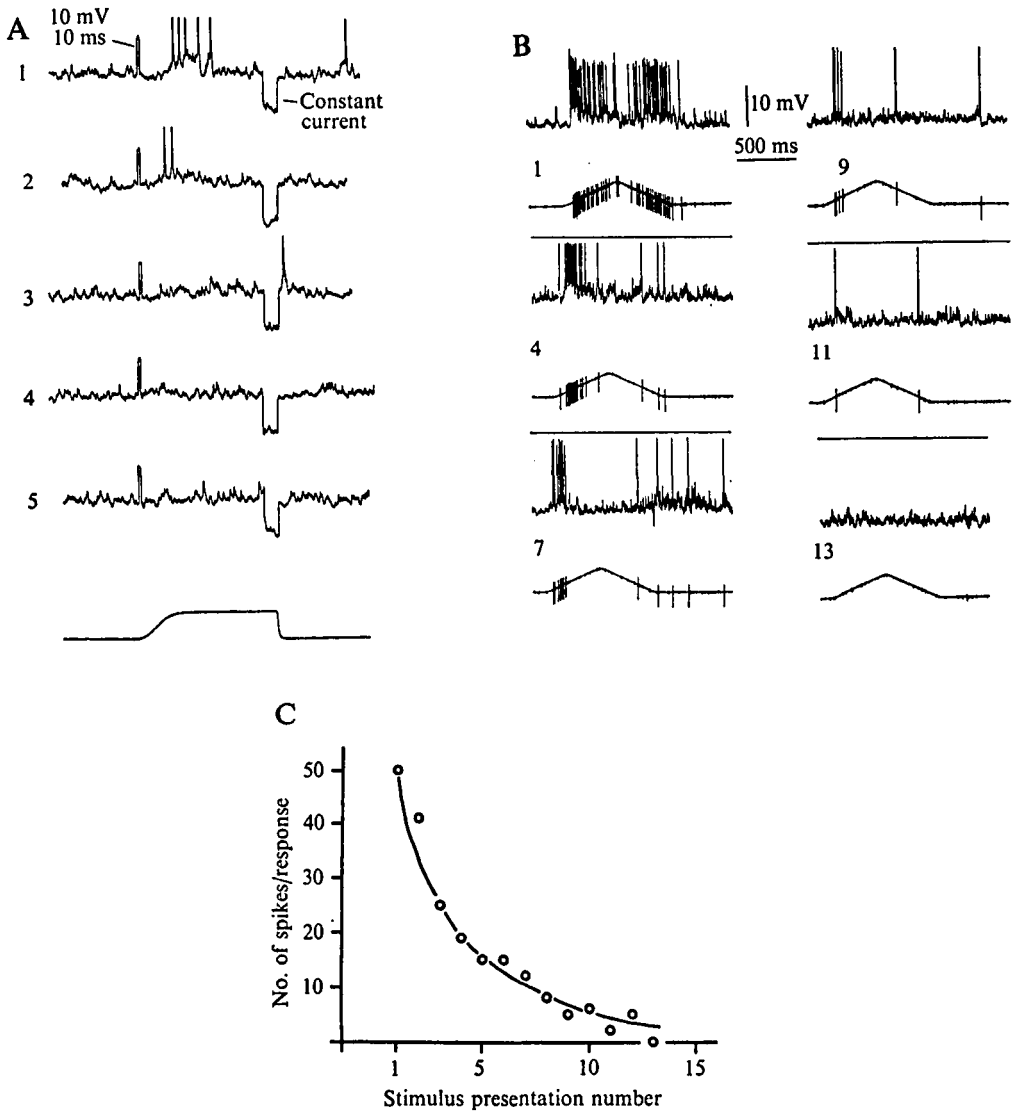
(ii) *Decrement*

The first possible mechanism of decrement which could operate prior to the point of convergence would involve a change in threshold of dendrite spikes. Such a mechanism would imply that the fan membrane is spike-supporting and that there are multiple sites of dendrite spike initiation within the fan, each being excited by only small areas of the receptive field. Under these conditions, a local increase in dendrite spike threshold would produce the kind of site-specific decrement observed in the MD system. With this possibility in mind, we performed experiments to discover whether spike initiation takes place in the fan, and whether the threshold changes as a result of repeated stimulation. Such a change has been reported to occur during repetitive synaptic activation of a cell in the abdominal ganglion of *Aplysia* (Stephens, 1973*a*, *b*), where it is the main factor in the decrement of spikes of the post-synaptic cell.

Intracellular records from the LGMD indicate that the fan membrane does not support spikes. The maximum amplitude of spike which we recorded in the most proximal parts of the fan was around 35 mV, with no overshoot; the membrane potential was −78 mV on exit. More distal penetrations record smaller spikes. When the electrode is inserted in the neurone between the base of the fan and thicker part of the axon (identified above as the site of spike initiation), larger amplitude spikes are recorded, and when recordings are made from the thick axon itself (O'Shea & Rowell, 1975) large over-shooting action potentials are seen. In some of these experiments the site of the penetration was confirmed by using a cobalt electrode. Typically, the site of penetration is indicated by a slight leakage of the marker; an example of such a penetration can be seen in Fig. 2 of O'Shea & Williams (1974). In addition to this evidence, the delay between the spike recorded intracellularly in the fan and extracellularly from the DCMD in the thoracic nerve cord is invariable, and is independent of the area of the retina stimulated. This too indicates that there is a single site of spike initiation and that convergence is complete at this point.

As a final and direct test of the hypothesis of alteration of spiking threshold, we injected constant current pulses (fractionally above the cell's current threshold for spiking) into the LGMD fan. Small changes in threshold were then revealed by averaging the number of spikes elicited by many repetitions of the electrical stimulus before and after repeated visual stimulation (Table 1). Even when visual responsiveness was drastically reduced by prolonged visual stimulation, we could detect no difference in the response to injected threshold currents. There is therefore no evidence to suggest that decrement in the MD system involves an increase in spiking threshold in the LGMD.

