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A CHEMICAL SYNAPSE BETWEEN TWO MOTION DETECTING NEURONES IN THE LOCUST BRAIN

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SUMMARY

1. The LGMD is the major source of visual input from the compound eye to the ipsilateral DCMD. Inactivating the LGMD or hyperpolarizing it, so it no longer spikes, abolishes the response of the DCMD to the visual stimulus.

2. Synaptic transmission between the LGMD and DCMD neurones is chemical. A spike in the LGMD terminals induces a postsynaptic potential in the DCMD dendrites, with a transmission delay of 1 ms. There is a conductance increase in the DCMD during an LGMD-mediated PSP. The conductance increase occurs at membrane potentials when the current/voltage relationship of the DCMD membrane is linear, and at several different membrane potentials.

3. The LGMD-mediated PSP within the dendritic region of the DCMD has a rise time of 1·3 ms, a half-time for decay of 2·2 ms and a total duration of 8·3 ms. In the cell body it has a rise time of 3·3 ms, a half-time for decay of 8 ms and a total duration of 21·3 ms.

4. The amplitude of the LGMD-mediated PSP depends on the membrane potential of the DCMD. The PSP amplitude is increased by membrane hyperpolarization and decreased by membrane depolarizations. At a membrane potential 30 mV more positive than resting potential the extrapolated size of the PSP is zero.

5. The synaptic efficiency of the LGMD–DCMD connection is usually 1·2.

$$\frac{\text{PSP size in DCMD dendrite at resting potential}}{\text{Spike size in LGMD terminals}} = \frac{18\,\text{mV}}{15\,\text{mV}}.$$

6. There is a threshold of 13 mV in the LGMD before synaptic transmission occurs. Currents less than 13 mV are not transmitted in either direction across the synapse although they do reach the synaptic region if they are injected at the extremities of the neurones within the brain.

7. Length constants for the LGMD are 0·36 mm between points *c* and *d* (see Fig. 4) in the protocerebrum and 0·63 mm between point *b* in the optic lobe and point *d* in the protocerebrum. The length constant measured between the dendrite region of the DCMD and its cell body is 1·34 mm.

8. DCMD spikes and PSPs follow spikes in the LGMD at a constant latency at frequencies up to 400 Hz. Usually a spike in the LGMD induces a spike in the DCMD.

Key words: Chemical synapse, brain neurones, motion detector, locust.

INTRODUCTION

In the locust two bilateral pairs of large movement detecting neurones have been identified. They are excited by small, novel stimuli moving within the visual field of one eye (Rowell, 1971*a*,*b*,*c*; O'Shea & Williams, 1974). One, the lobula giant movement detector (LGMD) has both its fan-like dendritic arborization and its cell body in the lobula behind the eye through which it is excited. The LGMD axon projects into the protocerebrum of the brain, where its branches abut on processes of the second neurone, the descending contralateral movement detector (DCMD). The DCMD has its cell body and dendritic arborizations on the side of the brain ipsilateral to the LGMD but its axon crosses to the contralateral side before projecting down the connective (O'Shea, Rowell & Williams, 1973). The following observations led O'Shea & Rowell (1975) to conclude that there was an electrical synapse between these two neurones, though this conclusion was not tested by measuring current flow between them directly. The DCMD follows spikes in the LGMD up to very high frequencies (320 Hz); it follows even when the brain is bathed in low Ca^{2+} saline or cooled with 8 °C saline. Also following an antidromically-induced spike in the DCMD a small depolarization occurs in the LGMD. Both the neurones and the connection between them are particularly interesting as they are involved in feature abstraction from the animal's visual world, and they show both lability of response and habituation. The output connections of the DCMD in the thorax are known in some detail (Burrows & Rowell, 1973; Simmons, 1980; Pearson, Heitler & Steeves, 1980; Steeves & Pearson, 1982), allowing a functional interpretation for the observed properties of the LGMD–DCMD synapse.

In this paper I show by direct evidence that transmission at the LGMD–DCMD synapse is mediated chemically and that in normal circumstances a spike is necessary in the presynaptic neurone for sufficient transmitter to be released to cause an EPSP in the postsynaptic DCMD. Transmission does not decrement at high spike frequencies and an LGMD-mediated excitatory post synaptic potential (EPSP) in the DCMD normally causes the DCMD to spike.

MATERIALS AND METHODS

Experiments were performed on male and female *Locusta migratoria* taken from our culture 10–25 days after their final moult. The locust's mandibles were removed, the wounds sealed with wax, and the insect was then mounted upright on a Plasticene block. An incision was made along the dorsal midline from the first abdominal segment to the anterior of the pronotum, the wings were pulled laterally and the gut removed. The brain was exposed by removing the top of the head capsule and, following removal of the overlying jaw muscles and oesophagus, the brain was supported by a plastic-coated stainless steel platform inserted frontally. Saline (Eibl, 1978) at room temperature (21–24 °C) was frequently washed over the preparations. The tracheal supply to the brain was not disrupted during the dissection and air was circulated by the animal's abdominal pumping movements.

Glass capillary microelectrodes filled with 3 M potassium acetate were used to record intracellular potentials from, and to inject current into, the two visu

neurones. The electrodes had d.c. resistances of 25–60 MΩ. They were connected to amplifiers which incorporated bridge circuits. Current was measured using a virtual ground amplifier. To stain the neurones, electrodes filled with 5 % cobaltous chloride or a saturated solution of cobaltic hexammine chloride were employed. Injected neurones were later intensified (Bacon & Altman, 1977). The DCMD and LGMD were identified using the positions of the electrodes in the brain, the response of the neurones to small objects moving within the ipsilateral visual field, and the relationships of the spikes recorded intracellularly to the spike recorded extracellularly from the DCMD axon in the contralateral connective. The LGMD and DCMD overlap in the median protocerebrum, so they were always distinguished in this region by the latency of synaptic transmission between them. Thus the LGMD was only conclusively identified in this region when the DCMD was also impaled intracellularly, and the synaptic delay between them was obvious. The position of the electrodes within the neurones was ascertained by reference to markers such as tracheae on the surface of the brain. Neurones characterized using these criteria were stained and the identifications and electrode positions confirmed.

To stimulate the neurones visually, an array of small (3·5×6 mm) green rectangular LEDs (light emitting diodes) were used. The 18 LEDs were positioned 3 cm from the head in a horizontal arc around the two eyes. Each LED was illuminated in turn. As one LED was extinguished and one of its neighbours became illuminated, the two neurones were excited. This method of stimulation usually excited at least one spike in each neurone, but avoided complete habituation. The timing of stimuli was displayed as stepping of voltage, each step corresponding to the time when one LED was extinguished and a neighbour became illuminated.

RESULTS

The LGMD as a source of visual input to the DCMD

Morphology

Within the protocerebrum, the terminals of the LGMD and the dendrites of the DCMD overlap (Figs 1, 2, 4). The LGMD terminals come into close contact with both the expanded primary neurite of the DCMD as it loops to cross the brain and also with the fine processes which project 100–200 μm either side of the neurite. The area of potential overlap occurs 60–100 μm below the surface of the brain.

Physiology

An LGMD spike caused an EPSP in the DCMD; usually this EPSP caused a spike in the DCMD. If the LGMD is the major source of visual (compound eye) input to the DCMD then: (1) spikes in the DCMD should follow those in the LGMD in response to the appropriate visual stimuli; (2) in the absence of a visual stimulus, injecting depolarizing current into the LGMD should produce spikes in both the LGMD and the DCMD; (3) inactivating or hyperpolarizing the LGMD so it no longer spikes in response to the visual stimulus should remove it from the pathway linking the compound eye with the DCMD, and the DCMD should no longer be excited by the visual stimulus. It has not previously been shown that the LGMD *is*

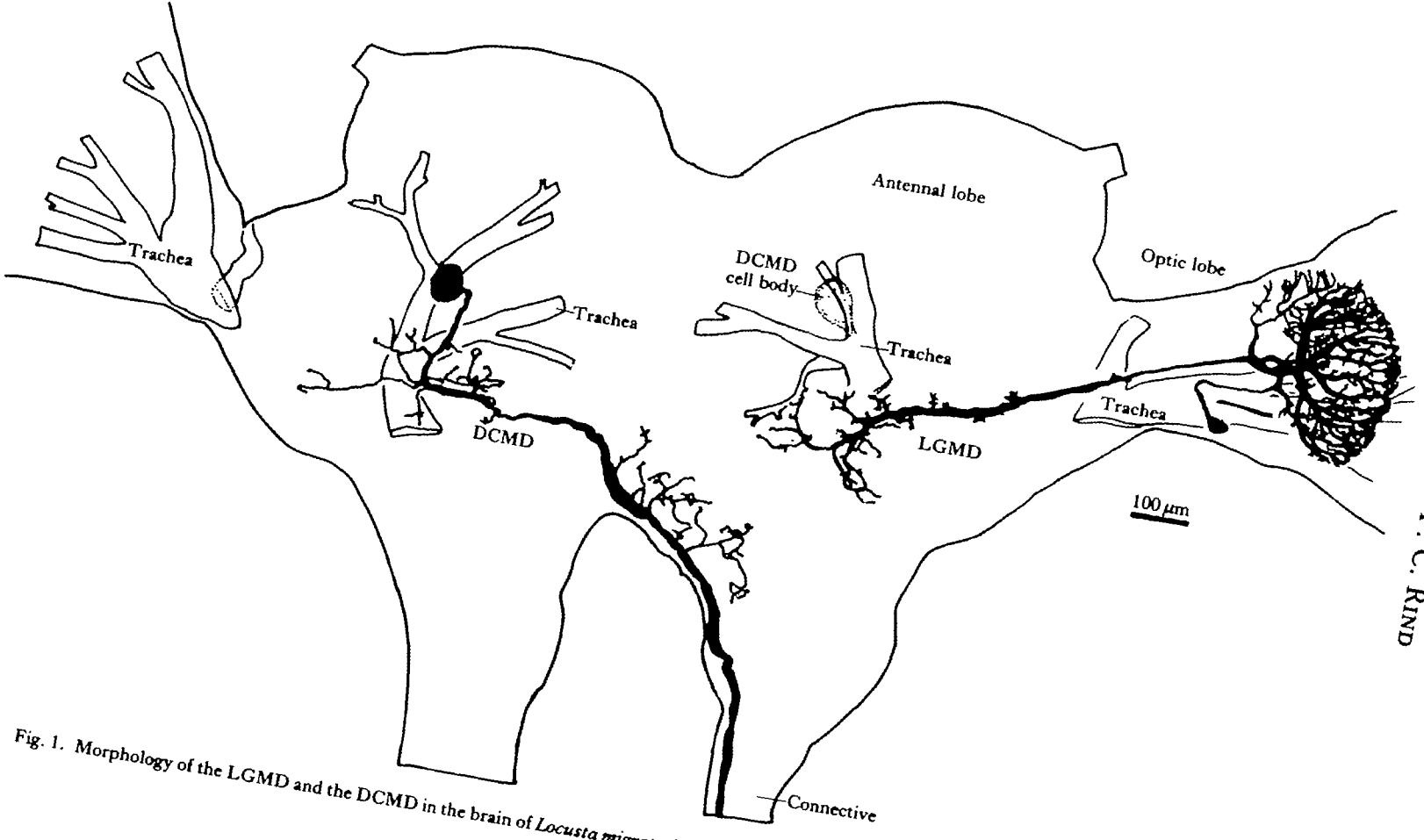

Fig. 1. Morphology of the LGMD and the DCMD in the brain of *Locusta migratoria*. Camera-lucida drawings from wholemounts of stained neurones; caudal view.

