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The Anatomy and Output Connection of a Locust Visual Interneurone; the Lobular Giant Movement Detector (LGMD) Neurone

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Summary. 1. The anatomy of a giant movement detector neurone in the locust lobula (the LGMD) is described on the basis of both intracellular injection of cobalt (Fig. 2) and the reconstruction of osmium-ethyl gallate and silver impregnated serial sections (Fig. 3).

2. It is shown that the LGMD has an anatomically complex junction with a previously described interneurone, the Descending Contralateral Movement Detector (Fig. 4), and that spikes in the LGMD precede 1:1 with fixed latency spikes in the DCMD (Figs. 1, 5).

3. Three separate dendritic subfields are seen in the lobula complex (Figs. 2, 3); these are tentatively ascribed to the three different classes of input to the cell.

4. A large part of the LGMD's terminal arborisation appears to serve only a single functional junction, that with the DCMD (Fig. 4).

Introduction

The abrupt movement of small objects in the visual field can initiate jumping or escape behaviour in the locust. The output connections made by giant visual interneurones, the descending contralateral movement detector (DCMD) and its ipsilateral homologue the DIMD, with metathoracic motoneurones known to be involved in jumping, were recently described (Burrows and Rowell, 1973). The anatomy of the DCMD, which has also been described (O'Shea, Rowell and Williams, 1974), can be accounted for in the metathoracic ganglion by its known connections with motoneurones involved in jumping.

Despite the extensive literature on the DCMD (Rowell, 1971a, b; Palka, 1972; Williams, 1972; Burrows and Rowell, 1973; see Rowell, 1971c for a review of earlier papers), the nature of its input from the optic lobe remained unresolved. It is possible to record with an extracellular tungsten electrode in the optic peduncle a conspicuous action potential, which precedes 1:1 the axon spike of the DCMD as recorded in the contralateral thoracic nerve cord (Northrop, pers. comm., and

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unpublished observations). Our anatomical description of the DCMD shows that its arborisation in the brain is confined to the protocerebrum. The ease of extracellular recording suggests, therefore, that the optic lobe spike derives from a relatively large axon belonging to a unit presynaptic to the DCMD. Subsequent work, reported here, confirms that conclusion and offers a description of the cell based on histological and electrophysiological techniques. In this and subsequent papers the neurone will be called the Lobular Giant Movement Detector (LGMD).

The DCMD, the DIMD and the LGMD are members of bilaterally symmetrical pairs of neurones.

Materials and Methods

Histological and electrophysiological techniques were used in the identification of the LGMD as the major presynaptic unit of the DCMD. The reconstruction of serial sections of stained material revealed a conspicuous neurone running from the lobula complex to the integrating segment of the ipsilateral DCMD. The subsequent penetration of that unit with an intracellular microelectrode while recording from the DCMD, and the injection of cobalt into it, confirmed the identification of the cell as the one which initiates spikes in the DCMD.

Adult specimens of two closely related species of locust, *Schistocerca vaga* and *Schistocerca gregaria*, were used in the study. No differences in the anatomy of either the DCMD or the LGMD, other than those associated with size variation among individuals, have been found between these species or between sexes.

a) Histological Techniques

The brains of both male and female adult locusts were prepared for light microscopy using Wiggleworth's osmium tetroxide-ethyl gallate method (Wigglesworth, 1957, 1959 and 1960) and the Chen modification of Bodian's protargol method (Chen and Chen, 1969). The former method was modified by fixing in phosphate-buffered 6.5% glutaraldehyde (pH 7.2) prior to osmication. All tissue was double embedded in celloidin and paraffin wax (Steedman, 1960). Horizontal, transverse and longitudinal consecutive series of sections were cut at 7 μm (Wigglesworth) and 15 μm (Bodian).

A Wild M20 microscope fitted with a drawing tube was used to make eight reconstructions of the LGMD and its relationship to the DCMD. Drawings were made at magnifications of 350 and 750X and were photographically reduced to a convenient size.

b) Electrophysiological Identification and Microelectrode Injection of Cobalt

A locust with mouthparts, legs and gut removed was mounted on a wax block, dorsal surface uppermost with the head rotated anteriorly, and firmly restrained with insect pins. The brain was exposed posteriorly and a solid glass platform was passed under the optic lobe; by raising the platform the optic lobe was supported and lightly stretched. Microelectrodes were filled with either 3 M potassium acetate or 1.5 M cobaltous chloride, and had resistances between 30 and 50 Megohms. Penetrations were made in the lobula and optic peduncle without removal of the neural lamella. A bipolar silver wire electrode was placed around the contra-

lateral thoracic nerve cord and connected to a differential preamplifier. This served to record the extracellular activity of the DCMD axon, which is the most conspicuous and easily identifiable unit recorded in this way. In some experiments extracellular stimulating electrodes were placed on the same nerve cord.