Intracellular records made from the LGMD fan during repetitive stimulation of a small area of the retina show that the fall in the number of LGMD and DCMD spikes is accompanied by a diminution in amplitude of the compound EPSPs (Fig. 3). This diminution continues beyond the point at which the spikes fail in the LGMD, showing that decrement is not in any way a consequence of the cell's spike output (e.g. by recurrent inhibition). EPSP amplitude and spikes are restored by rest, by dishabituation, or by stimulating a new area of the retina. We are confident that out electrodes record all potentials arising within the fan, as the space constant is very large; EPSPs evoked by retinal stimulation are, thus, always recorded, regardless of either the



ɾ. 3. Decrementing EPSPs produced in LGMD by repetitive visual stimuli.

(A) Response recorded by an intracellular electrode within the LGMD fan during repetitive stimulation of the eye by dimming a small (5°) area of the visual field. The time course of the intensity change is shown in the bottom trace, where up indicates a decrease in intensity. The amplitude of the change was approximately 2 $\log_{10}$ units. The interstimulus interval was 5 s. The response consisted initially of a compound EPSP which generated several spikes. With repetition, the amplitude of the PSP and the number of spikes it elicited decreased rapidly. The records shown are from five consecutive stimuli. A short pulse of hyperpolarizing current was injected into the cell through the recording electrode after each stimulus, and produces a constant deflexion throughout the series; there is no indication of a marked change in membrane resistance which might account for the changed efficacy of the EPSP.

(B) Upper trace as in (A) above (recorded intracellularly from the LGMD fan) while the eye is stimulated by the to-and-fro movement of a 10° black disc in the visual field. The triangular waveform corresponding to the movement of the disc is shown on the lower trace, and superimposed on it is the extracellular record of DCMD spikes, recorded in the ventral nerve cord. The interstimulus interval was 5 s. Stimulus presentation number indicated beside each trace. The initially large compound EPSP decreases in amplitude with repetition, and the number of spikes it elicits decreases likewise.

(C) A plot of the number of spikes elicited versus stimulus presentation number derived from the same experiment as is illustrated by selected intracellular records in (B) above. This is a typical example of response decrement in this system under these conditions; compare with, for example, Horn & Rowell (1968). The curve through the points is drawn by eye.

Table 1. *Numbers of spikes elicited by marginally supra-threshold current injection into the LGMD fan, before and after prolonged visual stimulation sufficient to produce profound response decrement*

	Before habituation	After habituation
Mean no. of spikes	$1{\cdot}6 \pm 0{\cdot}49$	$1{\cdot}4 \pm 0{\cdot}88$
	$N = 15$	$N = 15$

Probability of difference between these means occurring by chance, $\geqslant 0{\cdot}1$ (t test)

precise location of the electrode within the fan or the area of the retina selected. This is to be expected in a cell in which the site of spike initiation is some 200 μm from the base of the fan. Thus there seems no doubt that spikes are initiated by these EPSPs and that the reduction in spike number during habituation is a consequence of the decrement in PSP amplitude. We will now examine the possible causes of EPSP decrement.

The EPSP seen in the fan could decrease either as a result of processes occurring at the afferent/LGMD synapse (pre- or postsynaptically), or, less simply, as the result of a more distally located process. (For example, decrement could occur at the preceding synapse, the resultant potential changes being conveyed to the afferent LGMD synapse by a non-spiking, repeater, unit which transmits graded potentials. An alternative model, in which the non-spiking neurone is replaced by a spiking repeater neurone, is eliminated by the observation that one can record in the LGMD a smooth, stepless decrement in EPSPs lasting only a few milliseconds – too short to be the result of a large burst of spikes presynaptically). To distinguish between these possibilities we would have liked to record both pre- and postsynaptically at the afferent/LGMD synapse, and determine by inspection whether presynaptic activity declined during habituation of the MD response. To date we have failed to do this, presumably because of the small size of the chiasma afferents, the great majority of which are less than 1 μm in diameter. Instead, we stimulated the presynaptic area through fine, paired electrodes and found that single shocks produced decrementing PSPs in the LGMD fan. This indicates that the decremental process is located subsequent to the point of stimulation. We therefore have to establish that point anatomically and determine the pathway which connects it to the LGMD.

The shortest latency PSPs (2·25 ms) which we were able to produce (Fig. 4) came when the stimulating electrodes were placed in the distal part of the second chiasma. (Attempts to reduce this latency by moving the electrodes nearer to the lobula merely caused the LGMD to fire directly, presumably because of its much larger area and hence lower threshold to extracellular current than the minute chiasma afferents). As the chiasma itself is devoid of synapses, this result shows that the decremental process is located in the outer face of the lobula. The simplest interpretation of the histological and physiological evidence would be that the chiasma afferents make direct synaptic connections with the LGMD fan. In this case, the pathway from the stimulating electrode would be monosynaptic. Is this compatible with the observed latency?

The chiasma units are approximately 500 μm long, and between 0·1 and 1 μm thick. If they are spike-conducting, as their length might suggest, their conduction velocity will vary as a function close to the square root of the diameter (e.g. Pumphrey &