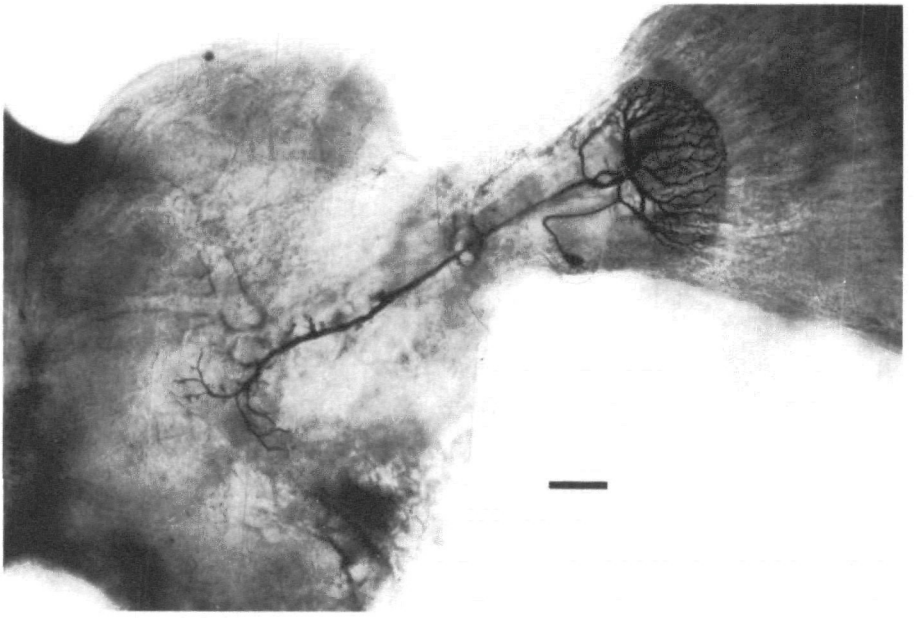

Fig. 2. Photomontage showing a caudal view of a stained LGMD neurone in the brain of *Locusta migratoria*. Scale bar: 100 μm.

major source of visual input to the LGMD and it is particularly important to be able to ascribe the excitation of the DCMD following the visual stimulus to the LGMD as it then becomes unnecessary to record from both neurones to identify the source of the excitatory input directly.

Both the LGMD and the DCMD spiked in response to the visual stimulus (Fig. 3A). When the LGMD was depolarized by current injection (Fig. 3A) it produced spikes which were followed one for one by spikes recorded simultaneously by a second intracellular electrode in the DCMD cell body and by a pair of extracellular hook electrodes around the DCMD axon in the contralateral connective. Conversely, gradual hyperpolarization of the LGMD, as in Fig. 3A–C, caused a failure of the LGMD to spike in response to the visual stimulus. When this happened (Fig. 3C) the DCMD also failed to produce an action potential in response to the visual stimulus. Inactivating the LGMD by excessive hyperpolarization or by poisoning with intracellular injection of hexammine cobaltic chloride (four experiments) also abolished the response of the DCMD to the visual stimulus. For example, after excessive hyperpolarization, the visual response of the LGMD of Figs 3A–C was inactivated for the duration of the experiment (2 h). The DCMD was not damaged and could be made to spike by injection of small depolarizing currents (Fig. 3E). Even when the DCMD was close to the threshold for spike initiation it no longer showed any response to the visual stimulus after the LGMD was inactivated. These results demonstrate conclusively that the LGMD is the main source of excitation in the DCMD following the visual stimulus.

The delay in transmission between the LGMD and DCMD

The measurement of the latency between LGMD and DCMD is particularly important as it is a good indicator of the type of synapse involved. Latencies less than 0·1 ms are considered indicative of an electrical synapse, whereas latencies above 0·4 ms are considered indicative of a chemical one. In order to extract an exact measurement of synaptic delay affected as little as possible by conduction delay, it was necessary to record the conduction velocities of spikes and PSPs at various regions in the two neurones, particularly those close to the synaptic regions. The conduction velocity of spikes and PSPs within and between both the DCMD and LGMD was measured by placing two electrodes simultaneously in different regions of the two neurones and monitoring the time taken for spikes and PSPs to pass from one electrode to the other. Latency was measured from initial deflection to initial deflection. This measurement is relatively little affected by the changing spike shapes which are due in part to different time constants, and to the presence of excitable membrane in the various regions of the LGMD and DCMD. Each region of the two neurones is assigned a letter of the alphabet (see schematic outline of the brain and the neurones in Fig. 4). Letter '*a*' denotes the dendritic fan of LGMD and letter '*g*' the DCMD cell body. The synaptic region occurs between regions *d* and *e* on the LGMD and region *f* of the DCMD. Examples of paired recordings from these regions are shown in Fig. 4B traces (i) to (vii). In each trace the first half shows activity in the two neurones on a slow time-base, in the second the sweep speed is increased and the tape is run *backwards* so the latency can be measured from initial deflection to initial deflection. The latencies measured between each electrode pair in Fig. 4Bi–vii are presented in

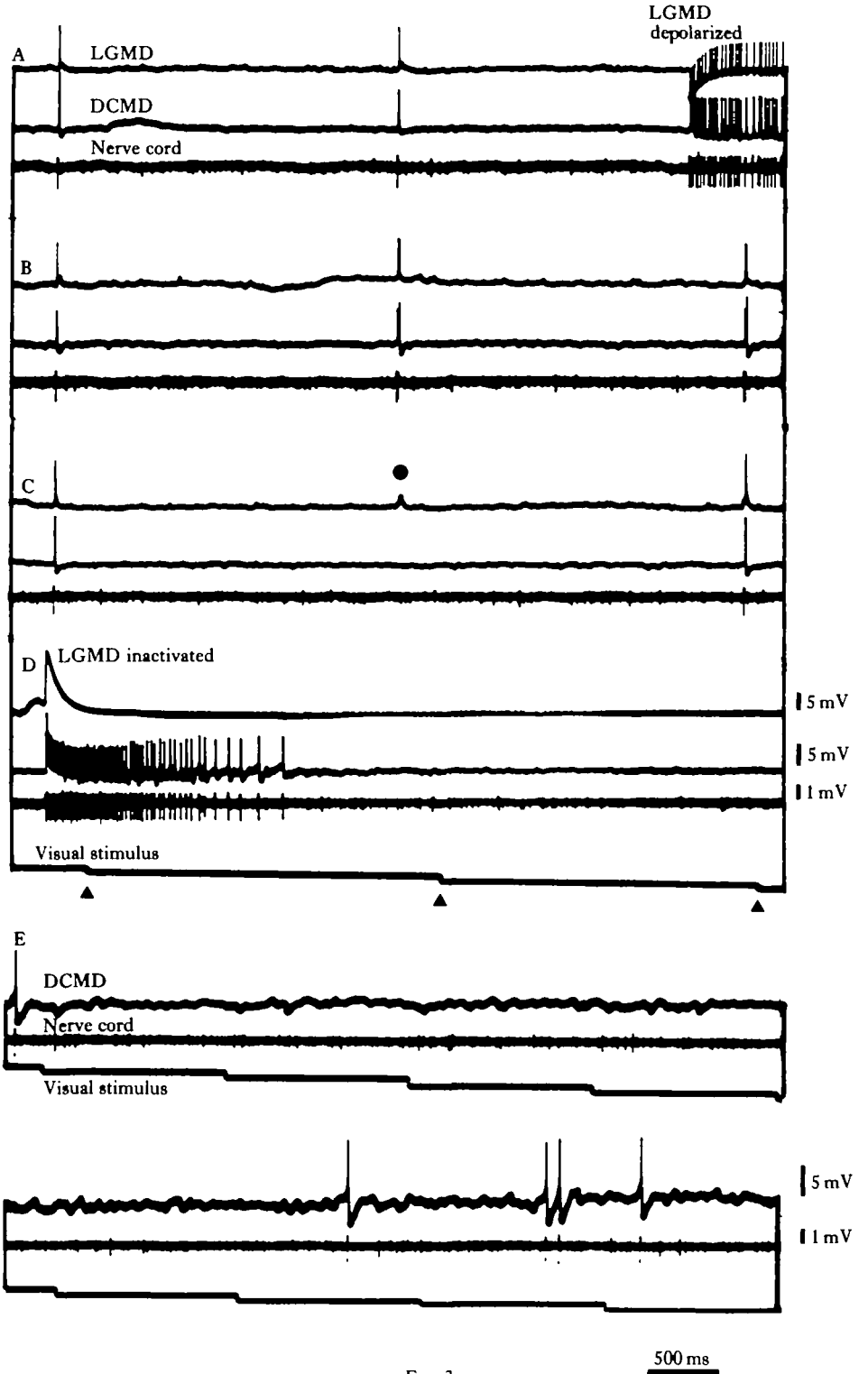
LGMD
depolarized
A
LGMD
DCMD
Nerve cord
B
C
D
LGMD inactivated
5 mV
5 mV
1 mV
Visual stimulus
E
DCMD
Nerve cord
Visual stimulus
5 mV
1 mV
500 ms

Fig. 3

ular form as an inset (Fig. 4C). The calculated delay between each point is shown at the base of the tabular inset. Note that in traces B (iii) and B (vii) the DCMD was hyperpolarized by 10 nA to reveal the LGMD-mediated PSPs underlying the spikes in the DCMD. There was no action potential in the DCMD axon following a PSP in the DCMD.