Impaled cells were injected with cobalt by passing depolarising pulses of 10^{-8} A and 500 ms duration at one per second through the microelectrode for as long as the intracellular penetration was maintained, usually no longer than half an hour. The cobalt ions were precipitated in the filled neurone by the application of a 0.05% solution of 44% ammonium sulphide in insect saline. The brain was removed from the head capsule, washed in saline, fixed in Carnoy's, dehydrated and cleared in methyl benzoate. This technique is essentially similar to that first described by Pitman, Tweedle and Cohen (1972).

For routine intracellular recording of the activity of the LGMD, microelectrodes were filled with 3 M potassium acetate. Action potentials were initiated in the cell by visual stimuli or by passing depolarising pulses of about 5×10^{-9} A and 0.2 ms duration through the microelectrode. Visual stimuli were provided by a disc mounted on a pen recorder carriage and driven through an arc of about 50° by a triangular waveform applied to the recorder. The disc subtended about 15° and its back-and-forward movement provided a potent stimulus for both the DCMD and the LGMD.

Results

a) Identification and Anatomy of LGMD

With a tungsten microelectrode placed either in the lobula or the optic peduncle, it is possible to record a unit with conspicuous extracellular impulses. Spikes in this unit precede 1:1, with constant latency, those monitored simultaneously in the DCMD soma and from the DCMD axon in the contralateral thoracic nerve cord (Fig. 1). This unit was penetrated with a cobalt-filled microelectrode by probing in the appropriate area of the lobula suggested by extracellular recordings. The injection of cobalt into it has shown that its spikes do not derive from a dendrite of the DCMD but that they belong to another neurone, which we call the LGMD. Such an injection is shown in Fig. 2, which is a stereo-pair photograph of the LGMD in *S. vaga*. Fig. 3 shows the same cell in *S. gregaria* as reconstructed from serial sections of stained material.

The soma of the LGMD, which varies between 50 and 90 μm diameter depending on sex and fixation, lies ventrally in the optic peduncle. There are two major zones of arborisation, one in the lobula complex and the other in the lateral protocerebrum. An 18 μm diameter axon connects these zones. The dendrites of the DCMD integrating segment are closely associated with one another, and this is presumably the area of synaptic contact between the two cells (Fig. 4).

The arborisation of the LGMD in the lobula complex consists of three distinct subfields; each is associated with a specific region of the lobula and they have been designated subfields A, B and C (see Fig. 3). The terms used to describe the regions of the lobula complex are borro-

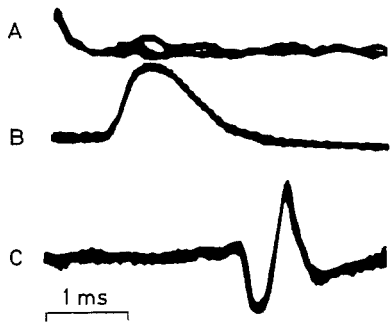

Fig. 1. Simultaneous recordings of the LGMD spike extracellularly from the optic peduncle (A), and of the DCMD spike as seen intracellularly from the soma (B) and extracellularly from the contralateral cervical connective (C). The oscilloscope is triggered from the LGMD action potential and about 30 sweeps are superimposed. Note that there is almost no variation in the latency between LGMD and DCMD spikes

wed and translated from Gouranton's description of the gross anatomy of that structure (Gouranton, 1964).

The largest subfield (A), both in terms of the number and size of its constituent elements, lies in the outer part of the "posterior capsule" of the lobula complex. It takes the form of an extensive, curved fan which lies in a plane approximately normal to the animal's longitudinal axis. The terminal branches of subfield A end abruptly at the distal surface of the posterior capsule and it is here that fibres from the whole of the second optic chiasma are received.

The second subfield (B) is also found in the posterior capsule, but a little anterior to the fan arborisation of A; it therefore appears in Fig. 2 to lie below A. The elements of subfield B do not have the obvious symmetry of A and they are thinner.