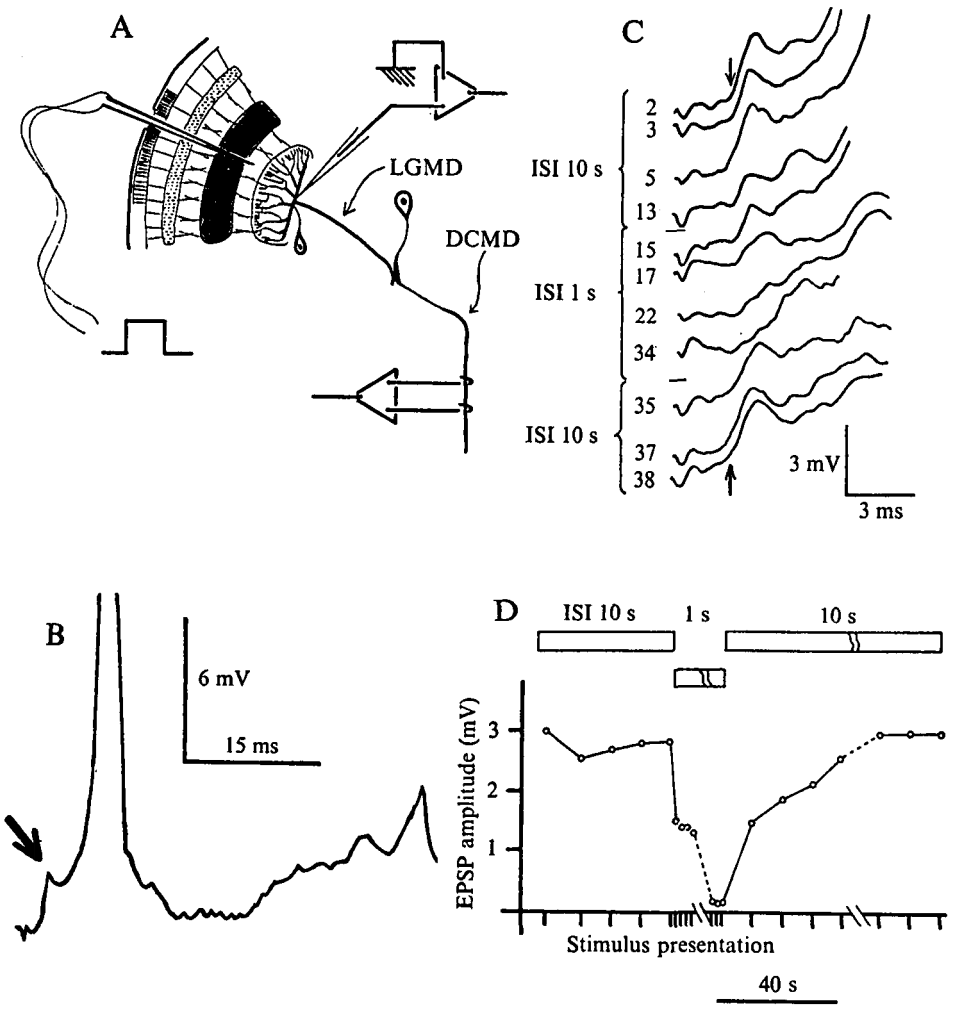


Fig. 4. Short-latency decrementing EPSP produced in the LGMD by focal electrical stimulation in the second optic chiasma.

(A) Diagram of experimental arrangement. An intracellular record is made from the input fan of the LGMD. Simultaneously, a pair of fine bipolar tungsten electrodes are introduced into the optic lobe through a hole in the retina. Their position in the lobe is monitored by measurement and subsequently confirmed histologically.

(B) Response to the first of a series of electric shocks to a site in the second chiasma. Compound EPSP with two major peaks, the first of which elicits spikes in the cell. Its first component (arrowed) has a particularly short latency.

(C) Examination of the early component, arrowed in (B), during repetitive stimulation. The negative inflexion at the start of each trace is a stimulus artifact. The latency of the first component is about 2·25 ms (arrows). It shows some decrement with repetition at an interstimulus interval (ISI) of 10 s and is rapidly eliminated by repetitive stimulation at an ISI of 1 s, after which it recovers when placed again on the slower stimulus regime. This decremental behaviour distinguishes it from the more resistant EPSPs illustrated in Fig. 5.

(D) Change in amplitude of the early component during the change-over in stimulus frequency from slow to fast and back to slow (ISIs of 10 s, 1 s, and 10 s respectively). Time scale below x axis, 40 s.

The divisions marked on the x axis represent stimuli.

Young, 1938; Hodgkin & Rushton, 1946; Burrows *et al.* (1965); Pearson, Stein & Malhotra (1970)). The 15 μm axons of LGMD and DCMD conduct at 3 m/s, and extrapolation suggests that the conduction time for the chiasma afferents would be 0·7–2·0 ms, depending on size. The EPSPs of the fan are apparently chemical in origin; they are augmented by hyperpolarization and have other properties unlikely in an electrical synapse. Transmission delay at a chemical synapse can be expected to be between 0·6 and 1·6 ms, according to our experience of other chemical synapses in locusts. The total latency expected for the postulated monosynaptic pathway is therefore between 1·3 and 3·6 ms. A disynaptic pathway would be approximately 1 ms slower ($\geqslant$ 0·6 ms synaptic delay + $\geqslant$ 0·1 ms axonal conduction delay). We conclude that any pathway with a latency of more than 3·5 ms is certainly polysynaptic, and that any less than 2·0 is certainly monosynaptic. In the intermediate range, 2·0–3·5 ms, the probability of a monosynaptic pathway is the greater the smaller the observed delay. Our minimal experimental values of 2·25 ms are thus not absolute proof of a monosynaptic pathway between the stimulating electrodes and the LGMD, but are strongly suggestive of it. They certainly preclude there being more than two chemical synapses; even they can only be accommodated by postulating the shortest delay synapses and the very thickest chiasma afferents.

If the pathway is monosynaptic, the site of decrement must be at the afferent/LGMD synapse. If the pathway is disynaptic, there is again a high probability that decrement is sited at that synapse, but also a possibility that it is located at the preceding synapse and transmitted via a graded potential to the afferent/LGMD synapse. There is no physiological or anatomical evidence to support this latter alternative, but it remains a possibility.