Initially there was a delay of 25 ms from the visual stimulus to a spike in the dendritic fan of the LGMD (region *a*). The spike, which was about 25 mV high (Fig. 4Biii) was conducted at 0·4 m s^{-1}, taking 0·25 ms to reach the LGMD axon at region *b*, the optic peduncle. An action potential at point *b*, was 13 mV high and was conducted to the peripheral boundary of the protocerebrum (region *c*) at 0·52 m s^{-1}, taking 0·25 ms. The axon of the LGMD dilates to about 17 μm in diameter between points *b* and *c* (Fig. 4A). An action potential recorded at point *c* was usually between 12–25 mV in amplitude, and showed a pronounced undershoot following the action potential but did not appear to be overshooting zero potential (see later section for measurement of LGMD resting potential). It was not possible to measure any conduction delay at all between points *c* and *d* on the LGMD axon, or between points *d* and *e*, in the LGMD: presumably it is here that synaptic contact with the DCMD occurs. LGMD spikes in regions *a* and *e* were usually between 6 and 15 mV in amplitude. In the DCMD an action potential was conducted from the dendritic region (*f*) to the cell body (*g*) at 1·2 m s^{-1} taking 0·25 ms. If the DCMD was hyperpolarized the PSP underlying the LGMD-mediated spike was revealed; this PSP conducted at the same speed as the spike from dendrites to cell body. A spike in the LGMD axon terminals at *e* took 1 ms to initiate a spike or a PSP in the dendrites of the DCMD at point *f*. The latency of this synapse was the same whether a spike was induced visually in the LGMD or by depolarizing intracellular current injection. The appreciable synaptic delay indicates the presence of a chemical synapse between the LGMD and DCMD neurones.

Conductance changes measured in the DCMD during LGMD-mediated PSPs

Conductance changes in the DCMD during an LGMD-mediated PSP were measured in two ways. First, the cell body of the DCMD was depolarized so that the DCMD produced spikes with a regular period between them. The LGMD was

Fig. 3. The LGMD as the source of compound eye input to the DCMD. The DCMD and the ipsilateral LGMD from one preparation are impaled simultaneously. (A) Visual stimulation (see stepping trace below D) causes a spike in the LGMD. Each LGMD spike is followed by a spike in the DCMD cell body and in the axon in the contralateral connective. At the end of the trace, depolarization of the LGMD causes both it and the DCMD to spike. (B) When the LGMD is hyperpolarized by 15 nA, spikes in the DCMD follow those in the LGMD during LED illumination. (C) When the LGMD is hyperpolarized by 25 nA, it occasionally fails to spike in response to the visual stimulus (▲). When the LGMD spike fails no spike or EPSP is seen in the DCMD. (D) After the LGMD is inactivated by excessive hyperpolarizing current (40 nA) it no longer spikes in response to visual stimulation. As the LGMD is inactivated the DCMD undergoes an initial period of intense spiking, which is unrelated to the visual stimulus, but it does not appear to be injured as resting potential remains unaltered and there are still small PSP-shaped potentials occurring and the DCMD can be induced to spike when current is injected into it. However, the DCMD no longer spikes in response to the visual stimulus after the LGMD is activated. (E) After the LGMD is inactivated the DCMD is depolarized close to the threshold for spike initiation but there is still no spiking in response to the visual stimulus. This indicates that the LGMD is the major, and possibly the only source of excitatory, ipsilateral compound eye input to the DCMD.

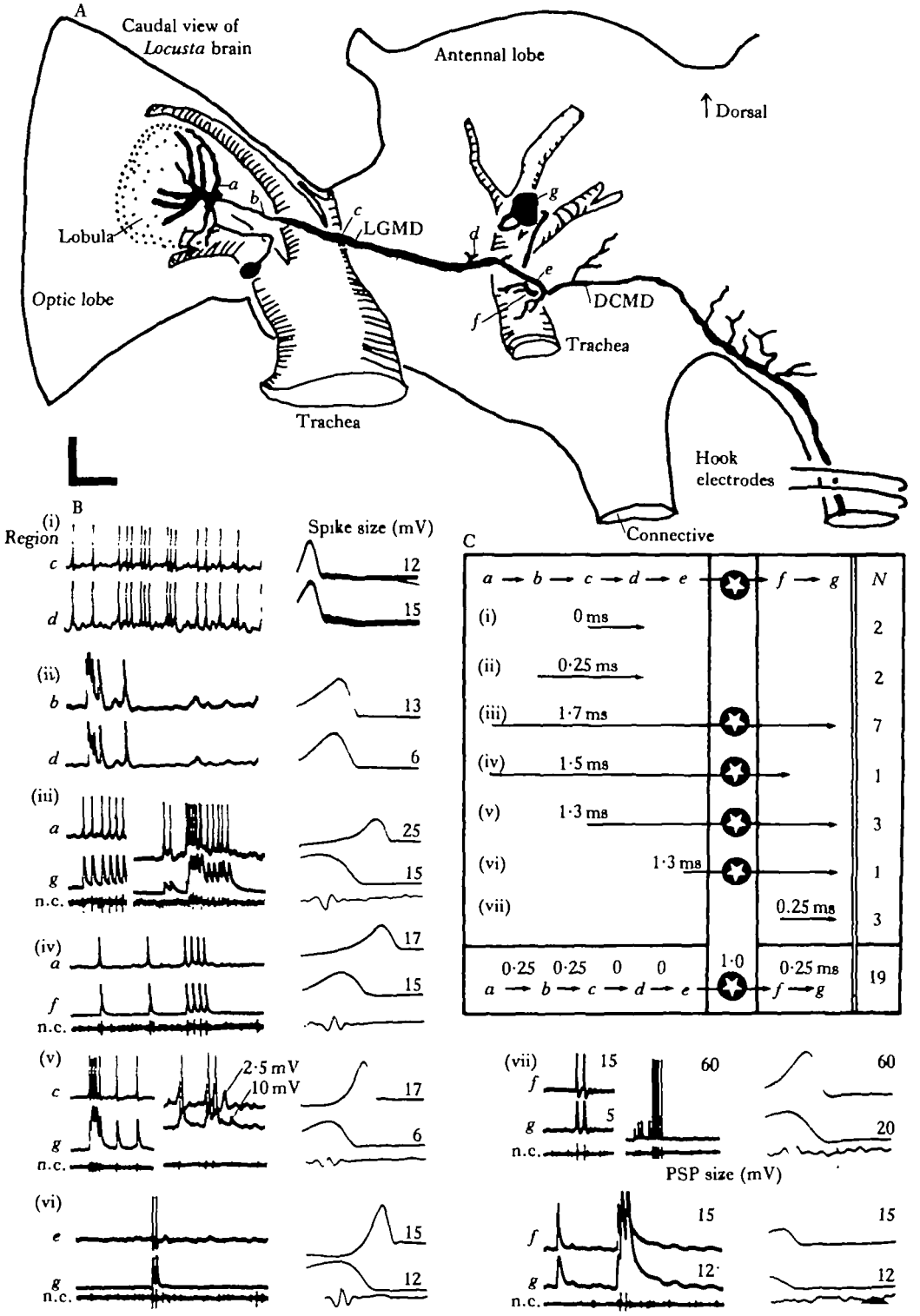
A
Caudal view of
Locusta brain
Antennal lobe
Dorsal
Lobula
Optic lobe
LGMD
DCMD
Trachea
Trachea
Hook
electrodes
Connective
B
Region
Spike size (mV)
(i)
c
d
12
15
(ii)
b
d
13
6
(iii)
a
g
n.c.
25
15
(iv)
a
f
n.c.
17
15
(v)
c
g
n.c.
2·5 mV
10 mV
17
6
(vi)
e
g
n.c.
15
12
C
a → b → c → d → e
f → g
N
(i) 0 ms 2
(ii) 0·25 ms 2
(iii) 1·7 ms 7
(iv) 1·5 ms 1
(v) 1·3 ms 3
(vi) 1·3 ms 1
(vii) 0·25 ms 3
0·25 0·25 0 0 1·0 0·25 ms 19
a → b → c → d → e f → g
(vii)
f
g
n.c.
15
5
60
60
20
PSP size (mV)
f
g
n.c.
15
12
15
12

Fig. 4

induced to spike either by depolarization *via* an intracellular electrode, or by visual stimulation. When the LGMD and depolarization-induced spikes were coincident (Fig. 5A) any conductance change associated with the arrival of the LGMD-mediated PSP was mirrored in the change in the depolarization-induced spike height. A conductance increase was observed. When a PSP and an experimentally induced spike coincided there was a 20 % reduction in spike height. Second, short hyperpolarizing pulses of current were injected into the DCMD *via* the intracellular recording electrode. LGMD-mediated PSPs were revealed by hyperpolarization of the DCMD, usually by about 10 nA. The source of the PSPs was identified directly by simultaneous recording from the LGMD or indirectly by their occurrence in response to visual stimulation (see above). All experiments showed that there was a conductance increase in the DCMD during an LGMD-mediated PSP (Fig. 5B–E). The PSPs progress from right to left across the trace. The conductance increased relative to that at resting potential, and was most obvious at the peak of the rising phase of the LGMD-mediated PSP, but there was also a slight conductance increase during the initial one-third of the falling phase. The conductance increase was not affected by manipulating the membrane potential of the DCMD to values more negative than resting potential. The conductance increase was measured at three different membrane potentials (−4 mV, −12 mV and −20 mV) by using the recording electrode to pass increasing amounts of hyperpolarizing current into the DCMD. These experiments indicate that the observed conductance increase is not due to any non-linearities of the current voltage relationship of the DCMD membrane during the course of a PSP itself. In Fig. 5D and E conductance changes are shown on a slow time-base to show conductance changes during summation of PSPs in the DCMD cell body and dendrites.

