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SYNAPSES UPON MOTONEURONS OF LOCUSTS DURING RETROGRADE DEGENERATION

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CONTENTS

Four interneurons of the ventral cord, the descending movement detectors (DMD) have symmetrical synapses upon the fast extensor tibiae (FETi) motoneurons on each side of the metathoracic ganglion. Each impulse in a DMD interneuron generates an excitatory post-synaptic potential (e.p.s.p.) of constant and similar amplitude in both FETi motoneurons of a normal locust. The symmetry provides inherent controls which makes this a convenient system to study the effect on inputs to a motoneuron caused by peripheral section of its axon. On the operated side the retrograde changes in the FETi motoneuron include, first an increased amplitude of the e.p.s.ps, then a brief period when they are variable, followed by a progressive reduction over a period of days. Other inputs to the FETi motoneurons from head, abdomen and tympanum also decline, but not at equal rates. Changes in e.p.s.p. amplitude are the opposite to those expected from simultaneous changes in the time constant. The observed changes in the e.p.s.ps are attributed to instability and then progressive loss of synapses upon the FETi motoneuron. The results show that the integrity of the motoneuron is essential for maintenance of its synaptic inputs.

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Introduction

Neurons of mammals are not individually identifiable and post-synaptic potentials (p.s.ps) recorded in their somata are summed potentials of many presynaptic fibres. To observe experimental effects upon the p.s.ps it is necessary to record from samples of neurons and even then it is difficult to distinguish between changes in the number or effectiveness of presynaptic fibres (Eccles, Libet & Young 1958). The locust, however, presents a convenient situation with neither of these disadvantages. The fast extensor tibiae (FETi) motoneuron of the metathoracic ganglion innervates the main jumping muscle of the hind leg by nerve 5, and in this ganglion has the largest soma, which is frequently visible through the sheath. A microelectrode in this soma records excitatory post-synaptic potentials (e.p.s.ps) that correspond 1:1 with impulses in four large descending visual interneurons, the descending movement detector, DMD, neurons, of the ventral cord which are excited by any novel movement in the visual field (Burrows & Rowell 1973). The synapses of these interneurons are distributed symmetrically upon the FETi motoneurons supplying the two legs. The e.p.s.ps which any of the fibres cause simultaneously in the two FETi motoneurons are of the same amplitude and distinct from the e.p.s.ps caused by impulses in the other fibres. This symmetrical arrangement is favourable for examining the effect of an experimental treatment on one side because the other side acts as a control against changes such as those due to ageing. We have examined the effect on these synapses of cutting the axon of the FETi motoneuron by removal of one leg, and found changes which can be related to the retrograde degeneration that has already been described histologically after section of the axon of insect motoneurons.

Section of an axon causes degeneration distal to the cut in the cockroach (Bodenstein 1957; Hess 1960) and in locust leg motoneurons (Rees & Usherwood 1972). The axon swells, then fragments and is replaced by glial cells, while the myelin sheath also disintegrates. Most interneurons in the neck connectives of the locust, however, show no degenerative changes 23 days after section (Boulton & Rowell 1969). The timing of the first histological changes varies greatly in different species, from only a few hours in crickets (Edwards & Palka 1971), 24 h in *Calliphora* (Boeckh, Sandri & Akert 1970) and 2 days at 30 °C in locust motoneurons (Rees & Usherwood 1972). Transmission at neuromuscular junctions of locusts persists for 9–24 days after section and ability to conduct an impulse distal to the section persists longer than neuromuscular transmission (Usherwood 1963). In crustacea, transmission can persist for more than 100 days after section (Hoy, Bittner & Kennedy 1967).

Changes also occur in the soma and dendrites when the axon is cut. Within 4 days the previously inexcitable soma membrane becomes electrically excitable in the cockroach (Pitman, Tweedle & Cohen 1972*b*) and a perinuclear ring of basiphilic granules forms at about the 3rd day (Cohen & Jacklet 1965; Jacklet & Cohen 1967) caused by a rapid synthesis of ribosomes which is interpreted as the first stage of regeneration (Young, Ashhurst & Cohen 1970). In the 'majority of cells examined' section of the axons of motoneurons (and the rest of the peripheral nerve) is followed by a marked reduction in the dendritic branches stained within the neuropile (Pitman, Tweedle & Cohen 1972*a*). These observations suggest that, at least in the cockroach, there is a retrograde degenerative process that is closely comparable to that described for vertebrate central and sympathetic neurons. Therefore it might be supposed that in insects there is a loss of synaptic inputs to a motoneuron when its axon is cut.

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Methods

One metathoracic leg was induced to autotomize in the last nymphal stage or in a teneral adult of *Schistocerca gregaria* of either sex reared in the laboratory. All operated locusts were then maintained at 23 °C. The locust was mounted ventral side uppermost with the metathoracic ganglion exposed for recording intracellularly from the somata of the FETi motoneurons as previously described (Hoyle & Burrows 1973). The FETi motoneuron to the intact leg was identified by evoking an antidromic impulse from stimulating leads implanted in the extensor tibiae muscle. On the operated side the antidromic impulse was evoked by stimulating the stump of nerve 5 and together with the characteristic position of the soma and the pattern of synaptic activity recorded gave unequivocal identification. As a further verification the motoneurons often were stained by injecting cobalt chloride through the recording microelectrode and subsequent precipitation of the black sulphide (Pitman *et al.* 1972*a*). Pairs of wire-hook electrodes on each of the pro-mesothoracic connectives recorded the impulses of the descending visual interneurons which were evoked by hand movements against the general laboratory background. Criteria for the identification of the impulses of the visual interneurons in recordings made from whole connectives have been given (Burrows & Rowell 1973). The DCMD has spikes of large amplitude elicited by small movements anywhere in the visual field of the eye contralateral to the connective from which the recording is made. The DIMD spike is smaller and can be recognized by the response features of the neuron, and by relating it to DCMD spikes in the opposite connective.