Making the assumption that decrement *is* associated with the afferent/LGMD synapse, the underlying process could be pre- or postsynaptic. One of the postsynaptic possibilities (a change in spiking threshold) has already been eliminated, and we can consider only those which would produce a decrementing EPSP. Incrementing recurrent inhibition has been eliminated (see above), and no IPSPs are associated with decrement, even in artificially depolarized cells, and decrement continues after spike output has ceased. Increase in membrane conductance consequent upon excitation, which would diminish the perceived size of the EPSP, cannot be demonstrated by direct measurement of potential change with injected current (see Fig. 3A). Further, if conductance changes were to account for decrement, they would have to be local, site-specific changes which do not interact; this is implausible, especially in a unit with so large a space-constant as the LGMD fan. A final telling piece of evidence against most postsynaptic mechanisms is the existence of a previously undescribed excitatory input which results in EPSPs in the fan which are resistant to decrement at all but the highest repetition rates (Fig. 5). These PSPs can be elicited by either whole-field visual stimulation or by electrical stimulation in the lobe, and have a shorter latency than the decrementing EPSPs associated with the response to small field stimuli. We will return to their function in a later paper. The only remaining candidate among postsynaptic mechanisms is that of receptor desensitization, and this we have not excluded. We currently favour the hypothesis that response decrement is due to a presynaptic process occurring in the excitatory terminals, and review the evidence in the Discussion.

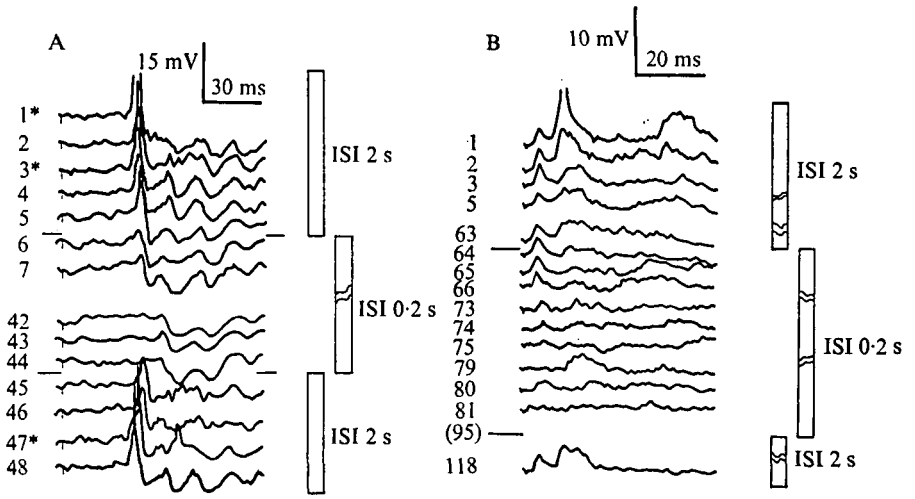


Fig. 5. EPSPs in the LGMD resistant to decrement, produced by visual or electrical stimuli.
(A) Intracellular recording from the LGMD fan. The small negative-going artifact at the start of each sweep represents a whole-field flash to the eye (electronic flash). This stimulus elicits an initial compound EPSP in the LGMD fan, and a subsequent compound IPSP. The IPSP will be discussed in detail in a further paper of this series, and is a characteristic of the response to large field stimuli. The EPSP shows little decrement at an ISI of 2 s (compare with the rapidly decrementing response to small-field stimuli at ISI of 5 s. Fig. 4). When the presentation rate is increased tenfold (ISI of 0·2 s) the EPSP is eliminated, but is rapidly regained within one or two trials at the old rate (ISI 2·0 s). Asterisks indicate spike responses.
(B) Intracellular recording from the LGMD fan. At the start of each sweep a small area of the lamina is stimulated electrically through a pair of fine tungsten electrodes, similar to the arrangement for stimulating the second chiasma shown in Fig. 3. Stimulus intensity is adjusted so as to elicit a single spike on the first presentation. The response consists of several excitatory components. The second of these, which gives rise to the first spike, shows a rapid decrement to a plateau level at an ISI of 2 s. The first component shows no decrement at this ISI, but can be reduced by a long train of stimuli at ten times the initial rate (i.e. ISI = 0·2 s). When the ISI is lengthened once again, both responses recover.

(B) *The nature of the excitatory afferents to the LGMD*

The LGMD is an ON/OFF unit (Palka, 1967*a*; Horn & Rowell, 1968; Rowell & O'Shea, 1976) and it seems clear this results from the convergence of separate ON and OFF afferent channels, both in sufficient density to cover the entire retina (see Introduction). The information on decremental processes given above allows us to begin to decide where this convergence occurs.

One possibility is that the convergence takes place on the LGMD fan; that is, there are two retinal projections to the LGMD, one of OFF units and one of ON units. The alternative hypothesis is that the convergence takes place at some earlier stage, and that the LGMD receives only one, ON/OFF, retinotopic excitatory projection. It is now possible to decide between these hypotheses on the basis of the response of the system to habituating stimuli, given that the afferent synapses to the LGMD fan, or an immediately preceding synapse in the lobula, is the site of decrement (see Fig. 7). There would be a generalization of habituation between ON and OFF channels if the projection were of a single class of ON/OFF afferents, but not if the ON and the OFF channels each projected via separate synapses onto the LGMD. In the latter case there would be no interaction, and stimulating an area of the retina with say ON