The third subfield (C) lies in the "dorsal lobe" of the lobula complex. It is less extensive than the others and appears in sectioned material to receive its input from an uncrossed bundle of fibres which run from the inner surface of the medulla, over the dorsal part of the posterior capsule, to the dorsal lobe of the lobula.

b) Output Connection

Intracellular recordings from the LGMD with potassium acetate-filled microelectrodes, show that it responds in the same way to visual stimuli as the DCMD (Fig. 5). Spikes in the LGMD can be initiated by intracellular stimulation with depolarising pulses of 5×10^{-9} A, and the DCMD axon spike follows them 1:1, with constant latency up to 320 Hz,

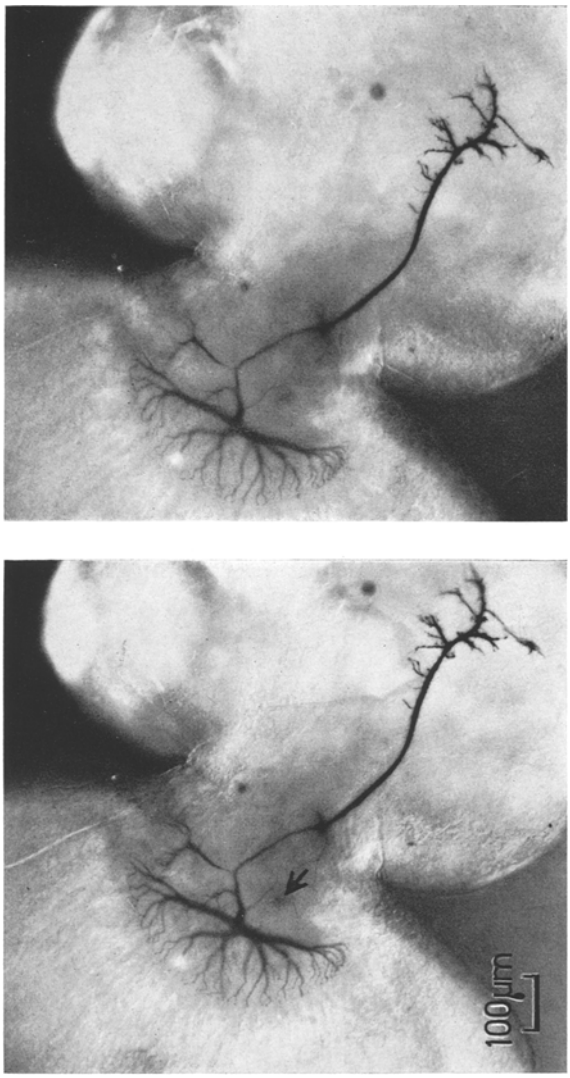

Fig. 2. The LGMD of *S. vaga* as revealed by the intracellular injection of cobalt. The soma is out in the plane of focus and its location is indicated by an arrow. Note the three zones of arborisation in the lobula and the extensive branching of the axon in the lateral protocerebrum. When viewed with stereo-viewers (65 mm separation), the fan-like arborisation (subfield A) in the lobula has a slight convex curvature and subfield B lies behind it; compare with Fig. 3. The site of injection can be seen in the optic peduncle where there is a small amount of leakage from the axon

which is the maximum frequency at which spikes can be driven by intracellular stimulation. When injured, the LGMD will spike at frequencies up to 400 Hz for several seconds and again the axon spikes of the DCMD follow them 1:1. The DCMD axon can be stimulated in the thoracic nerve cord and the antidromic spike recorded in the soma. It is not possible, however, to initiate action potentials in the LGMD by such stimulation nor by intracellular stimulation of the DCMD soma.

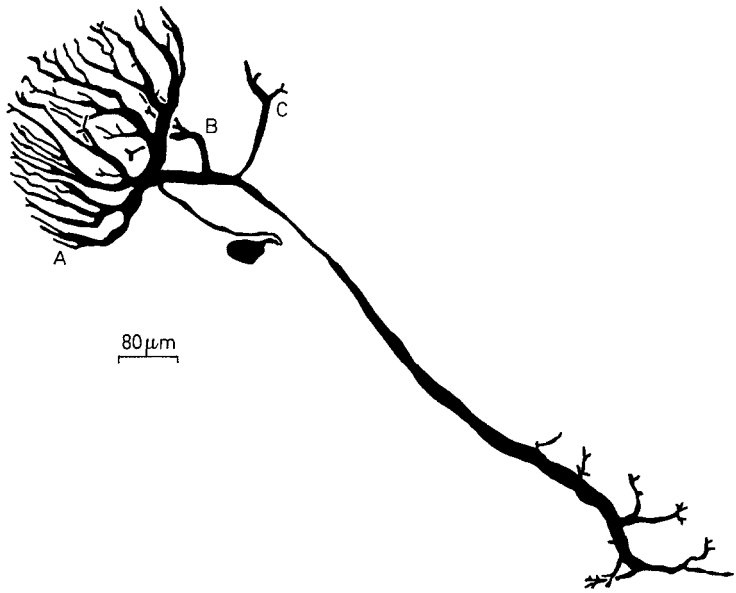

Fig. 3. A reconstruction from serial sections of the LGMD in *S. gregaria.* The three subfields of the cell's branching pattern in the lobula complex are marked A, B and C (see text for explanation and compare with Fig. 2)