Size and shape of the PSP

The size and the shape of the LGMD-mediated PSPs depended both on the part of the DCMD neurone from which they were recorded and on the membrane potential

Fig. 4. Estimates of conduction velocity within the LGMD and DCMD and synaptic delay between them. (A) Camera lucida drawing of an LGMD and a DCMD neurone from separate individuals. The neurones have been superimposed using the outline of the brain as a guide. Each region of the two neurones from which recordings were made is denoted by a letter of the alphabet (*a–g*). The synapse between the LGMD and the DCMD occurs between region *e* of the LGMD and region *f* of the DCMD. (B) Recordings were made using two electrodes inserted into the LGMD, LGMD and DCMD, or the DCMD. Traces i–vii are divided into two sections, the first showing activity recorded from each region of the neurone on a slow time base and the second showing the passage of a spike or a PSP between the two electrodes on a much expanded time base. The tape has been run backwards so the second part of the trace progresses from right to left. This is in order to record the initial inflection of the spike recorded at each region. Recording from the LGMD. (i) At regions *c* and *d*, note the common PSPs in the two regions. (ii) At regions *b* and *d*. (iii) At region *a* and DCMD in region *g* (cell body). Two traces are shown on a slow time base, in the second the DCMD has been hyperpolarized by 10 nA to reveal the EPSPs following a spike in the LGMD. Note that during the EPSP there is no spike in the axon of the DCMD in the contralateral connective. n.c., nerve cord. (iv) In region *a* and the DCMD in region *f*. (v) At region *c* and DCMD in region *g*. Note the lack of correspondence in PSPs in the two neurones shown at higher gain in the second part of the trace. (vi) At region *e* and the DCMD at region *g*. (vii) Recording from the DCMD at *f* and *g* during passage of a spike or an EPSP between the two electrodes. (C) The table sets out each of the latencies measured in the order they were listed in Bi–vii. The number of times the latency between two regions was measured is indicated to the right of each measurement. At the base of the table the time taken for a spike to travel between each region and across the synapse has been calculated, using the data presented in the Table. The synapse is denoted with a star.

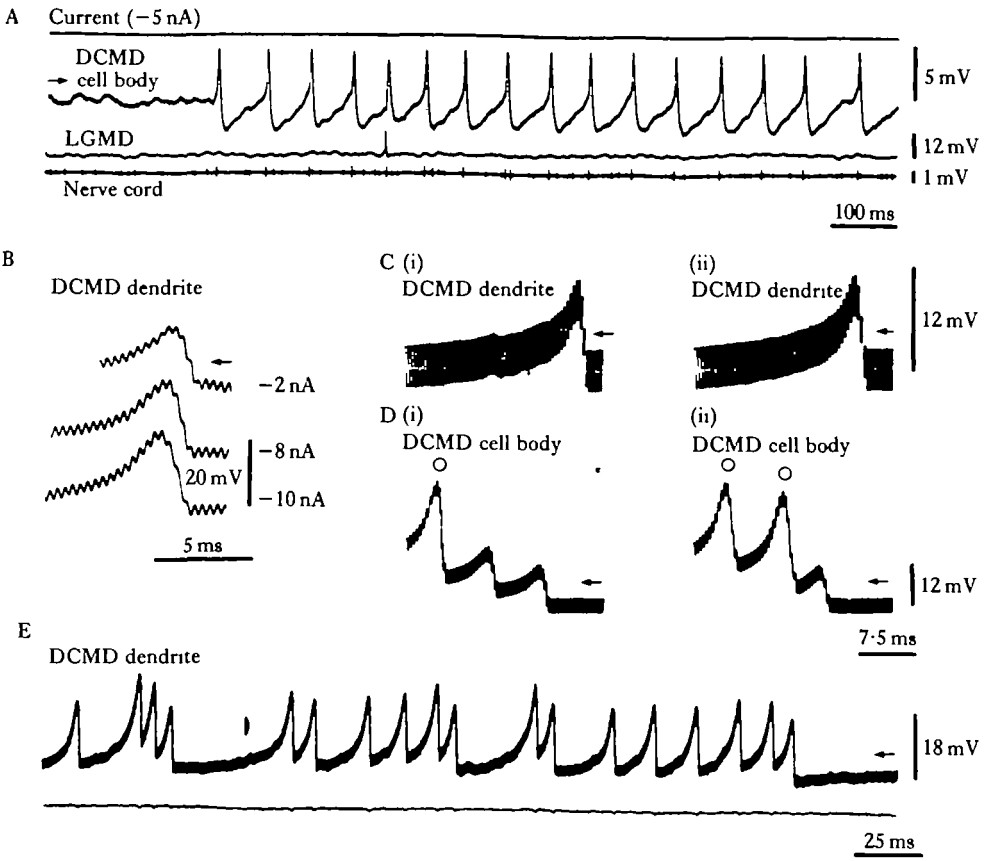

Fig. 5. conductance changes underlying an LGMD-mediated PSP in the DCMD. In records B–E forwards is from right to left across the figure. (A) A single intracellular electrode was used to depolarize the DCMD cell body and cause the DCMD to produce a regular pattern of spikes. A coincident spike in the LGMD, monitored intracellularly, produces a PSP in the DCMD which causes the depolarization induced spike to be reduced in size by 20 %. (B) Using a single intracellular electrode, short hyperpolarizing pulses of current were injected into the DCMD during an LGMD-mediated PSP. The current pulses are reduced in height during the rising phase and the initial one-third of the falling phase of the PSP. The reduction in the amplitude of the hyperpolarizing pulses during the LGMD-mediated PSP was not affected by a gradual hyperpolarization of the DCMD by passing a steady −2, −8 or −10 nA current through the recording electrode in the DCMD dendritic region. (C) During an LGMD-mediated PSP in a second DCMD neurone, hyperpolarizing pulses of current were injected, and membrane potential recorded *via* a single electrode. The conductance changes measured during the entire time course of the PSP (i and ii). (D) Conductance changes measured by the same method as above were monitored during LGMD-induced spike (○) and PSPs in the DCMD cell body. Two examples are shown (Di and Dii). (E) Conductance changes, measured as detailed in section A, are shown during a long train of PSPs in the dendritic region of the DCMD. Records E and B are from the same experiment.

of the DCMD. The PSPs recorded from the expanded neurite which bears dendritic branches rose and decayed more sharply than those recorded in the cell body (Figs 5C, D; 6A, B). In the dendritic region of the DCMD the PSP rise time was 1·3 ms compared with 3·1 ms in the cell body. The half-time for decay was similarly extended, being 2·2 ms in the dendritic region and 7·4 ms in the cell body region of the DCMD. The amplitude of the PSP in the dendritic region was 1·4 times that in the cell body (Fig. 6B). The amplitude of the LGMD-mediated PSP showed a linear relationship t

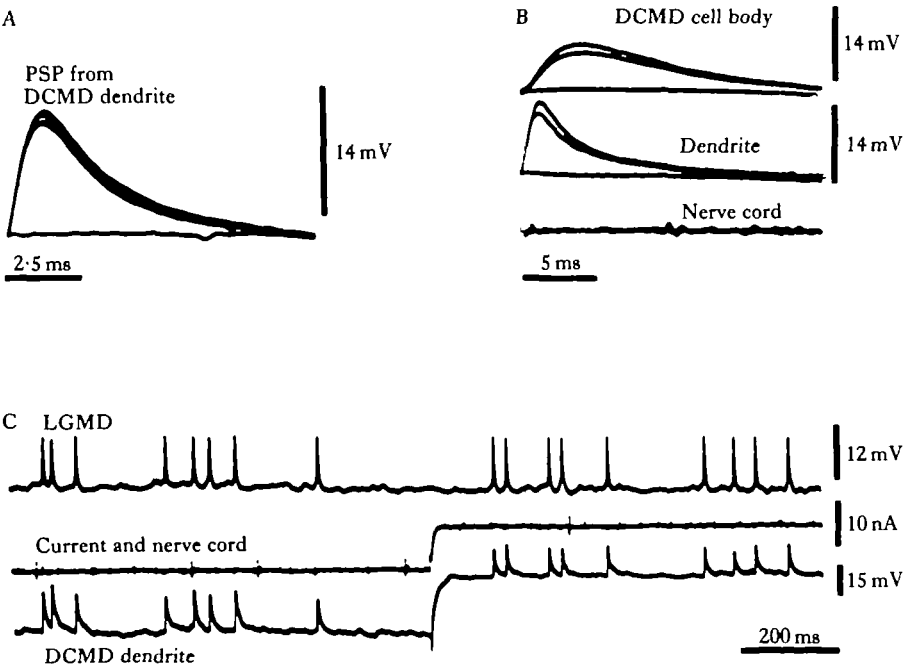

Fig. 6. Characterization of the LGMD-mediated PSP. (A) The PSP recorded from the dendritic region of the DCMD has a rise time of 1·3 ms and a half-time for fall of 2·2 ms. The whole PSP lasts about 8·3 ms. (B) This contrasts with the same PSP recorded simultaneously by a second electrode in the DCMD cell body which has a rise time of 3·3 ms and a half-time for fall of 8 ms. The whole PSP lasts 21·3 ms. (C) The size of the LGMD-mediated PSP increases as the DCMD is hyperpolarized. At the beginning of the record shown in C the DCMD has been hyperpolarized by injection of a steady −10 nA current through the recording electrode. The hyperpolarization ends in the middle of the record and the LGMD- mediated PSP is reduced in size.

membrane potential/current over the range tested. The PSPs increased in amplitude as the DCMD was hyperpolarized and decreased as it was depolarized (Figs 6C, 7, 8).

In the experiment shown in Figs 7 and 8 current was passed into the DCMD first from the recording electrode in the dendritic region (Fig. 7) and then from a second electrode in the cell body, while the recording electrode in the dendrites measured DCMD membrane potential and the LGMD-mediated PSP amplitude (Fig. 8). This enabled plots to be made of PSP amplitude *vs* current injected, and of PSP amplitude *vs* membrane potential. Extrapolating the graph of PSP amplitude *vs* membrane potential gave a reversal potential for the PSP at 30 mV more positive than resting potential. A 10 mV change in membrane potential produced a 5·6 mV change in PSP amplitude. PSP amplitude at resting potential was about 15 mV. This data could then be used to calculate the reversal potential from the graph shown in Fig. 7 as the recordings were made from the same neurone:

Fig. 8. 10 mV membrane potential shift..... 5·6 mV amplitude change.
Fig. 7. 13·6 nA current..... 5·6 mV amplitude change.
i.e. 13·6 nA current..... 10 mV potential shift.

Reversal potential from Fig. 7 is +28 nA, i.e. 20·6 mV more positive than resting potential.

From Fig. 7 PSP reversal would have occurred after injection of +28 nA. depolarization of 28 nA resulted in a membrane potential 20·6 mV more positive than resting potential. Two factors made the calculation of the actual PSP reversal potential difficult. First, there was considerable variation in the size of individual LGMD-mediated PSPs, independent of DCMD membrane potential (Table 1). Second, measurement of the resting potential of the DCMD was prevented by the large field potential which made it difficult to judge when the electrode was just inside the DCMD.