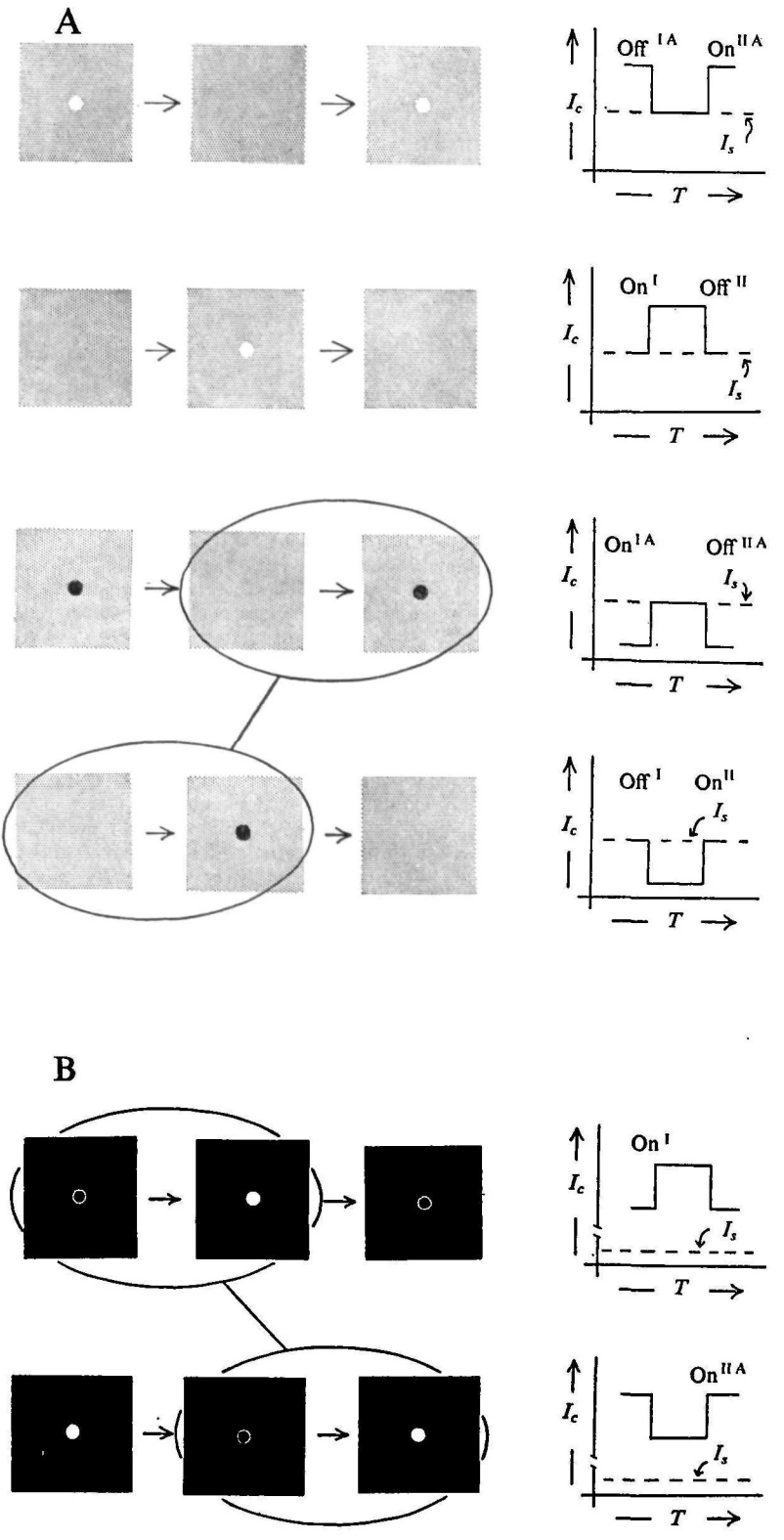


Fig. 6. For legend see opposite.

stimuli until the response habituated would not affect the response of that same area to OFF stimuli.

To demonstrate generalization of habituation, our basic technique was to change the illumination of a small (5°) portion of the retina in a step-wise manner for 2 s, and then return it to the original level. In this way we produced either 'brightening pulses', in which there was a local increase in illumination, or 'dimming pulses', in which there was a decrease in illumination. We recorded the phasic response of the MD system (extracellular DCMD spikes, recorded in the pro/mesothoracic connective) to both the ON and OFF transients of these pulses. Thus a 'brightening pulse' would evoke an initial ON response (ON^{I}), followed by a subsequent OFF response (OFF^{II}), and a 'dimming pulse' an initial OFF response (OFF^{I}) and a subsequent ON (ON^{II}), response. Fig. 6 illustrates this terminology. In principle, it should be possible to compare initial and subsequent ON or OFF responses, and demonstrate whether or not the subsequent response is the smaller; if so, this would imply that responsiveness was reduced as a result of the preceding, and different, initial response. In practice, the existence of a tonic lateral inhibitory network peripherally in the visual pathway (see the subsequent paper, Rowell & O'Shea, in preparation) complicates the experimental design, as only responses which share identical centre/surround relations can be compared legitimately. Thus, in the example referred to above, it is invalid to compare OFF^{I} and OFF^{II}, as the OFF^{I} response is generated by elements which are subject to moderate inhibition from the surround, whereas OFF^{II} responses are generated by elements which are inhibiting the surround and are thus themselves effectively disinhibited. To circumvent this problem, matched pairs of stimuli with identical centre/surrounds must be selected, as illustrated in Fig. 6 (further treatment of this will be provided in our forthcoming paper on lateral inhibition). The hypothesis, that the labile synapse is fed by an ON/OFF afferent (and that there is generalization of habituation between ON and OFF stimuli) then predicts that the ratios OFF^{II}/ON^{IA}, ON^{IIA}/ON^{I}, OFF^{IIA}/OFF^{I}, and ON^{II}/ON^{IA} will all be less than unity. In these ratios, both the numerator and denominator responses share identical centre/surround

Fig. 6. Generalization of habituation between ON and OFF responses. The principle is to compare, say, an OFF response, with an identical response evoked shortly after an ON response. If the latter is smaller than the former, it indicates that the ON and OFF responses share a final common pathway at or before the site of response decrement.

The response to a change in light intensity over a small area is to a large degree determined by the relative intensity of illumination of the area surrounding the test area due to the existence of a tonic lateral inhibition network. To compare responses it is, therefore, necessary that they share identical 'centre/surround' conditions. This can be achieved by the experimental arrangement summarized in (A); from the eight responses elicited by this experimental design, four pairs can be selected which are affected by lateral inhibition. One such pair is indicated on each diagram, and the others are listed in the text and in Table 2.