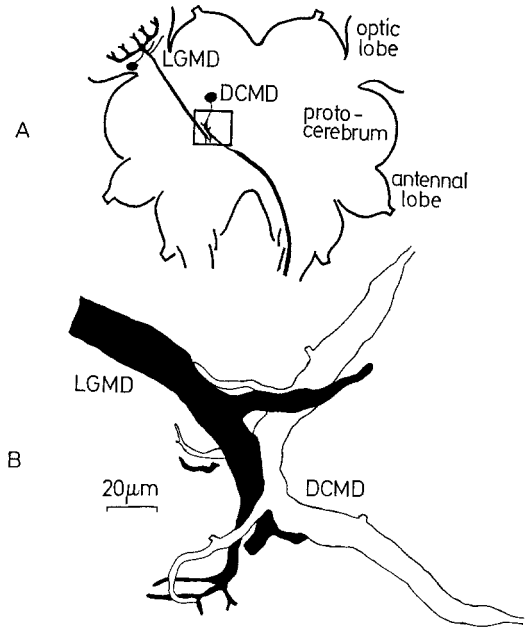

Fig. 4. Drawing A is a diagrammatic representation of the brain and both the LGMD and DCMD. The area indicated in the rectangle was reconstructed from serial sections at high magnification, and the close association between the LGMD and the integrating segment of the DCMD is revealed (B)

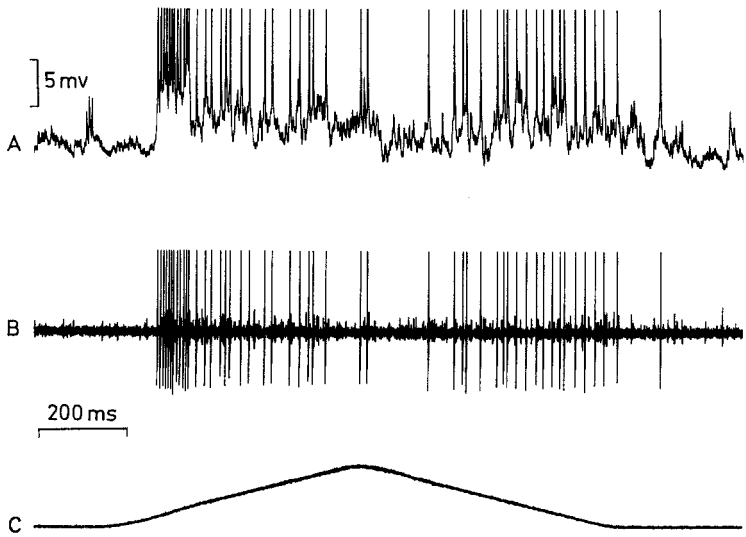

Fig. 5. Simultaneous recordings intracellularly from the LGMD in the lobula (A), and extracellularly from the DCMD in the contralateral thoracic nerve cord (B). The lower trace (C) is the output from a servomotor which drives a black disc subtending about 15° at the animal's eye, in a back-and-forward motion through an arc of 50°. The spikes in the LGMD precede 1:1 the DCMD axon spikes. Note that synaptic potentials are readily recordable in the LGMD

It is possible, by making the assumption that the conduction velocity of the LGMD axon is the same as that of the DCMD (they have similar diameters) and by measuring the distance between recording sites on the two cells, to estimate the synaptic delay at the LGMD-DCMD junction. Such calculations are subject to error but it seems likely that the delay is less than one-half a millisecond.

There is clearly a very reliable excitatory synapse between the LGMD and DCMD and the anatomy suggests that many of the LGMD dendrites in the protocerebrum are associated with the DCMD integrating segment (see Fig. 4). The protocerebral branching of the LGMD is, however, extensive and not all the dendrites can be accounted for by the LGMD's known connection with the DCMD. Nothing is yet known of the connections made by the LGMD with other neurones.