The linear relationship between membrane potential of the DCMD and LGMD-mediated PSP amplitude, and the apparent reversal potential of the PSP are not in themselves diagnostic of a chemical synapse between the LGMD and DCMD, but in conjunction with the earlier observations of 1 ms synaptic latency and a conductance increase during an LGMD-mediated PSP they can be taken as illustrating features of the chemical synapse between the two neurones. The features of this synapse were further investigated in the following experiments. In particular, tests were made for

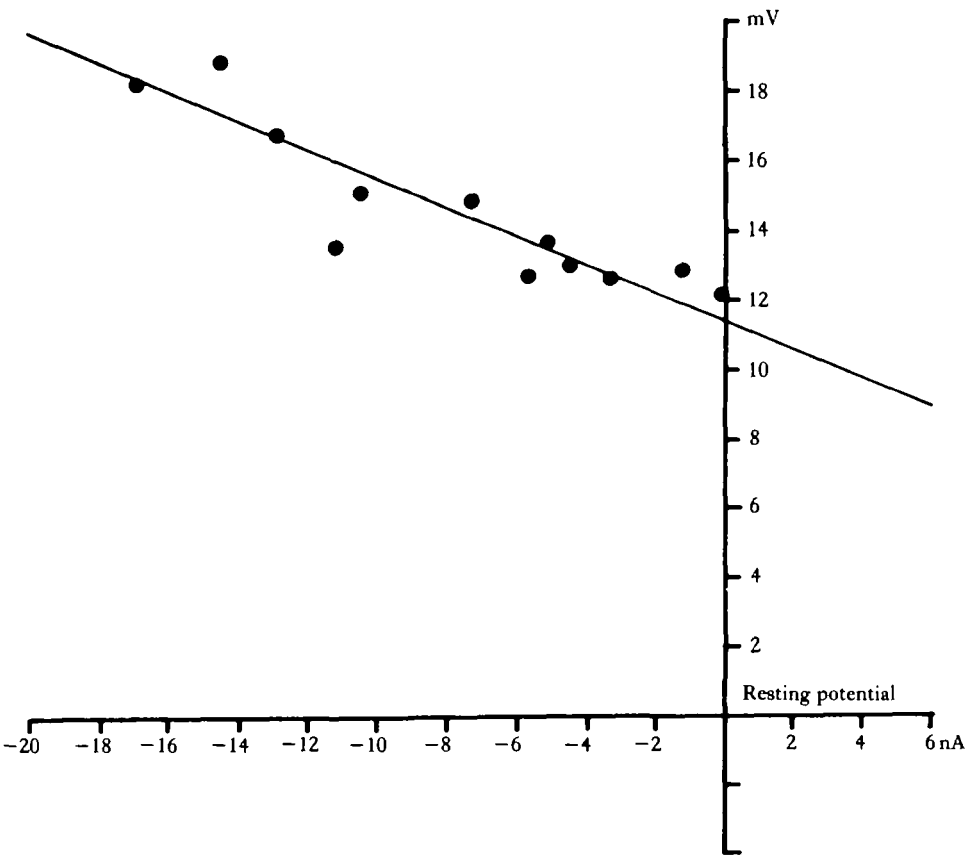

Fig. 7. The average amplitude of five LGMD-mediated PSPs are shown plotted against strength of hyperpolarizing current. Current is injected through, and membrane potential recorded by a single electrode in the dendritic region of the DCMD.

presence of a rectifying electrotonic synapse, a synapse which could partially explain the above results (Furshpan & Potter, 1959; Giaume & Korn, 1983).

Electrotonic and graded transmission across the LGMD–DCMD synapse

Intracellular electrodes were placed in both the LGMD and the DCMD simultaneously, current was injected through one and the resultant change in membrane potential recorded at the other. A spike, of at least 13 mV in amplitude, was necessary in the LGMD before an EPSP was produced in the DCMD. No evidence was found for current flow in the opposite direction from DCMD to LGMD.

Electrotonic graded transmission

Depolarizing and hyperpolarizing currents were injected into both LGMD and DCMD to investigate current flow across the synapse. The threshold for spike production in the LGMD was very close to resting potential, so only small amounts of presynaptic depolarizing current could be used without the intervention of action

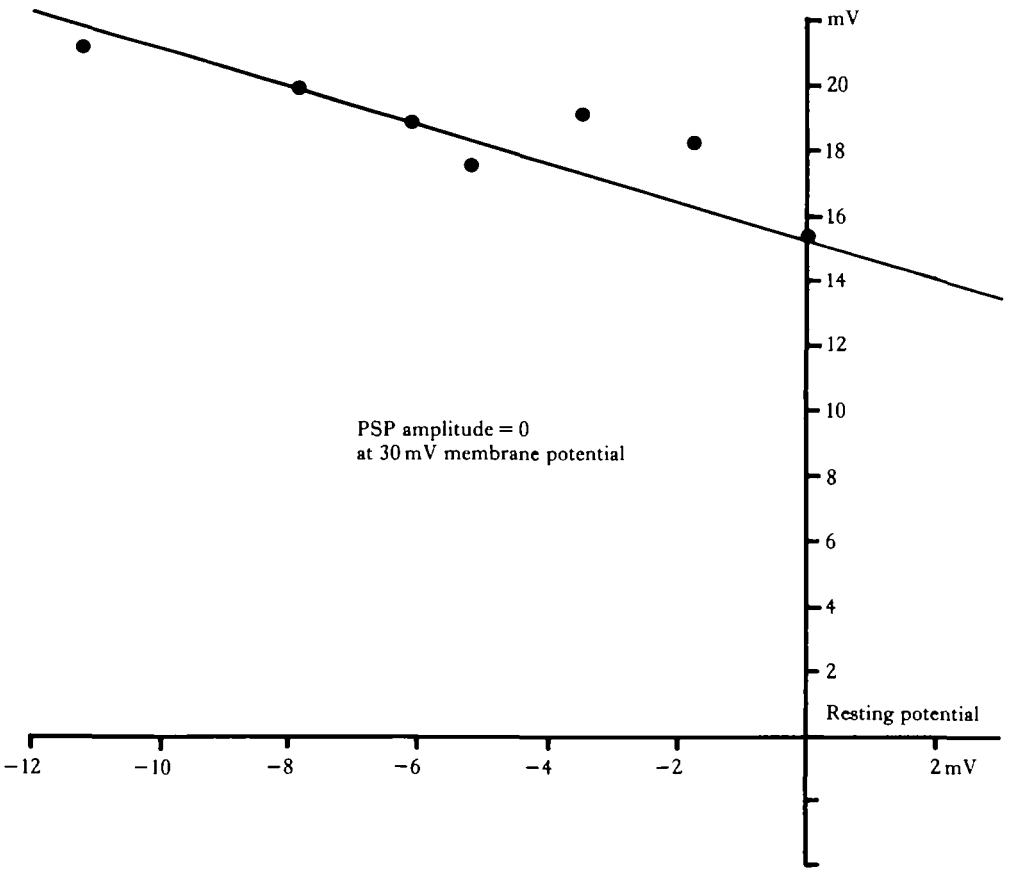

Fig. 8. From the same experiment as Fig. 7 except current is injected from a second electrode placed in the cell body of the DCMD. The average amplitude of five LGMD-mediated PSPs is shown plotted against DCMD membrane potential. Extrapolating the line, drawn by eye through these points, back to zero amplitude of PSP gives a reversal potential 30 mV more positive than resting potential.

Table 1. *Mean and standard deviations in the amplitude of the LGMD-induced PSP, recorded in the DCMD dendrite, during the injection of either depolarizing or hyperpolarizing current through the recording electrode*

Current (nA)	+9·2	+8·5	+6·9	+6·2	+5·4	+4·62	+3·8	+3·1	0·0	−3·1	−4·6	−6·2	−6·9	−7·7	−8·5
Mean PSP size (mV)	18·6	19·5	18·9	17·8	19·8	17·9	19·7	21·0	26·3	24·5	30·0	27·1	27·5	32·1	30·8
S.D.	3·1	1·1	2·4	2·2	2·7	1·6	1·3	1·6	2·9	1·4	2·9	2·4	1·4	1·1	2·3

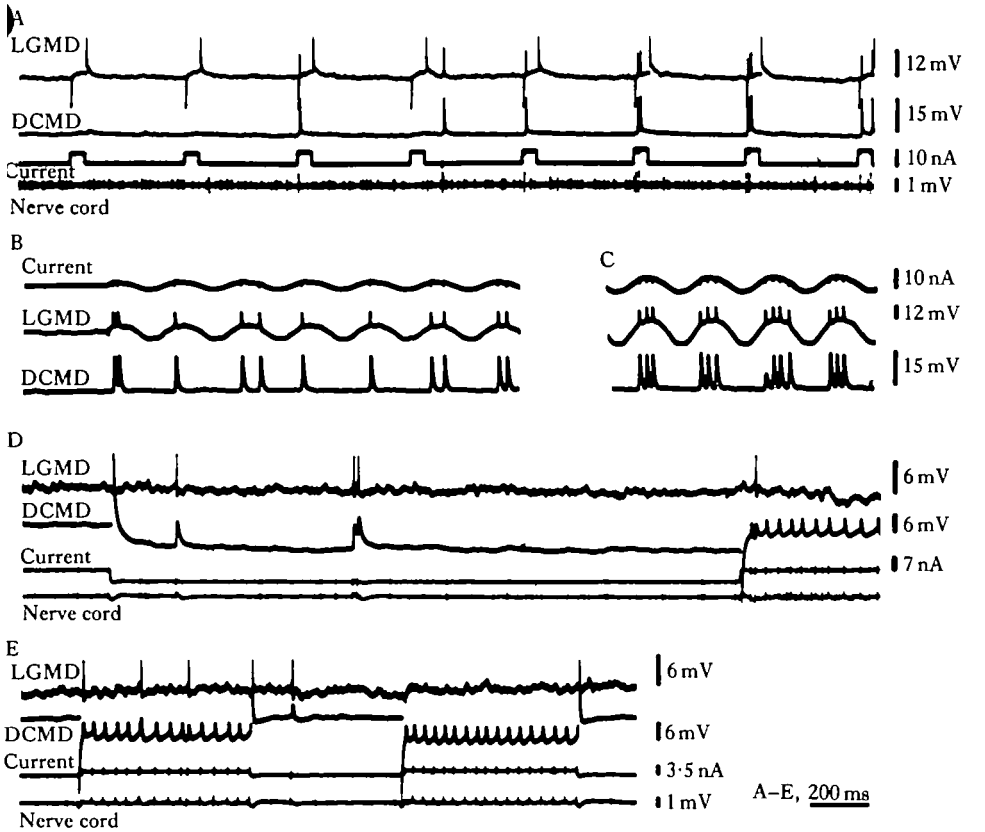

Fig. 9. Transfer of graded potentials between the LGMD and DCMD. The LGMD and the DCMD are impaled simultaneously, each with a single electrode. The recordings shown in A and B were made from the dendritic fan of the LGMD (region *a*) and the cell body of the DCMD (region *g*), in the same preparation. Those shown in C and D were made from the terminals of the LGMD in the protocerebrum (region *e*) and the DCMD cell body in the same preparation. (A) The LGMD is held hyperpolarized by 10 nA of current throughout the experiment to prevent spiking in response to the small amounts of depolarizing current injected into it. No changes in DCMD membrane potential were seen during small depolarizations of the LGMD. A spike in the LGMD evoked a spike in the DCMD. (B) Superimposing small amplitude waves of depolarization then hyperpolarization on the resting membrane potential of the LGMD did not produce corresponding fluctuations in the membrane potential of the DCMD. (C) Increasing the amplitude of the waves of depolarization and hyperpolarization still produced no corresponding fluctuations in DCMD membrane potential. More spikes were produced by the LGMD during the depolarizing phase of the oscillations and these were followed one to one by spikes on the DCMD. (D) Injection of a long pulse of hyperpolarizing current into the DCMD produced no potential change in the LGMD terminals. (E) Similarly, injection of depolarizing current into the DCMD did not produce any membrane potential change in the LGMD terminals.