Results

Normal responses of the FETi motoneurons

Each FETi motoneuron has a number of responses that are independent of inputs from its own leg, so that symmetrical tests of normal inputs can be made on the two sides of the locust.

Synapses from visual interneurons

Descending from the brain in each connective are the large diameter axons of two interneurons which respond to any movement of a contrasting object in the visual field. These are the descending movement detector (DMD) interneurons (Burtt & Catton 1954; reviewed by Rowell 1971). Movement seen by the left eye excites impulses in the descending contralateral fibre (DCMD) in the right connective and usually fewer impulses in the descending ipsilateral fibre (DIMD) in the left connective. The terms ipsi- and contralateral therefore are relative to the eye which sees the stimulus. The impulses in the interneurons have constant and distinguishable spike heights and all excite the FETi motoneurons on each side causing one e.p.s.p. for each impulse (figure 1). The responses of the normal FETi motoneuron are therefore an indication that all inputs to the operated side are functional as far as the ganglion. The e.p.s.ps are clearly distinguishable from all other p.s.ps in the FETi motoneurons by their size, shape and relation to the stimulus, but we always took the precaution of recording the descending impulses in both connectives so that irregularities in transmission from the presynaptic impulse to the e.p.s.p. were not missed. In normal locusts in which impulses can be recorded in the interneurons without corresponding e.p.s.ps in the FETi motoneurons, we suppose that the electrodes on the connectives block the impulse transmission (figure 1*c*, *d*). In operated locusts this failure in the experimental procedure is revealed because e.p.s.ps are lacking on the control

side. The e.p.s.ps caused by impulses in either DCMD are of the same amplitude on the two sides with similar summation properties. Often the first e.p.s.p. of a group is larger than those that follow (figure 1*a*). The e.p.s.ps of the two DIMD fibres are also similar on the two sides and are usually of larger amplitude than those of the DCMD. The time constant of the e.p.s.ps caused by both types of interneurons is 20–25 ms.

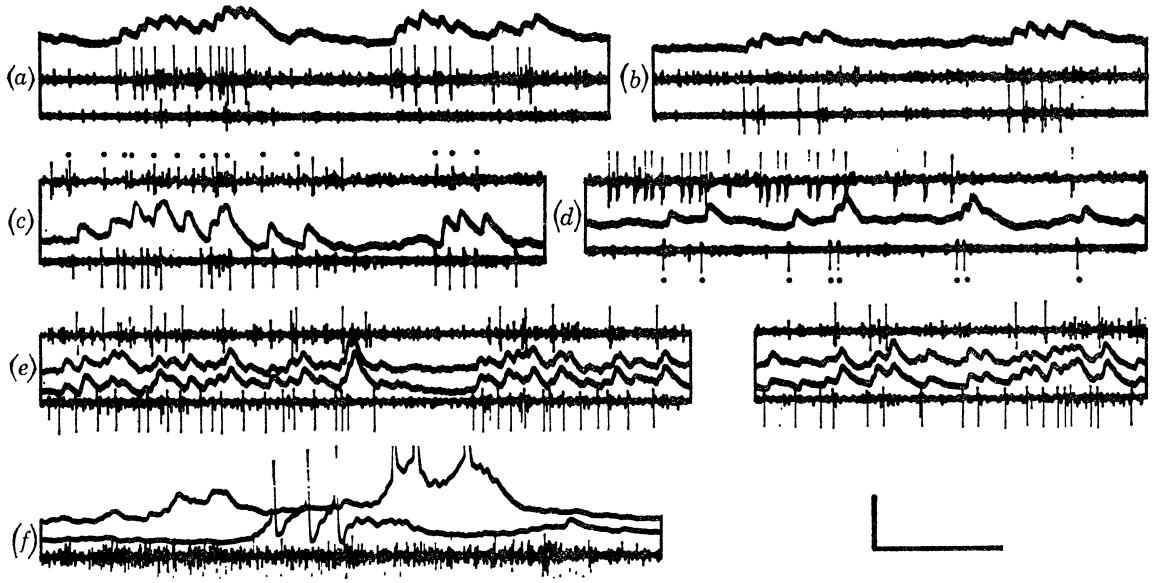

FIGURE 1. Connexions of the descending movement detector interneurons with the fast extensor tibiae (FETi) motoneurons of the metathorax. (*a*, *b*) The e.p.s.ps caused by spikes of the DCMD interneurons in the connective ipsilateral (second trace (*a*)) or contralateral (third trace in (*b*)) to the FETi motoneuron. (*c*, *d*) The e.p.s.ps caused by spikes of the DIMD interneuron (dotted) in the ipsilateral (first trace in (*c*)) or contralateral (third trace in (*d*)) connective. (*e*) The amplitude of the e.p.s.ps from a particular interneuron is the same in both FETi motoneurons (traces 2 and 3). (*f*) Touching the ventral surface of the first few abdominal segments evokes spikes in both motoneurons (traces 1 and 2). Calibration: vertical (*a–e*) 2 mV, (*f*) 10 mV; horizontal 100 ms.

That the interaction between the four visual interneurons and the FETi motoneuron is monosynaptic is a reasonable inference from two constant physiological features of a large number of normal locusts (Burrows & Rowell 1973). First, the relation between the interneuron spikes and the e.p.s.ps is one-to-one up to a frequency of 200 Hz. Secondly, the latency is 0.9 ± 0.1 ms between the presynaptic spike recorded at its entry to the metathoracic ganglion and the onset of the e.p.s.p. We lack the ultimate anatomical demonstration that a third neuron is not involved, but none of the arguments would be affected if a third neuron were intercalated, for the experiments refer to the input to the motoneuron. The anatomy, so far as it can be interpreted, does not contradict the idea that the relation is monosynaptic (see below).