In (B). a single example of a modified experimental arrangement is shown, in which the surround intensity is at all times much darker than that of the test area. By this means, the inhibitory effect of the surround is reduced, and the weakest responses to illumination changes of the test area are appreciably increased. This allows a better test of the hypothesis of generalization of habituation. It was a design of this sort which produced the numbers given in Table 2.

In all these experiments the various steps in illumination are of one $\log_{10}$ unit. In the experiments figured in (B) surround intensity was 1 $\log_{10}$ unit below the lowest value of the test area. The small graphs plot the intensity of illumination of the test area (I_c) against time (T), and also indicate the illumination level of the surround area (I_s).

Table 2. *Generalization of habituation between* ON *and* OFF *responses*

(For terminology and discussion, see text and Fig. 6. The principle is to compare OFF responses with ones which were preceded 2 s earlier by an ON response and, similarly, to compare ON responses with those preceded by an OFF response. If there is significant response decrement due to the earlier response, then the ratio of the two ON or OFF responses will be significantly different from unity. Two different ratios are provided for each class of response, to compensate for differing lateral inhibition conditions generated by the test procedure, as explained in the text. The data are derived from three different animals, each contributing ten measurements to each value given below.)

Mean numbers of spikes/response.

Stimulus ...	ON^{I}	OFF^{II}	OFF^{I}	ON^{II}	OFF^{IA}	ON^{IIA}	ON^{IA}	OFF^{IIA}
$\bar{x}$	6·1	7·6	10·1	3·1	13·7	4·2	3·8	5·3
S.D.	2·7	4·5	3·7	1·7	4·1	2·4	1·7	2·9

$N = 30$ throughout.

Ratios between means

$\frac{OFF^{II}}{OFF^{IA}}$	$\frac{OFF^{IIA}}{OFF^{I}}$	$\frac{ON^{II}}{ON^{IA}}$	$\frac{ON^{IIA}}{ON^{I}}$
0·55	0·52	0·81	0·69

Significance of difference between numerator and denominator in above ratios:

$P = < 0{\cdot}001$	$< 0{\cdot}001$	*ca.* 0·1	$< 0{\cdot}01$

The lack of significance of the ratio ON^{II}/ON^{IA} is attributed to the low level of response obtained in both these conditions: for further discussion, see text.

relations, and are subject to the same degree of lateral inhibition. In the first experiments performed, all four ratios were indeed less than unity, but only the two OFF ratios were significantly so. The ON responses of the MD system are, however, always less than the OFF responses (Rowell & O'Shea, 1976), and response decrement cannot be demonstrated if the evoked test response is too small. In a second series, we therefore lowered the surround intensity so that it was always at least 1 $\log_{10}$ unit below the minimum test area intensity (Fig. 6B); this reduces the effect of surround inhibition on all responses, and increases the number of spikes that each evokes. Under these conditions, the results (Table 2) confirm the predictions generated by the hypothesis, (i.e. an ON response reduces the response to an immediately following OFF response, and vice versa). This result demonstrates that convergence of ON and OFF channels takes place prior to the labile synapse, and indicates that the LGMD fan is fed by a retinotopic projection of ON/OFF excitatory afferents.

DISCUSSION

We have shown that the LGMD fan is the site of convergence of the retinotopic projection in the MD system, that the excitatory afferents to the fan are ON/OFF units, and that the decremental process responsible for habituation and novelty preference in the MD system is located in or between ON/OFF units in the lobula. We have also presented strong, but not conclusive, evidence that the chiasma afferents synapse directly on to the LGMD. In this case these afferents are themselves ON/OFF units and the decremental process is localized at the afferent/LGMD synapse, which suggests that the decremental process is located presynaptically. Two uncertainties remain: first, whether or not the chiasma afferents synapse directly with the fan

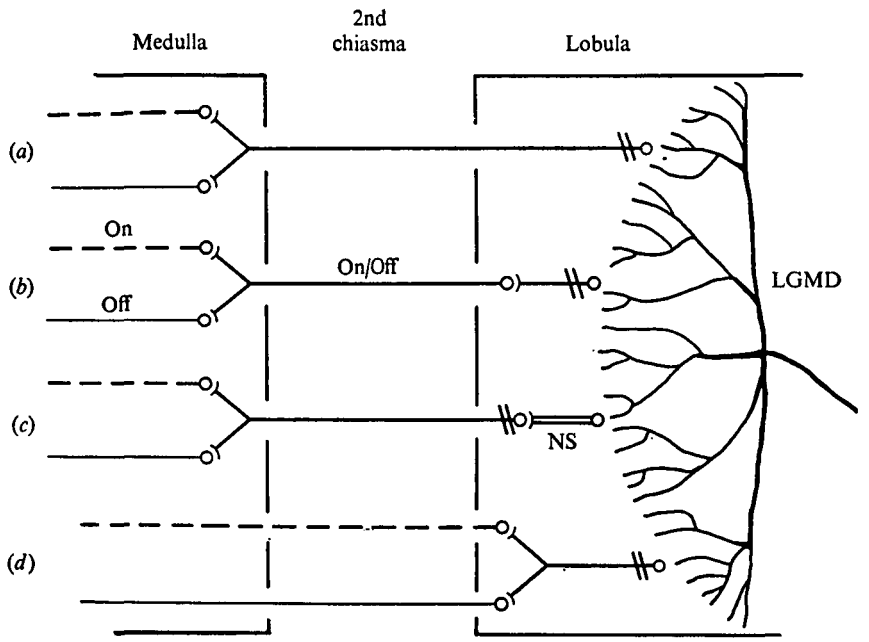


Fig. 7. Diagram showing the four arrangements of neurones which are consistent with the present results on the retinotopic projection to the LGMD fan. All synapses are excitatory and chemical; those preceded by oblique cross-hatchings are subject to response decrement. Heavy continuous lines indicate ON/OFF units, light continuous lines represent OFF units, and heavy dashed lines represent ON units. The unit drawn with a double line is a non-spiking ON/OFF unit. No units are known to traverse the medulla from its distal to its proximal face without a synapse (Strausfeld, 1976, and personal communications), and separate ON and OFF units are known to exist at the proximal face of the medulla (Rowell, O'Shea & Williams, in preparation). Further details of the diagrams are dictated by the results reported in the text. Of the various alternatives shown, (*a*) and (*d*) are respectively the most likely and next most likely to occur.

of the LGMD and, secondly, whether decrement at that synapse could be other than a presynaptic process. These possibilities will be discussed and considered with two other matters: the significance of the site of response decrement, and the origin of the previously described sensitivity gradient in the receptive field of the MD neurones.