potentials. This problem was alleviated by applying short depolarizing pulses to the LGMD at point *b*, while it was hyperpolarized by about 10 nA (Fig. 9A). The membrane potential of the DCMD cell body was not altered during the injection of depolarizing pulses into the LGMD unless the LGMD spiked in response to current injection. Injection of small sinusoidal waves of hyperpolarizing, then depolarizing, current into the LGMD, at point *b*, had no effect on the membrane potential of the DCMD cell body, unless the LGMD spiked (Fig. 9B). Increasing the strength of the

current only increased the number of spikes produced by the LGMD during the depolarizing phase of the wave; there was still no sign of sinusoidal modulation of DCMD membrane potential (Fig. 9C). Current flow from the DCMD to the LGMD was investigated by injecting hyperpolarizing and depolarizing pulses of current into the DCMD cell body and recording the potential change in the LGMD, at point *e*, (Fig. 9D, E). Neither hyperpolarizing nor depolarizing current, applied at a variety of DCMD membrane potentials, had any affect on the LGMD membrane potential.

Threshold for transmitter release

The threshold for release of transmitter at LGMD terminals on the DCMD was normally exceeded by a spike in the LGMD (Fig. 10). Occasionally there were large PSPs or small spikes in the LGMD which did not exceed the threshold for transmitter release but they did allow an estimation of the release threshold (Fig. 10A, Bi, ii). The threshold spike size for transmission at the LGMD–DCMD synapse, as recorded in the terminals of the LGMD at point *e*, was about 13 mV. The resting potential of the LGMD was measured as −53 mV (Fig. 10E). (Note that the response of the DCMD was monitored extracellularly during penetration of the LGMD and showed no change in responsiveness to the visual stimulus.) This means that the actual threshold for transmitter release was 13 mV depolarization from −53 mV, which is −40 mV. The high threshold of synaptic transmission coupled with the low threshold for spike production in the LGMD means that it is not possible to look at the input/output characteristics of this synapse without the intervention of pharmacological agents, such as tetrodotoxin, to block spike production.

Transmission across the synapse

Once the LGMD had produced a spike the DCMD followed one to one with unvarying delay up to frequencies of 400 Hz (Fig. 10Ci, ii, Di, ii). These high following frequencies could be maintained for several seconds at least (Fig. 10Ci, Di). The fixed delay with which the spikes in the DCMD followed those in the LGMD further suggests that the DCMD is not responding to a general level of depolarization but rather to each action potential in the LGMD.

One reason for the apparent inability to measure any transmission of graded or maintained potentials across the LGMD–DCMD synapse may be the distance of the current-passing recording electrode from the synapse. To test this, current/voltage curves were constructed for both the LGMD and the DCMD. Electrodes were placed in a variety of regions in each neurone and current was passed between them.

Current/voltage (I/V) relationship in different regions of the LGMD

The I/V relationships shown for the LGMD in Fig. 11 were plotted from measurements made by placing two intracellular electrodes in the LGMD and using one to pass 300-ms pulses of current and the other to monitor membrane potential. When current was passed into the LGMD below the dendritic fan (point *b*) and membrane potential monitored 400 μm away near the terminals of the LGMD in the protocerebrum (point *d*), the I/V relationship was linear for all currents used. The slope of the line gives a membrane resistance of 0·52 MΩ between these two points (●, Fig. 11). Similarly when two electrodes were placed more closely together, one in the

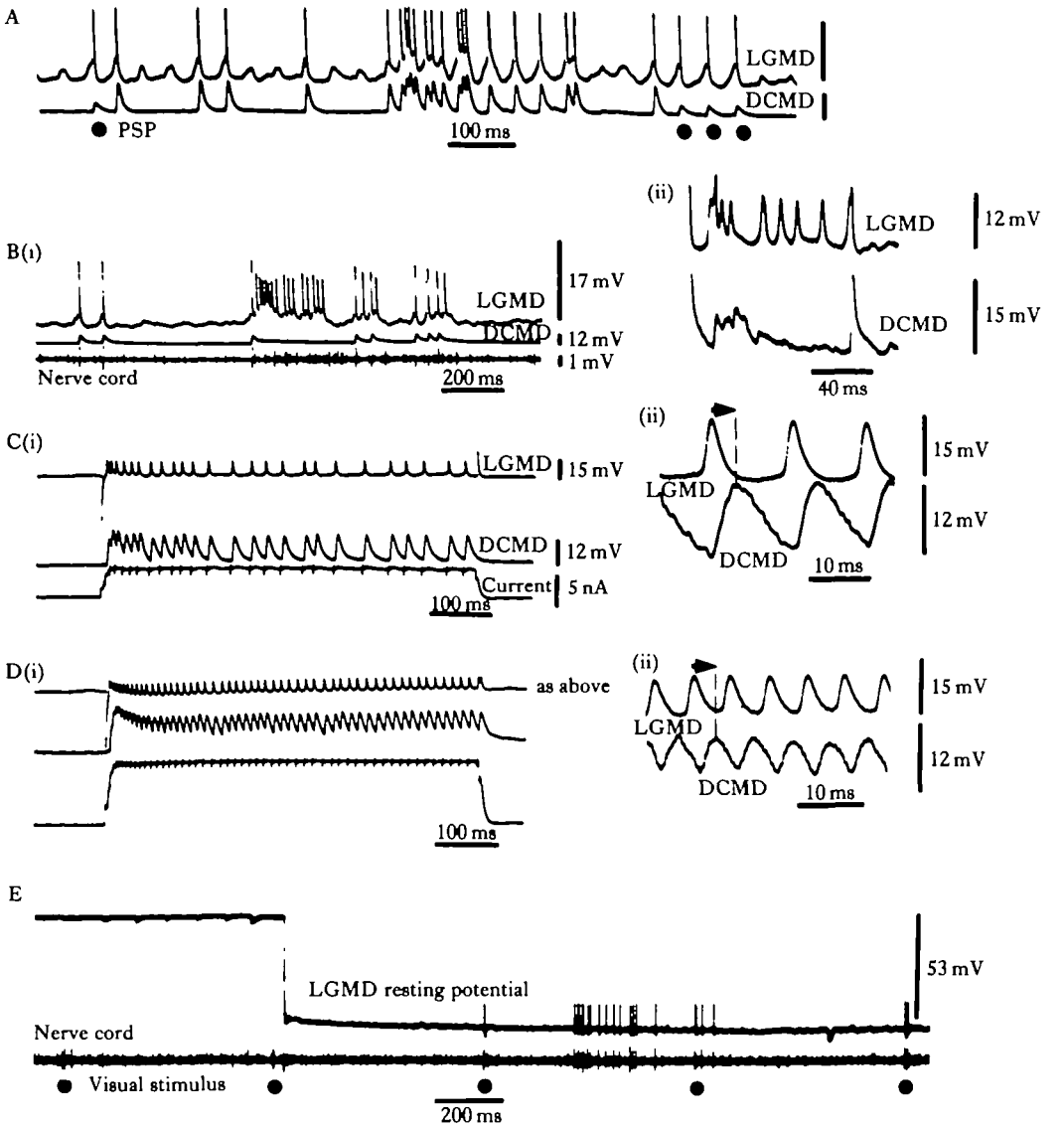

Fig. 10. Transmission of large depolarizing potentials and spikes across the LGMD–DCMD synapse. In the LGMD a depolarization of at least 13 mV above resting potential is necessary before transmission across the LGMD–DCMD synapse is detected. Once the threshold is exceeded spikes in the DCMD follow those in the LGMD, at a fixed latency up to a frequency of at least 400 Hz, for a period in excess of 1 s. Recordings were made using pairs of intracellular electrodes, one inserted in the dendritic fan of the LGMD (region *a*), the other in the cell body of the DCMD (region *g*). (A) Spikes in the LGMD are followed by either spikes or PSPs (●) in the DCMD. However potential fluctuations of 4–5 mV in the LGMD are not reflected in the membrane potential of the DCMD. (B) Spikes greater than 13 mV in amplitude and smaller spike-like potentials occur in the LGMD. Spikes in the DCMD follow LGMD spikes greater than 13 mV in amplitude but not the smaller spike-like potentials. (C), (D) Increasing strengths of depolarizing current are injected into the LGMD, causing it to spike, these LGMD spikes are followed one to one by spikes in the DCMD. The peak to peak latency between an LGMD, and a DCMD spike is 5 ms, irrespective of the frequency of LGMD response (Cii, Dii). (E) Recording during penetration of the LGMD. A resting membrane potential of −53 mV is measured. Note that impaling of the LGMD does not alter the response of the DCMD to visual stimulation, monitored extracellularly from its axon in the contralateral connective.

LGMD axon in the brain at point *c* and one 100 μm away near the terminals at po[illegible] *d*, and hyperpolarizing current passed into the axon, the I/V relationship was linear, with a slope representing a membrane resistance of 0·83 MΩ between the two points (○, Fig. 11). These values suggest that 10 nA of current injected intracellularly just below the dendritic fan of the LGMD (the recording site used for Fig. 9) would give rise to a 5 mV shift in membrane potential at, or near, the synapse; i.e. 1/2 to 1/3 the size of a spike in the region.