Auditory and abdominal inputs

Several auditory interneurons run up the connectives from the metathoracic ganglion. Any sharp sound causes a brief depolarization of both FETi motoneurons but there is not a one-to-one correspondence of p.s.ps with the ascending impulses that are recorded in the thoracic connectives. The FETi responses are unlikely to be caused directly by auditory sensory fibres entering the metathoracic ganglion because they habituate more rapidly than the primary responses and cobalt filling fails to show any motoneuron or sensory neuron arborizations

which cross the mid-line. The interneuron impulses in the thoracic connectives, however, are a guarantee that the auditory excitation has passed through the metathoracic ganglion. In normal locusts excited by a sudden loud noise, the response in the two FETi motoneurons is symmetrical but we did not test the effect of section of one auditory nerve or explore further its mechanisms.

A delicate touch to the abdomen is a strong stimulus causing depolarization of both FETi motoneurons via ascending interneurons of the abdominal connectives. The response is mainly a smooth depolarization of the FETi motoneurons and where there are distinguishable unitary p.s.ps they do not correspond with impulses in the connectives. The response is part of a complex movement by which the hind leg fends off any object touching the abdomen, and the depolarization is apparently mainly contributed by local metathoracic interneurons which control groups of motoneurons (Burrows & Horridge 1974). A feature of the response is that spikes are readily elicited in the FETi motoneurons (figure 1*f*), whereas they are not by auditory and visual inputs.

Antidromic spikes

The antidromic spike invades the soma passively with an amplitude of 10–24 mV and an exponential decay with a time constant that ranges from 4 to 12 ms in different locusts. In a normal locust the amplitude and time constant of the antidromic spike is similar in the two FETi motoneurons. This potential can therefore be used to observe any changes in the membrane properties, for example resistance changes of the motoneuron as a consequence of axotomy. This is essential when differences in the amplitude of p.s.ps on the operated as compared to the control side are to be interpreted, because a reduction in time constant over the critical range could account for a reduction in the amplitude of the p.s.ps observed. An electrode in the soma, however, observing changes in the time constant of the antidromic spike gives no information about relative changes of resistance of subsynaptic compared to non-synaptic membrane, but merely the change within range of the electrode.

The part of the axon proximal to the cut continues to propagate impulses for as long as our tests continued, up to at least 30 days after removal of the leg. This persistance was an essential component of our method of identifying the FETi motoneuron on the operated side by its antidromic spike.

Responses after removal of the leg

Early changes on the operated side

During the first 24 h the e.p.s.ps caused by both DCMD fibres are of larger amplitude in the FETi motoneuron on the operated side (figure 2*a*). E.p.s.ps caused by the DIMD fibres, however, remain the same amplitude on the two sides (figure 2*b*). The response to an auditory input is augmented on the operated side (figure 2*d*). The amplitude of the e.p.s.ps on the operated side caused by DCMD or auditory input is increased although the time constant of the FETi on that side is invariably shorter than on the control side (figure 2*e*, *f*). A shorter time constant, other factors being equal, would be expected to reduce the amplitude of the e.p.s.ps, therefore changes in the postsynaptic membrane alone cannot be the cause of the observed changes. Moreover, a change in the postsynaptic membrane would affect all inputs, but e.p.s.ps from the DIMD are unchanged in amplitude.

The abdominal stimulus causes typical responses in which impulses are elicited in the FETi motoneurons on both sides (figure 2*c*) for 24 h after the operation, but thereafter spikes are