(*a*) *The afferent pathway to the LGMD*

The observed latencies are just sufficient to accommodate two chemical synapses in the pathway between the chiasma afferents and the LGMD, assuming the most rapidly transmitting synapses known in insects and the thickest (and thus fastest) of chiasma afferent axons. On a purely probabilistic argument, this alone suggests a monosynaptic pathway, but the alternative possibilities for a disynaptic pathway are shown in diagrammatic form in Fig. 7. Perhaps the most striking aspect of the alternatives is their relative complexity and, the cumulative evidence of parsimony in the construction of arthropod nervous systems inclines us to favour the monosynaptic connexion. The parsimony argument must, of course, be used with caution, until the exigencies of the rest of the visual system are known. For example, Kien (1974*a*, *b*) has produced a model circuit to explain the optokinetic lobula cells of *Schistocerca*

which requires separate ON and OFF projections to the lobula. In the absence of electrophysiological recording from chiasmatic units or of further anatomical study of the inputs to the LGMD fan, these alternatives must all be considered as compatible with the present results. It will be noticed that of the four circuits in Fig. 7, only one places the site of decrement other than at the afferent/LGMD synapse, and involves the rather unlikely postulate of a non-spiking follower interneurone of no known function. The other three models all require that decrement be at the afferent/LGMD synapse.

(*b*) *Pre- or postsynaptic decremental processes*

It has not so far been possible to record from the units presynaptic to the fan, and the negative results of tests for postsynaptic correlates of decrement are, by themselves, inconclusive. There may, for example, be local conductance changes which are too small or too remote to be recorded (see Zucker, 1972*b*). We were also unable to test directly the possibility that decreased sensitivity of receptors of the postsynaptic membrane could cause decrement. However, Rall & Rinzel (1973) have suggested that synaptic potency could be adjusted in a site-specific manner by altering the stem resistance of dendritic spines. Postsynaptic processes are known, therefore, which could account for decremental properties of the LGMD response and which we have not excluded. There is, fortunately, an earlier observation which makes it almost certain that presynaptic processes are responsible for the decrement.

Dishabituation of the MD system occurs in response to arousing stimuli and counteracts the initial decremental process. Unlike habituation, dishabituation is not site-specific. It affects all of the retina simultaneously, and it does not enhance the response of previously unstimulated areas of the retina above control levels (Rowell & Horn, 1968; Rowell, 1971*b*, *c*, 1974). During dishabituation, one sees a restoration of the EPSP amplitude which was lost during habituation (O'Shea & Rowell, in preparation). There is virtually no electrophysiological event in the fan which can be recorded during the dishabituation process; occasionally one sees a very small (< 1 mV) and slow hyperpolarization, presumably the result of a very distant input elsewhere on the neurone. Unless there is an unsuspected mechanism by which a neurone could reset the sensitivity of postsynaptic receptors at the synapse between two other neurones, this finding suggests that dishabituation is a presynaptic process. It is highly unlikely that a presynaptic process could operate in such a way as to compensate for a postsynaptic decrement, without incrementing the response to non-habituated areas of the retina (see Rowell, 1971*b*).

Direct pre- and postsynaptic recording in a central decrementing synapse has been possible so far in only a single preparation in which it is shown that presynaptic events are responsible for the decline (Model, Highstein & Bennett, 1975; Highstein & Bennett, 1975). Less direct methods applied to other synapses have usually produced the same conclusion (Zucker, 1972*b*; Castelucci & Kandel, 1974), although occasionally postsynaptic processes have been implicated, such as receptor desensitization (Wachtel & Kandel, 1971) or a rise in threshold of spike initiation (Stephens, 1973*a*, *b*). The more common presynaptic events include both a decrease in the number of quanta released by the presynaptic terminal, and, in the first example, a decrease in quantal content. As yet we cannot determine what presynaptic process

underlies response decrement in the MD system, as our preparation does not allow us adequately to examine the statistical nature of synaptic transmission. This is basically a function of the large size of the unit and the very large number of afferents it receives (the retinotopic projection, for example, probably contains one axon corresponding to each of 9000 ommatidia). As a consequence, there is very considerable synaptic noise, the contribution of an individual unit is unrecognizable and only compound EPSPs are usable. The statistical techniques for testing the applicability of a particular distribution to the amplitudes of such PSPs are highly unreliable and fraught with untestable assumptions (Zucker, 1972*b*).