Current/voltage relationships within the DCMD

When the electrodes were placed in the DCMD cell body, the I/V relationship was linear over the entire range of hyperpolarizing currents injected, and with up to 3 nA of depolarizing current (Fig. 12). The relationship gave a membrane resistance for the DCMD cell body of 2·2 MΩ. Hyperpolarizing current was passed into the DCMD through an intracellular electrode in the dendritic region, and membrane potential was monitored simultaneously in the cell body with a second electrode, to produce an

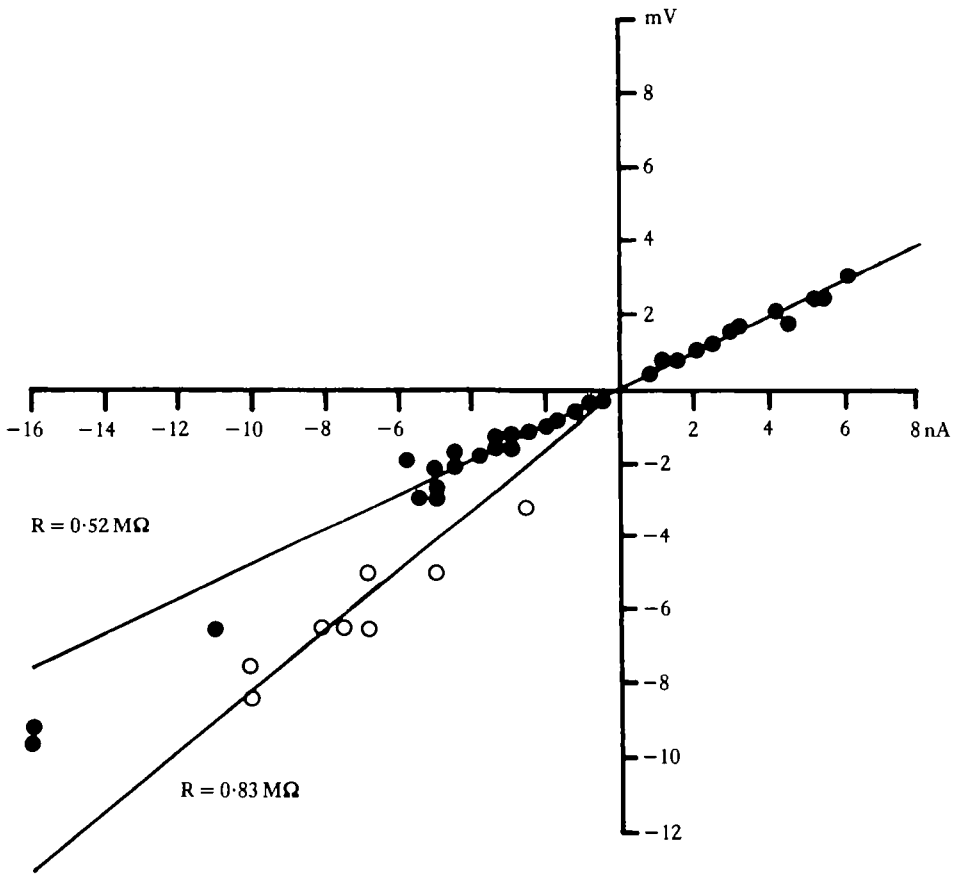

Fig. 11. Current/voltage relationships of the LGMD. Pairs of intracellular electrodes were inserted at different points along the length of the LGMD. Current was passed into the LGMD's dendrites within the optic lobe at point *b* (●) or into the axon in the brain at point *c* (○), and the resultant shift in membrane potential recorded in the brain at a point close to the LGMD synapse with the DCMD (point *d*).

V relationship (Fig. 12). The calculated membrane resistance between the DCMD dendrites and cell body is 2·4 MΩ. This compares with a value of 1·09 MΩ resistance when hyperpolarizing current was injected into the cell body of the DCMD and membrane potentials were monitored in the dendrites of the DCMD. Each of these experiments was performed on a different preparation.

In the DCMD, injection of 3 nA of depolarizing current into the dendritic region led to the recording of a 6 mV deflection in membrane potential measured at the cell

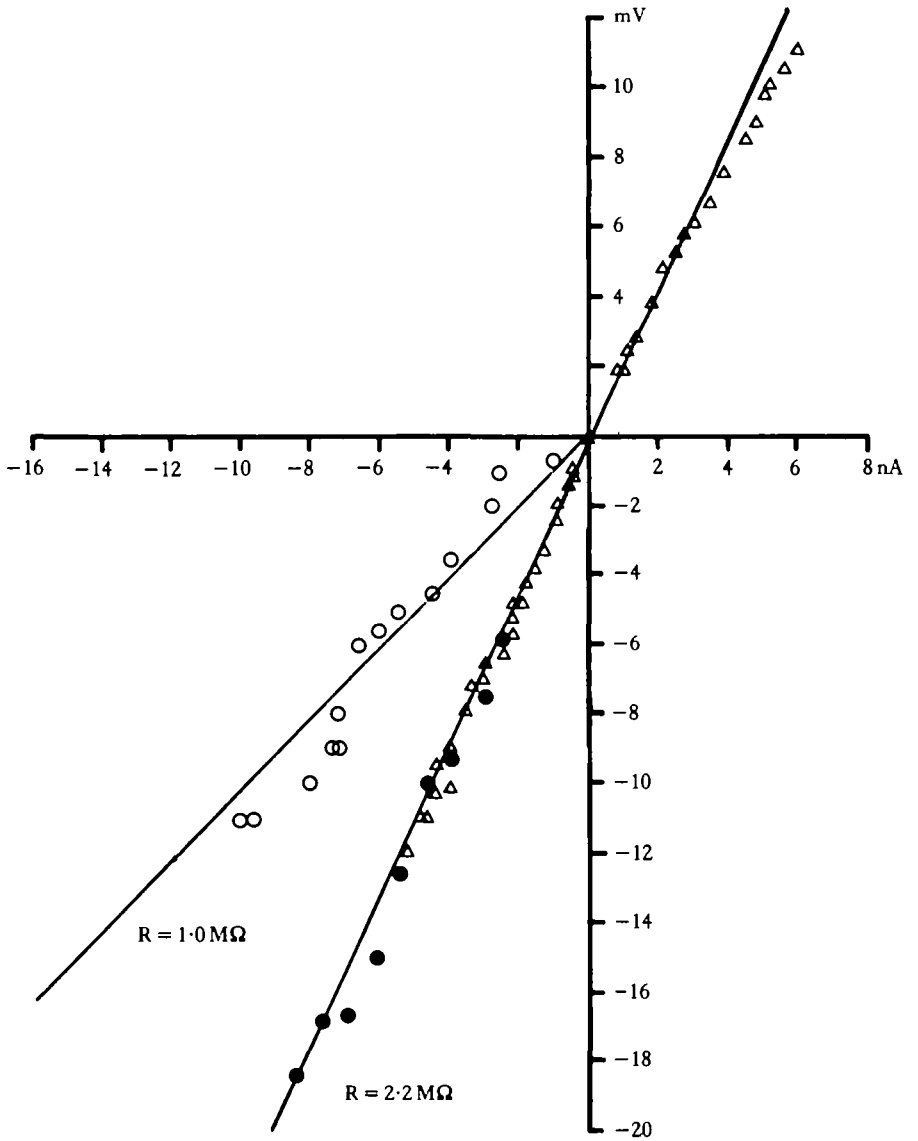

Fig. 12. Current/voltage relationships of the DCMD recorded using pairs of electrodes. Current was passed through an electrode in the cell body and membrane potential recorded by a second electrode in the cell body (△) or by a second electrode in the dendritic region (○). Current was passed into the dendritic region and membrane potential recorded in the dendritic region (●).

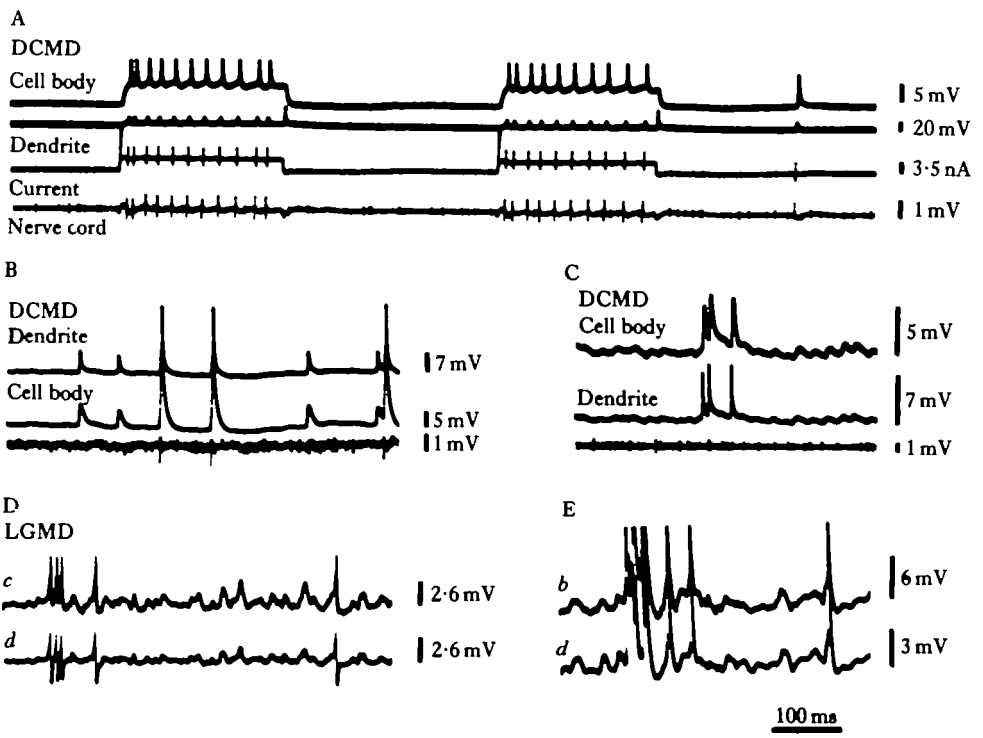

Fig. 13. Pairs of electrodes placed at different sites along the large processes of the DCMD or LGMD record common unitary PSPs which are conducted electrotonically between the electrodes. The size of the PSPs at the two electrodes can be used to calculate the length constant of the DCMD or LGMD. (A) Small amounts of current injected through one intracellular electrode in the dendritic region of the DCMD result in a recordable change in membrane potential at a second electrode in the cell body. 3·5 nA of depolarizing current injected into the DCMD dendritic region results in a 5 mV depolarization at the cell body. (B), (C) In the DCMD, LGMD-mediated PSPs are readily recorded throughout the large processes in the brain. When two electrodes are placed 450 μm apart one in region *f* in the DCMD dendrites and the other in region *g*, the DCMD cell body, the LGMD-mediated PSPs are 7 mV high in the dendrites and 5 mV high when measured simultaneously in the cell body. This gives a length constant of 450 μm/(ln 7 mV/5 mV) = 1·34 mm for this region of the DCMD. (D) When electrodes are placed 250 μm apart in regions *c* and *d* of the LGMD within the protocerebrum the size of these electrotonically conducted potentials measured at the two electrodes are 3·4: 1·7 mV respectively. This gives a length constant of 250 μm/(ln 3·4/1·7) = 0·36 mm. (E) When electrodes are placed 4·50 μm apart in region *b*, in the optic lobe and region *d*, in the protocerebrum, the sizes of the PSPs measured at the two electrodes are 2·6 mV: 1·3 mV. This gives a length constant of 450 μm/(ln 2·6/1·3) = 0·65 mm.