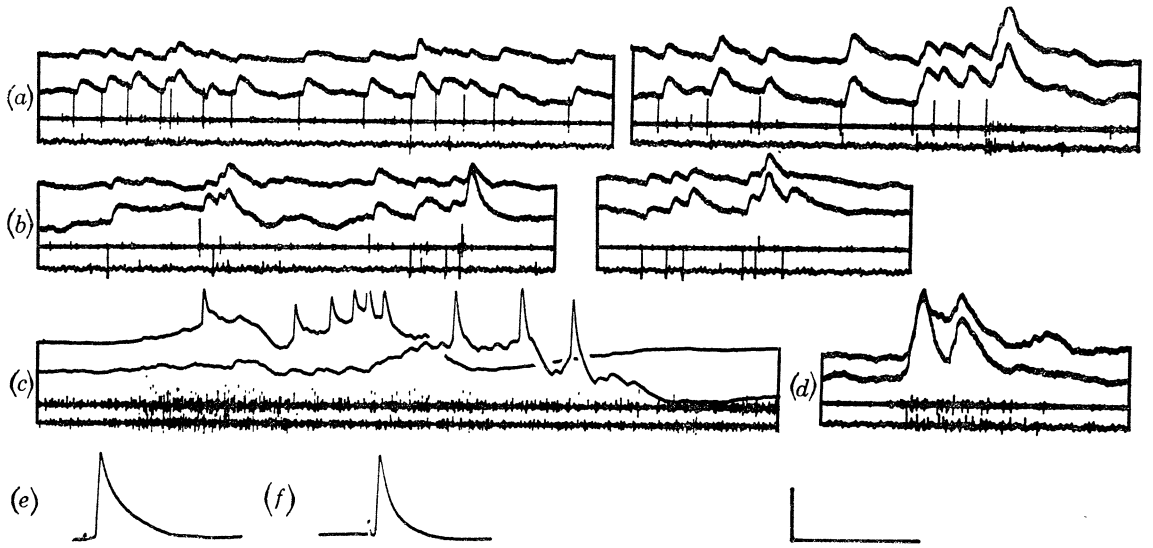

FIGURE 2. Enhancement of the e.p.s.ps in the FETi motoneuron 20–30 h after axotomy. (*a*, *b*) The increased size of the e.p.s.ps from DCMD (*a*) or DIMD (*b*) spikes in the FETi motoneuron axotomized for 24 h (2nd trace). Trace 1 shows the normal FETi, trace 3 the connective ipsilateral, and trace 4 the connective contralateral to the axotomized FETi motoneuron. (*c*) Touching the abdomen evokes spikes in both FETi motoneurons. (*d*) A loud clap causes a synchronous response in both. (*e*, *f*) The antidromic spike in the axotomized motoneuron (*f*) has a shorter time constant than the control side (*e*). Records (*a*, *b*, *d*–*f*) are from the same locust, (*c*) from another. Calibration: vertical (*a*, *b*, *d*) 2 mV, (*c*, *f*) 10 mV; horizontal (*a*–*d*) 100 ms, (*e*, *f*) 40 ms.

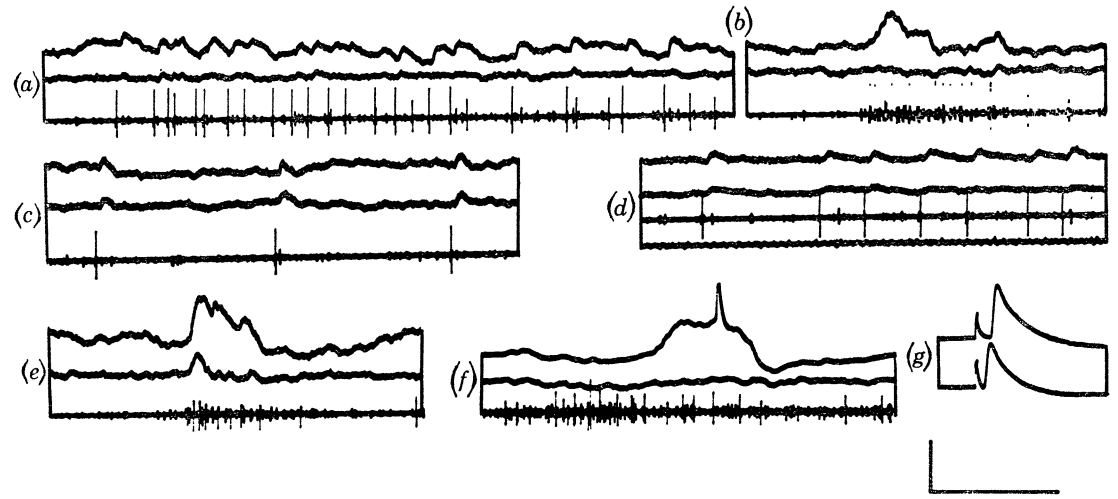

FIGURE 3. Variability in the amplitude of the e.p.s.ps 40–50 h after axotomy. (*a*) In a long sequence of DCMD spikes in the connective ipsilateral (trace 3) to the axotomized FETi motoneuron (trace 2) the occurrence and amplitude of the e.p.s.ps is variable. (*b*) With a high frequency of DCMD spikes the e.p.s.ps are absent. (*c*) Later in the same preparation the e.p.s.p. was visible clearly in response to a low frequency of DCMD spikes. (*d*) A different locust showing the failure of the e.p.s.ps upon repetition of the DCMD spikes. (*e*) The response to a loud clap is reduced. (*f*) Touch to the abdomen fails to evoke spikes and even e.p.s.ps. (*g*) The time constant of the axotomized motoneuron is somewhat shorter than the control. The locust in (*a*–*c*, *e*–*g*) had its leg removed for 42 h, that in (*d*) for 51 h. Calibration: vertical (*a*–*e*) 2 mV, (*f*) 20 mV and 10 mV, (*g*) 15mV; horizontal (*a*–*f*) 100 ms, (*g*) 40 ms.

not generated on the operated side. The locust remains capable, however, of making a one-legged evasive jump. As the FETi motoneuron provides the extensor thrust for a jump, and clearly a missing leg cannot participate in the jump, there may be a plastic change that is unrelated to the others reported here. No mechanism is known, however, for the suppression after 24 h of the ability of the FETi motoneuron to initiate orthodromic impulses, at a time when at least some of its inputs generate e.p.s.ps larger than usual.