(*c*) *Significance of the site of response decrement*

There are few examples in which the locus of behaviourally significant response decrement has been established (cf. Krasne, 1976). In the best known of these, for example, the crayfish tail-flip circuit (Zucker, 1972*a*, *b*), the withdrawal of the gill complex in *Aplysia* (Castellucci *et al.* 1970) and the response of crickets and cockroaches to mechanical stimuli to the cerci (Callec *et al.* 1971; Zilber-Gachelin & Chartier, 1973; Murphy & Matsumoto, 1976) all or part of the decremental process has been localized at the primary afferent synapses. There has been a tendency to generalize from these results, and to suggest that the primary afferent synapse is the logical site for the process. In the MD system, however, this synapse is not the site of decrement. It seems probable that in any particular instance the site of decrement will be determined by at least two factors: (*a*) the amount of sensory processing required to abstract significant information from the receptors and (*b*) the number of independent systems to which the receptors contribute. Both these qualities are likely to be at their lowest in the examples cited above, which are initiated by phasic mechanoreceptors of little other function. By contrast, in the visual system the retinal cell output is processed and abstracted over many synaptic levels. The same initial output is differentiated for many other purposes apart from supplying the MD system: form, movement, direction, spatial relationships are preserved in some and not in others. Each point on the retina is represented in the second chiasma by possibly as many as 50 different units in the fly (Strausfeld, 1976), which stresses the multiplicity of uses to which visual information is put by insects. The primary afferent synapse is clearly an unsuitable place for decrement in a sensory system serving many different functions, some of which show no decrement. The lobula contains many other fan-shaped cells of the same general form as the LGMD, at least some of which are known to be the outputs of other forms of visual information derived from the retina. Not all of these systems habituate. For example, those fan cells which are sensitive to directional movement of large fields and drive optomotor or optokinetic systems show no decrement (Kien, personal communication, in *Schistocerca*; Dvorak, Bishop & Eckert (1975), Hausen personal communication, in dipterous flies).

(*d*) *The origin of the sensitivity gradient of the MD neurones*

The MD system does not respond equally to identical stimuli at all points on the retina. Instead, there is a rather complex gradient of sensitivity in which responsiveness is greatest to stimuli presented to the centre of the eye and declines most rapidly at the extreme edges of the visual field (Palka, 1967*a*; Rowell 1971*a*). In addition, there

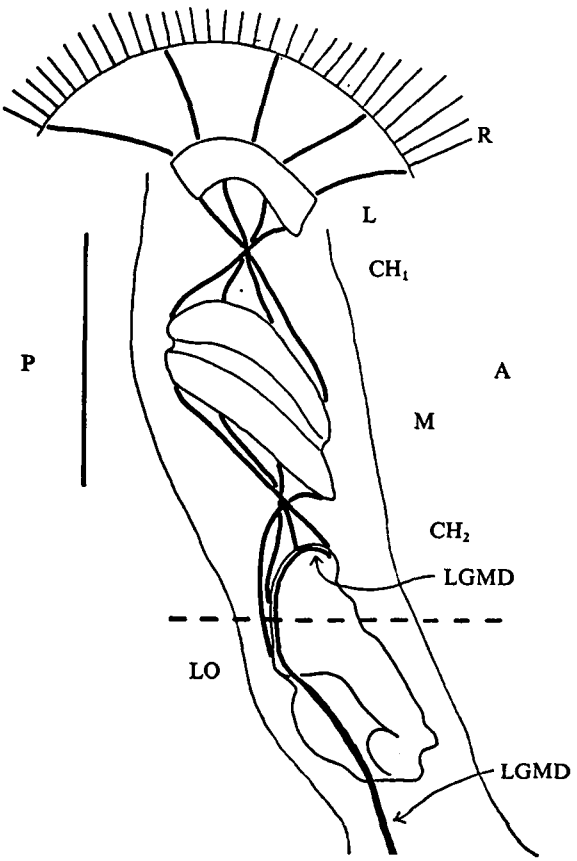


Fig. 8. A schematic diagram of the optic lobe of *Schistocerca*, based on a horizontal section The heavy lines represent the course of the lobular giant movement detector neurone in the lobula, and a few of the many axons of the retinotopic projection which runs from retinula cells to lamina, lamina to medulla via the first optic chiasma, and medulla to lobula via the second optic chiasma. Note that the anterior edge of the retina projects ultimately to the extreme distal part of the LGMD fan, and the posterior edge to the most proximal part of the fan. The dotted line represents the approximate level of the parasagittal section shown in Fig. 1. R, Retina; L, lamina; M, medulla; LO, lobula; CH_1 and CH_2, first and second chiasmata; LGMD, lobular giant movement detector neurone. Scale, 0·5 mm. A, anterior; P, posterior.

is a gradient in the horizontal direction such that anterior parts of the retina are less stimulating than posterior ones. Such variation in sensitivity could be produced by varying the properties of the various components of the retinotopic projection, but we would suggest that it is possible to produce it without such differences. The shape of the LGMD fan and its relationship to the site of spike initiation suggests a mechanism which might underlie gradients of sensitivity both in the LGMD and in other interneurones with wide receptive fields. Identical units synapsing with the fan will have a relative potency dependent on their proximity to the zone of spike initiation and the internal geometry of the fan. The retinotopic projection in the locust, as in other insects, is twice laterally reversed in the two chiasmata (Fig. 8), which results in the retina being mapped on to the fan in the following way. The anterior parts of the retina map on to the most distal parts of the fan dendrites, the posterior retina maps on to the

proximal parts of the dendrites, and the dorso-ventral retinal axis maps dorso-ventrally on to the fan. This organization predicts a symmetrical, dorso-ventral, gradient of sensitivity (i.e. most sensitive in the centre and least sensitive at the dorso-ventral periphery of the visual field) and another which increases from the anterior to the posterior margin of the visual field. The prediction is more or less fulfilled by the measurements described above. However, a major discrepancy exists. At the extreme posterior margin of the visual field responsiveness falls markedly. This fall-off is not obviously due to a sudden increase in retinal curvature in this area, which might result in fewer ommatidia scanning a given area; indeed, almost the opposite is the case, as can be seen in plate 26(*a*) of Burtt (1967). If there is an effect which is related to optical curvature, it would be expected to reinforce the posterior–anterior gradient already attributed to the fan, not to oppose it. It may be that the very thick proximal segments of the fan, to which these posterior regions project have a reduced input impedance, or that there is heterogeneity among the small-field afferent units. It seems likely that most or all of the sensitivity gradient in the receptive field of the MD neurones is determined by the LGMD rather than by differences between otherwise similar afferents, as these are performing other visual functions in which a sensitivity gradient might well be inappropriate.

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