body (Figs 12, 13A). Small potential fluctuations recorded by an intracellular electrode in the DCMD, near the LGMD synapse onto the DCMD, were also recorded by an electrode in the cell body (Fig. 13B, C). LGMD-mediated PSPs recorded at resting potential had the size ratio 5 mV: 7 mV when recorded simultaneously by electrodes in the cell body: dendrites of the DCMD. It seems unlikely, based on these observations (see Fig. 13D, E for transmission of small potentials within the LGMD) and on the current/voltage relationship of the LGMD, that current passed into the LGMD dendrites was not reaching the LGMD–DCMD synapse, or that small potentials produced postsynaptically were not being recorded in the cell body. In addition the LGMD-mediated postsynaptic depolarizations on which conductance measurements were made fal[illegible]

the linear parts of the DCMD current/voltage curve. This leads further support to the conclusion that the change in size of the hyperpolarizing pulses injected into the DCMD during the LGMD-mediated PSPs is due to a chemically-mediated conductance increase at the LGMD–DCMD synapse rather than a voltage-sensitive conductance change in DCMD membrane (due to the depolarization during the PSP itself).

DISCUSSION

A number of lines of evidence in the data presented here indicate that the synapse between the LGMD and DCMD is chemical, rather than electrical as had been previously proposed (O'Shea & Rowell, 1975).

The latency from a spike in the terminals of the LGMD to a PSP in the dendrites of the DCMD is an order of magnitude slower than that in spike transmitting electrical synapses such as that between crayfish giant fibre and giant motoneurone (Furshpan & Potter, 1959; Giaume & Korn, 1983), or that between the hatchet fish giant fibre and motoneurone (Auerbach & Bennett, 1969*b*). In addition, the duration of the PSP seen at these electrical synapses is much shorter than that at the LGMD–DCMD synapse, or at spike-transmitting chemical synapses between Mauthner neurones and giant fibre in the hatchet fish (Auerbach & Bennett, 1969*a*), between Muller neurones and interneurones in the lamprey (Martin & Ringham, 1975) or at the squid giant synapse (Bullock & Hagiwara, 1957). Further conclusive evidence for chemical transmission comes from the demonstration of a conductance increase in the DCMD during the LGMD-mediated PSP. This conductance increase occurs over the range of membrane potentials where the current/voltage curve for the DCMD is linear, and was observed at several different membrane potentials. There did not appear to be any electrical component to the transmission, as neither hyperpolarizing nor depolarizing pulses of current were transmitted in either direction across the synapse.

Previous workers (O'Shea & Rowell, 1975) did not record from the pre- and postsynaptic neurones simultaneously but concluded that the LGMD–DCMD synapse is electrical because: it was able to transmit at very low temperatures; in low calcium salines; at very high impulse frequencies; with very little latency between a spike in the LGMD and one in the DCMD and, following an antidromic spike in the DCMD a small potential could be recorded in the LGMD. When the LGMD–DCMD synapse was tested for transmission in the cold, or in low calcium saline, no controls were used to test if, under the same stimulus conditions, chemically transmitting synapses would also function. The LGMD–DCMD synapse is located deep within the protocerebrum, and this could prevent the cold or low calcium saline penetrating to it. There is no doubt that it is unusual for a chemical synapse to sustain transmission of high frequencies of spikes. However, it is known for synapses that normally use graded chemical transmission to maintain a constant output for several seconds or minutes (e.g. Bennett, 1968; Burrows & Siegler, 1978; Siegler, 1981*a*,*b*; Blight & Llinás, 1980; Simmons, 1981, 1982*a*,*b*). The apparently instantaneous transmission between the LGMD and DCMD, reported by O'Shea & Rowell (1975), is clearly in error as the observed synaptic delay, when measured in my work, is in excess of 1 ms. The method employed here was to measure synaptic delay and conduction velocity directly with independent electrodes within the neurones, eliminating the problems

associated with estimating conduction times. The small potential seen by O'Shea & Rowell in the LGMD, following an antidromic spike in the DCMD is probably due to electrical coupling between the two electrodes, as no current of either sign could be passed across the synapse from DCMD to LGMD, indicating that no rectifying electrical synapse existed between them.

What are the special features of the LGMD–DCMD synapse?

Normally inputs and outputs are both spikes – that is the LGMD produces a spike, which produces a PSP in the DCMD, triggering a spike. The 1 ms latency between a spike in the LGMD terminals and a PSP or spike in the DCMD dendrites is the same irrespective of the frequency of LGMD spikes, up to an observed maximum of 400 Hz. The DCMD follows high frequencies of spiking in the LGMD for as long as the LGMD is induced to spike at that frequency. The DCMD does not show any evidence of synaptic depression or facilitation.

In Table 2 the features of the spike-transmitting synapse between the LGMD and DCMD are compared with other spike-transmitting synapses occurring between neurones which have themselves been well characterized. The size of the LGMD 'spike' is relatively small, and was not observed to overshoot zero potential at any point in the LGMD. The efficiency of transmission for the LGMD–DCMD synapse, at resting potential, is very high when compared with other spike-transmitting synapses (see Table 2). In general, synapses which transmit graded potentials and release transmitter tonically at rest have higher synaptic efficiency than those that normally transmit spikes. For example, ocellar receptors to second order interneurones in the dragonfly have a synaptic gain of 9 (Simmons, 1982*b*), locust ocellar 'L' neurone excitatory outputs to other 'L' neurones have a gain of 0·25 (Simmons, 1982*a*), locust ocellar 'L' neurone output to third order interneurones have a gain of about 0·5 (Simmons, 1981*b*), fly compound eye photoreceptors to first order interneurones have a gain of 4 (Dubs, Laughlin & Srinivasan, 1981) and crab muscle receptor organ to motoneurone synapse has a gain of 0·43 (Blight & Llinás, 1980). Not all synapses which transmit graded potentials release transmitter tonically at rest. In the lobster stomatogastric ganglion (Graubard, 1978), non-spiking neurones do not release transmitter at rest onto the postsynaptic gastric mill neurones; there is a 13 mV threshold to be exceeded before transmission occurs. At this synapse the synaptic gain measured to the peak response of the gastric mill neurone is about 0·12. In contrast, spiking neurones within the stomatogastric ganglion release transmitter tonically at resting potential and show graded synaptic transmission. The gain for graded transmission at one of these synapses is 0·18 at +10 mV presynaptic potential. This gain is higher than that observed at most of the other spiking chemical synapses mentioned in Table 2. The gain of any synapse is greatest when the presynaptic neurone is depolarized well above the threshold for transmitter release (Katz & Miledi, 1967), as is the situation in many of the synapses which normally transmit graded potentials and several which transmit spikes. Thus it is even more remarkable that the LGMD–DCMD synapse has such a high gain when the threshold for transmitter release is 13 mV above normal resting membrane potential. The gain for the LGMD–DCMD synapse is high even when the DCMD is hyperpolarized by 10 nA (i.e. 20 mV) so that the PSP does not evoke an

Table 2. *Comparison of the features of spike-transmitting synapses with those of the LGMD–DCMD synapse*

	Transmission: electrical (E) chemical (C)	Resitance (MΩ)	Length constant (mm)	Resting potential (RP) (mV)	Threshold for synaptic transmission (mV)	PSP reversal potential (mV)	Gain, PSP size: spike size	PSP duration (ms)	PSP rise time (ms)	PSP half-time to decay (ms)	Synaptic delay (ms)
Crayfish, giant fibre to giant motoneurone (Furshpan & Potter, 1959)	E	Pre 0·10 Post 0·0	1·1	−91 −75			19/82 = 0·24		0·45	1·4	0·12
Fish, giant fibre to motoneurone (Auerbach & Bennett, 1969*b*)	E	0·5 0·9	3·0	−70			8/90 = 0·09	0·63	0·21	0·21	
Fish, Mauthner to giant fibre (Auerbach & Bennett, 1969*a*)	C	0·74 0·52	3·1 3·0	−70	RP + 30	RP + 90	8/70 = 0·11	1·3	0·5	0·42	0·3–0·4
Lamprey, Muller to interneurone (Martin & Ringham, 1975)	C	1·0 6·0	1–1·7	−70 −55	RP + 50		2/85 = 0·02	40·0	5·0	20·0	
Squid, giant synapse (Bullock & Hagiwara, 1957; Hagiwara & Tasaki, 1958; Katz & Miledi, 1966, 1967)	C			−70 −70	RP + 25–30	RP + 60	2/50 = 0·02	3·5–4·5	1·1–1·5		1·5–2·0
Locust, LGMD to DCMD (present investigation)	C	0·5–0·7 1·0–2·2	0·5 1·3	−53	RP + 13	RP + 30	18/15 = 1·2	8·3	1·3	2·2	1·0

Threshold for synaptic transmission and PSP reversal potential are measured relative to resting potential.

regenerative activity (i.e. falls within the linear portion of the DCMD current/voltage curve).

The presence of a chemical synapse between the LGMD and the DCMD means that relatively small diameter processes of the LGMD can depolarize the DCMD. It is a combination of the amount of transmitter released from the LGMD and the potential gradient across the DCMD which can interact to influence the size of PSP induced in the DCMD. This situation contrasts with that at electrical synapses when the presynaptic neurone must have a greater charge density as this is the sole motive force for transmissions of the PSP across the synapse. The presence of a chemical synapse between the LGMD and DCMD reduces the current load on the LGMD and allows more integration to occur within its terminals.

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