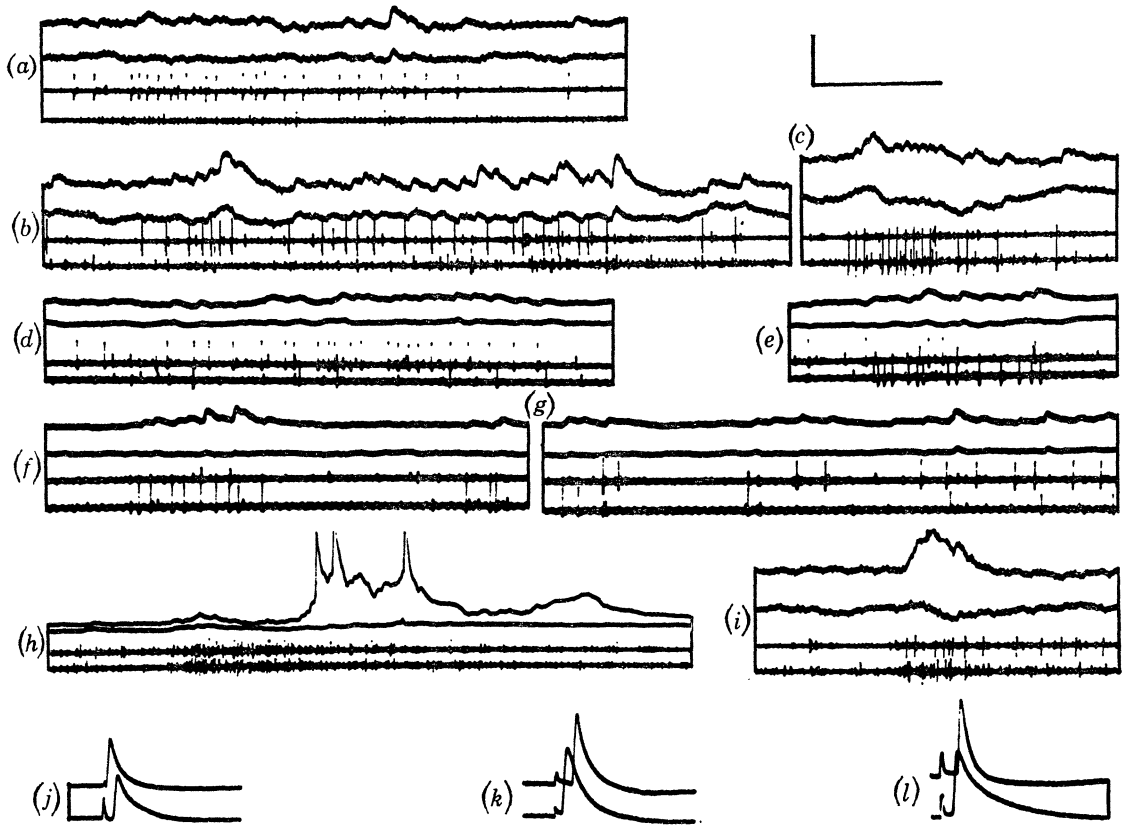

FIGURE 4. Reduction in the amplitude of the e.p.s.ps 70 h or more after axotomy. (*a*) 72 h after axotomy; only the DIMD in the connective ipsilateral (trace 3) to the injured FETi motoneuron (trace 2) produces an e.p.s.p. but this is of reduced amplitude. (*b*, *c*) 96 h after axotomy; DCMD spikes produce e.p.s.ps of reduced and variable amplitude which fail at high frequency. (*d*, *e*) 192 h after axotomy; e.p.s.ps to both DCMD interneurons have disappeared, but e.p.s.ps caused by the DIMD neurons remain at a reduced amplitude. (*f*, *g*) More than 192 h after axotomy; the same picture remains as in (*d*, *e*). (*h*) Touching the abdomen of the same locust as in (*a*) fails to cause e.p.s.ps or spikes. (*i*) Same locust as (*b*, *c*); a loud clap fails to evoke any response. (*j–l*) The antidromic spike in the axotomized motoneuron (lower trace) now has a longer time constant than the control. Times after axotomy are: (*j*) 192 h, (*k*) 72 h, (*l*) 120 h. Calibration: (*a–c*, *i*) 2 mV, (*d–g*) 6 mV, (*h*) 15 mV, (*j–l*) 10 mV; horizontal (*a–i*) 100 ms, (*j–l*) 40 ms.

Variability on the operated side

About 2 days after removal of the leg the amplitude of the e.p.s.ps caused by the visual interneurons becomes variable (figure 3). The variability appears at about 27 h, is most common at 40–70 h and is less obvious later. It takes many forms: (*a*) A hit and miss occurrence of the e.p.s.p. in relation to the presynaptic spikes. For example the first and the last spikes of a sequence may evoke e.p.s.ps but those in the middle fail (figure 3*a*). (*b*) A group of spikes at high frequency may fail to evoke e.p.s.ps (figure 3*b*) but a subsequent low frequency causes e.p.s.ps of a consistent amplitude (figure 3*c*). (*c*) The amplitude of the e.p.s.ps may decline upon repetition

(figure 3*d*). The e.p.s.ps occurring at the same time upon the control side show no such variability.

The auditory and abdominal inputs have less noticeable variability, but change relative to the control. The amplitude of the auditory response is reduced but remains of a similar form to that on the control side (figure 3*e*). The abdominal input is ineffective in evoking spikes and may fail to cause a depolarization (figure 3*f*). The time constant of the antidromic spike lengthens so that it approaches once again that of the control side. Therefore the variability in the DMD responses is most easily attributed to presynaptic instability rather than postsynaptic oscillations of responsiveness.