Discussion

a) Relationship between LGMD and DCMD Activity

The responses of the DCMD to visual stimuli have been extensively described from extracellular recordings and the cell must today be

regarded as one of the most thoroughly studied of interneurones. It is clear from the present study that spikes in the LGMD are normally transmitted 1:1 to the DCMD and our knowledge of the LGMD's response characteristics is therefore also extensive.

Both the DCMD and its ipsilateral homologue the DIMD respond to abrupt movement of small dark objects in the visual field. The DCMD is not excited by visual stimuli which affect the whole visual field; its activity is therefore stabilised against the animal's own movement. The response of the DCMD to repeated stimulation has been well documented. Rapid habituation occurs when the same part of the retinal array is repetitively stimulated (Horn and Rowell, 1968). Decrement of the response is limited to the stimulated area, and Rowell (1971a) has shown that complete recovery of the response to a moving target results from a 2° deflection of its axis. Recovery from habituation is prolonged, usually taking more than an hour. Rapid recovery, however, i.e., dishabituation, can occur in response to a great variety of sensory stimuli and to spontaneous changes in the animal's arousal state (Rowell, 1971a).

It is clear that the decrement observed in the response of the DCMD is a reflection of a process occurring at least one synapse peripheral to that made by the LGMD onto the DCMD. Further, the site-specific nature of habituation is evidence that decrement occurs prior to convergence of primary sensory input (Rowell, 1971a). Subfield A of the LGMD is the final site of convergence in the pathway to the DCMD and decrement must therefore be occurring more peripherally than it. Our results suggest, and we are hopeful that, the afferent synapses on to the LGMD are the primary sites of the lability observed in the response of the DCMD. Synaptic correlates of habituation can be recorded in the LGMD and the results of these experiments are being prepared for publication (O'Shea and Rowell, in prep.).

The estimated delay of <0.5 ms between the LGMD and DCMD spikes and the high following frequency of the DCMD suggests an electrical coupling between the cells. The electrophysiological evidence for the presence of an electrotonic synapse between the LGMD and DCMD, and the mechanism of spike initiation in the latter will be considered in detail in a later publication when the function of the DCMD integrating segment will be described.

b) Possible Afferent Connections of the LGMD

Considerable information processing is implicit in the response characteristics of the LGMD, and an extensive antecedent chain of interneurones between it and the retinula cells may be assumed. This conclusion is supported by its long minimum response latency (25 ms)

to small field stimulation. Further, the anatomy suggests that subfields A and C are connected to the more distal parts of the optic lobe by different pathways; subfield A receives input from the whole of the retinal projection via the second optic chiasma, and subfield C appears to receive its input from an uncrossed bundle of fibres which run from the inner surface of the medulla. The results of focal electrical stimulation in the retina and lamina show that there are two excitatory pathways onto the LGMD, one with a short latency of $\leq$10 ms and another with a latency similar to the neurone's response to movement (i.e., 20–25 ms), and also an inhibitory pathway. The afferent connections of the LGMD appear therefore to be complex; there are at least three classes of input each with different response characteristics and different decremental properties. As a working hypothesis we suggest that these inputs may be localised on the three dendritic subfields.

c) Functional Aspects of the Movement Detector System

We know of only one certain postsynaptic unit of the LGMD (the DCMD) and one other which is probably postsynaptic (the DIMD). At first sight, the complex morphology of the LGMD termination in the brain hints at many other unknown connections. Reconstruction of the junction between the LGMD and DCMD, however, shows that many of the LGMD terminals appear to be involved in this one junction, presumably to increase its safety factor. There are grounds for hoping that the actual connectivity may be much simpler than the anatomy, and the extensive arborisation of the LGMD in the protocerebrum may in fact have few postsynaptic connections.

The output connections made by the DCMD and its ipsilateral homologue, the DIMD, with metathoracic motoneurones are consistent with the hypothesis that they are concerned with jump initiation (Burrows and Rowell, 1973). Further, it is likely that the function of the DCMD is limited to escape; its anatomy in the metathoracic ganglion can be accounted for entirely by its known connections with motoneurones involved in jumping (O'Shea, Rowell and Williams, 1974). With the addition of the LGMD to the circuit, we now have a sequence of two bilaterally symmetrical pairs of interneurones, the anatomy and connectivity of which is well understood, which is excited by abrupt movement of small objects in the visual field and whose output is to a group of identified metathoracic motoneurones. It is our intention now to proceed with the analysis of the escape system to the more peripheral parts of the optic lobe in order eventually to construct a complete circuit.

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