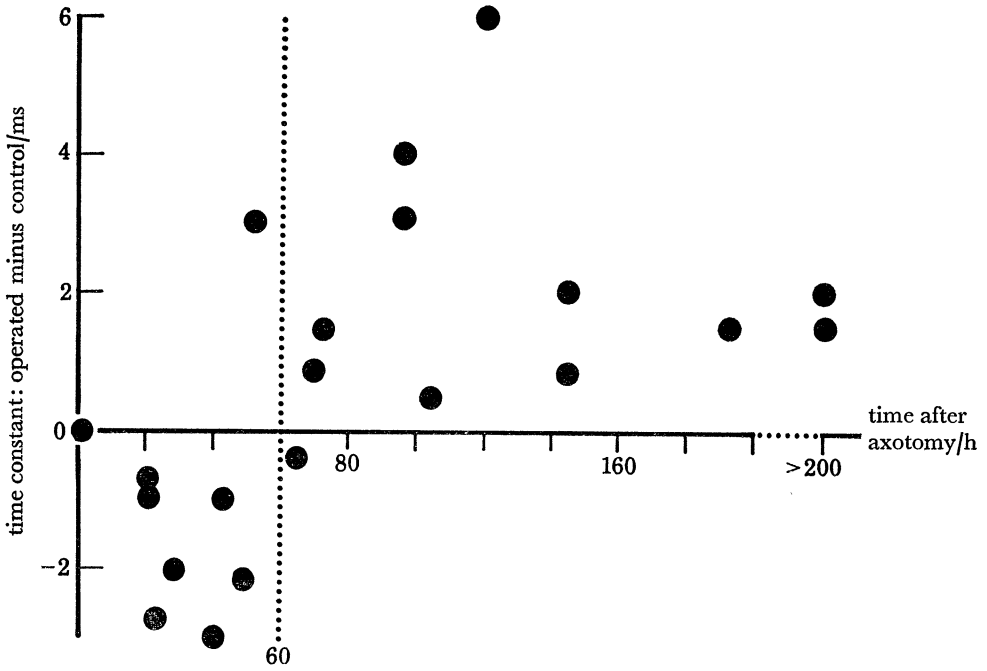

FIGURE 5. Graph to show the difference in time constant of the FETi motoneuron of the control side and the axotomized FETi on the opposite side of the ganglion. Initially the operated FETi motoneuron has a shorter time constant but 60 h or more after the operation has a longer one.

Later changes on the operated side

Sixty hours after removal of the leg the general pattern is a progressive decline in the amplitude of the inputs upon the FETi motoneuron on the operated side (figure 4). The inputs to the normal side are of the same amplitude as those in an unoperated locust. The DIMD inputs are notably more persistent than the DCMD inputs (figure 4*a*, *b*): DIMD inputs fall to only about half normal height even 200 h postoperatively, whereas DCMD inputs have fallen to a tenth of that on the control side by about 72 h (figure 4*d*–*g*). In locusts examined 200 h after leg amputation, e.p.s.ps caused by the DCMD neurons could often be distinguished, though usually they were barely visible above noise. Nevertheless this is an indication that the presynaptic impulses reach the membrane of the FETi motoneuron.

Auditory inputs fall progressively from 40 to 120 h postoperatively and disappear to nothing (figure 4*i*). Abdominal inputs have already fallen to zero by 60 h (figure 4*h*). Impulses recorded in the thoracic connectives show that auditory and abdominal stimuli cause excitation that

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TABLE 1. SURVEY OF 20 SCHISTOCERCA IN WHICH RECORDINGS WERE MADE FROM BOTH FETi MOTONEURONS AND BOTH PRO-MESOTHORACIC CONNECTIVES

Numbers in columns 2–5 and 7 show the ratio of the amplitude of the p.s.ps on the operated to those on the normal side

operated (h)	DIMD ipsi.	DIMD contra.	DCMD ipsi.	DCMD contra.	auditory normal	auditory operated	abdominal normal	abdominal operated	spike height/mV	
									normal	operated
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
20	1	1	abs.	1.2	g	1	g	g, spikes	15	13
20	1	1	3	1	g	1	g	g	12	11.5
24	1	1	1.5	1.5	g	2	g	g, spikes	13	12.2
27	1	1	1.5	abs.	g	2	g	p	12	14
40	1	1	abs.	0.8	g	1	g	p	13.5	15.5
42	0.1	0.1	0–0.3	0	g	0.5–1.0	g	p	17	14
48	0.5	0.5	0–0.7	0.3–0.7	g	0.6	g	p	21	19
51	1.5	1–2	0.3–1.5	0.7–1.5	g	1.0	g	p	18	24
66	0	0	0–0.5	0–0.3	a.c.s.	a.c.s.	g	o	12	10
70	0.5	0.3–0.5	a.c.s.	0.5	g	0.3	g	o	15	13
72	abs.	0.5	0.1	0.1	g	1	g	p	18.3	17.5
96	0.6–0.8	0.6–0.8	0	0	g	0.5	g	o	14.5	14.0
96	1	0	0.5	0.3	g	0	g	p	24	12
96	0.3	0.1	0.5	0.5	g	0.5	g	p	7	11
120	0–0.3	0	0	0	g	0.3	g	p	12	10
144	0.6–0.8	0.6–0.8	0.1	0.1	a.c.s.	a.c.s.	g	o	14.5	14
144	0.6	0.6	0.1	0.1	g	0	g	o	14	13.3
192	0.5	0.5	0.1	0.1	a.c.s.	a.c.s.	g	o	17	16.3
200	0.5	0.5	0.1	0.1	g	0	g	o	13	12.5
200	0.1	0.1	0.1	0.1	g	0	g	o	9	16

abs., absent on both FETi motoneurons.
a.c.s., absent on control side, therefore comparison impossible.
g, good input visible.
p, poor input.
o, no input visible.

passes through the ganglion, so presumably the failure to see e.p.s.ps on the FETi motoneurons is a specific effect on certain synapses or local interneurons only.

The time constant of the antidromic spike continues to increase (figures 4*j–l*, 5). The increased time constant would be expected to enhance the amplitude of the e.p.s.ps. The observation that the e.p.s.ps decrease in amplitude implies that changes in the properties of the postsynaptic membrane are not an adequate explanation of the changes; the same conclusion reached for the early changes. In no locust have we observed a changed transmembrane potential in the FETi motoneuron on the operated side. In no locust did axotomy cause an increased electrical excitability of the membrane of the soma as happens in the cockroach (Pitman *et al.* 1972*b*).

All physiological results are summarized in table 1.

Morphology

The DCMD interneuron has been filled by electrophoresis with cobalt ions and its arborization in the metathoracic ganglion described (O'Shea, Rowell & Williams 1974). The main axon runs in a straight line towards the posterior of the ganglion from its entry on the dorsal side of the anterior connective. A lateral branch runs to each region of nerve 5 motoneurons. Another symmetrical pair of thin branches run transversely in the abdominal part of the ganglion, and there is an arborization of a single branch in the anterior mid-line of the metathoracic ganglion.

This pattern of branching is unchanged at the gross level a month after the removal of the leg (figure 6*d*).

The most obvious morphological effect of removing the leg is the thinning of nerve 5 as the sensory fibres, with somata in the leg, disappear. This thinning is not obvious in the first few days but a month after the operation nerve 5 is no more than a quarter of its normal thickness. In these animals the somata of the motoneurons of nerve 5 persist in their proper places but are reduced in size (figure 6*c*). Unlike motoneurons of vertebrates, locust motoneurons do not die when their axon is cut, and we use the transmission in the cut axon to generate the antidromic spike by which the motoneuron is identified.

The FETi motoneuron has a dendritic pattern that is the most restricted in locality of any of the larger motoneurons of this ganglion. Shortly after the axon leaves the soma there is a mass of extremely fine dendrites and a few thicker ones (figure 6*b*).

The dendrites of the FETi motoneuron do not disappear when the leg is removed, but the inconsistency in the filling of fine branches by cobalt ions does not allow a more definite statement about morphological changes.

There are situations in which the anatomy of neurons at the light-microscope level can be directly related to the physiological interactions between the same neurons. For example, when two neurons are clearly in contact at few large anatomical synapses, it is a ready assumption, but still difficult to prove, that physiological synapses are located at these places. On the other hand when two neurons are nowhere in contact it is clear that a physiological interaction must be attributed to other neurons lying between. When the two neurons, however, have numerous fine branches that occupy the same region of neuropile, and when the method of staining them is likely to be incomplete, the physiological interaction is more reliable than the anatomy as an indicator of change. The terminal arborizations of the DCMD interneuron suffer no changes at the gross level after removal of the leg, and the dendrites of the FETi motoneuron do not disappear. The tendency of the finest dendrites to fill with the cobalt ions may change on

removal of the leg. For these reasons, therefore, we consider anatomical details at the light microscope level to be unable to demonstrate that anatomical synapses change in number. Possibly electron microscopy of identified neuron branches would show correlations with the physiological changes.

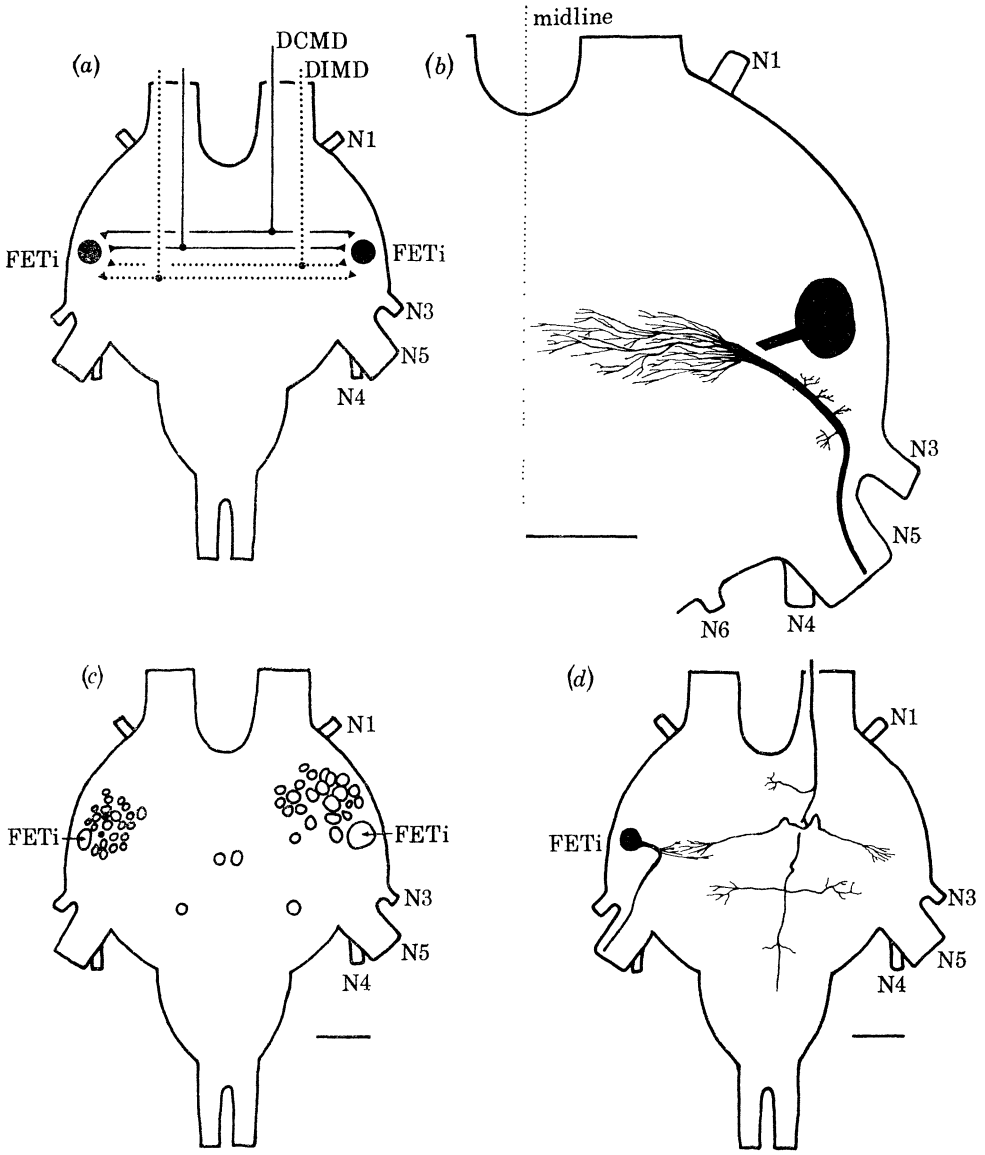

FIGURE 6. Summary of the anatomy as seen from the ventral side. Scale bars 200 μm. (*a*) Schematic representation of the symmetrical connexions of the four descending visual interneurons (DCMD and DIMD) with the fast extensor tibiae (FETi) motoneurons on the two sides of the metathoracic ganglion. (*b*) Camera lucida drawing of an FETi motoneuron which has been stained by injecting cobalt ions through an electrode inserted into its soma. This procedure causes some swelling of the soma. The dendritic branches are profuse and not all are drawn. (*c*) Drawing of the metathoracic ganglion in which nerve 5 was treated with cobalt ions which diffused into motoneurons including the FETi, with axons in this nerve. On one side (the left of the figure) the leg had been removed 4 weeks previously. (*d*) Drawing of a preparation in which cobalt ions were diffused through nerve 5 of a leg removed 2 weeks previously and at the same time through the meso-metathoracic connective on the other side. Only one of the DMD neurons is drawn and the profile of the FETi motoneuron is indicated.

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Discussion

The constant height and shape of the e.p.s.ps show that there is a constant relation between the synaptic input from the DMD neurons to the FETi motoneuron, as recorded by a microelectrode in the soma, but it is not possible to infer how this arises. There may be one unique synapse or a constant pattern of them, but the physiological properties have a basis in constancy of anatomy and release of transmitter. If it were not so the variations caused by removal of the leg would not have been so clear.

One might suppose, from a study of the scanty literature on the topic, that the morphological changes observed in the somata of insect motoneurons when their peripheral nerve is cut are caused by the severance of the axon (Cohen & Jacklet 1965). Removal of a leg, however, causes the degeneration of all the sensory fibres which have their somata in the periphery, as well as cutting the axons of the motoneurons. The sensory input to the FETi motoneuron from its own leg appears to be indirect, via interneurons of the ganglion (Burrows & Horridge 1974). We do not have, therefore, a situation in which the effects of cutting the motor axon are complicated by the degeneration of some of the synaptic terminals upon the motoneuron. The situation is more complicated in the mammalian cord, where it has been found that loss of some inputs to motoneurons (Eccles, Krnjević & Miledi 1959) and other central neurons (Kjerulf & Loeser 1973) causes changes, usually hypersensitivity, of the postsynaptic neuron to stimulation of surviving inputs. Exactly this question, however, could be examined in the locust by cutting one thoracic connective and testing the FETi responses to the DMD fibres in the other connective after various intervals, but the bilateral symmetry would no longer provide a control on one side. The mechanisms of synaptic changes following presynaptic or postsynaptic damage to neurons are important because several medical conditions such as pain following neural lesions, tic douloureux and focal epilepsy have been attributed to changes of this type.

A variety of hypotheses can be found for the cause of the physiological synaptic changes reported here. The change may be in the failure of the FETi motoneuron to respond, or in the failure of the presynaptic arborizations to transmit, or both. The explanation that we favour is that the axon arborizations of the DMD interneurons that are presynaptic to the FETi dendrites become detached, although the evidence is slender.

A general feature of all the preparations was the change in the time constant of the FETi motoneuron on the operated side, which is reasonably attributable to a change in membrane resistance, but the changes recorded should have the effect of altering the amplitude of the postsynaptic potentials in the opposite direction to that observed. There is no indication from the height of the antidromic spike of a change in membrane potential on the operated side so that the changes in e.p.s.p. height cannot be attributed to this cause. The main feature, however, which rules out changes in the FETi as the main cause of the e.p.s.p. changes is that some of the synaptic inputs fail before others, and visual, auditory and abdominal inputs fail to different extents in the same locust. Explanations involving parts of the operated FETi motoneuron, such as supposing that some of its dendrites fail before others, requires additional assumptions about the limitation of some dendrites to particular inputs. For the same reason, of economy of hypothesis, we do not propose that the postsynaptic potentials are amplified by dendritic spikes and that what we observe is a failure in the conversion process. Two observations suggest that the potentials recorded in the soma are p.s.ps and not the remnants of dendritic spikes. First, the amplitude of the potentials can be increased by hyperpolarizing the soma and are not blocked

abruptly. Secondly, about 3 days after leg amputation, the amplitude of the potentials caused by the visual interneurons is variable. This would not be expected of dendritic spikes. Therefore the most acceptable hypothesis is that the failure of the synaptic input on the operated side is a progressive disappearance of numerous anatomical synapses which have a fixed anatomical arrangement in the normal locust, or at least a progressive physiological failure of fixed synapses to transmit. The changes are therefore an effect of the damaged FETi motoneuron upon the fibres that are presynaptic to it, and their surrounding glia. In comparison it is known that when the axon of vertebrate motoneurons is cut the synapses are physically swept off the soma surface by proliferating microglia cells (Blinzinger & Kreutzberg 1968). The strong adhesive which normally holds pre- and postsynaptic membranes together, even when the tissue is homogenized (Gray & Whittaker 1960), must be released.

The nature of this failure can be approached by the irregularity of the postsynaptic potentials on the third day after the operation. At this stage, and at no other, the effectiveness of the interneuron impulses waxes and wanes, sometimes producing a full-sized e.p.s.p. on the operated FETi motoneuron but at other times being ineffective, even in a rapid burst. The inference is that there is some instability in the milieu of the interneuron terminals; either the anatomical synapses are in process of falling off and are in motion, or nearby glial cells cause changes in the ionic milieu so that the interneuron terminals are at times inexcitable. Anatomical studies at this stage may throw further light on the nature of the loss of physiological synaptic inputs.

This work was done while one of us (G. A. H.) was a visiting fellow of Balliol College, Oxford, with facilities in the Department of Zoology, Oxford University.

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