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STRUCTURE AND SYNAPTIC ACTIVATION OF THE FAST COXAL DEPRESSOR MOTONEURONE OF THE COCKROACH, *PERIPLANETA AMERICANA*

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INTRODUCTION

Now that behavioural descriptions of walking in the cockroach are available (Delcomyn, 1971) attempts are being made to elucidate the neuronal mechanisms involved in leg movements. Innervation of some of the muscles utilized in walking has been studied and the patterns of activity of motoneurones to both coxal levator and depressor muscles have been described in the intact, free-walking animal (Pearson, 1972) and in dissected preparations in which sensory input from the legs has been interrupted (Pearson & Iles, 1970). The basic features of the normal pattern are retained in the de-afferented preparation leading to the conclusion that walking movements are to some extent centrally programmed. Further study of this central system requires intracellular recording from motoneurones within the thoracic ganglia and examination of their synaptic inputs.

The general organization of cells and fibres in the cockroach metathoracic ganglion has been described (Guthrie & Tindall, 1968; Pipa, Cook & Richards, 1959) but few details are yet available for single identified cells. A method of injecting individual crustacean motoneurones with the fluorescent dye Procion Yellow was introduced by Stretton & Kravitz (1968). This technique allows detailed reconstruction of cell structure to be made and has since been applied to insect neurones (Bentley, 1970; Hoyle & Burrows, 1970), leech motoneurones (Stuart, 1970) and various mammalian spinal neurones (Jankowska & Lindstrom, 1970*a*, *b*, 1971). In this paper a modified technique which avoids the necessity of locating and penetrating the cell with a microelectrode (Iles & Mulloney, 1971) is used to fill the largest coxal depressor motoneurone of the cockroach with Procion Yellow. This cell is active during leg depression when a cockroach jumps or is running rapidly (Pearson, 1972). The histological detail obtained from dye injection has facilitated an analysis of the jumping response.

A stimulus to the anal cerci of a cockroach induces a jump followed by running (Roeder, 1959; Pearson & Iles, 1970). Afferents from receptors in the cerci are known to excite giant axons which originate in the last abdominal ganglion (Hess, 1958; Harris & Smyth, 1971; Milburn & Bentley, 1971). The giant axons ascend in the abdominal cord to the thoracic ganglia and have been assumed to synapse with leg motoneurones completing an 'evasion reflex' (Roeder, 1948, 1962). However, it has recently been suggested that axons smaller than the giant fibres (but also excited by

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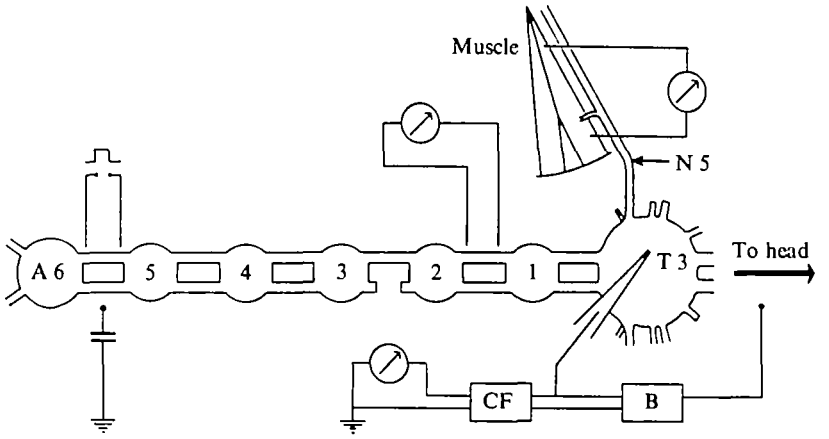

Fig. 1. The experimental agreement for microelectrode recording from metathoracic motoneurones (ventral view). A1–A6, the abdominal ganglia; T 3, metathoracic ganglion; N 5, nerve 5; CF, cathode follower; B, bridge circuit.

cercal stimuli) are responsible for transmission in the abdominal cord to thoracic motoneurones (Dagan & Parnas, 1970), and the function of giant fibres is unclear. In the present paper microelectrode recording from thoracic motoneurones has failed to detect any post-synaptic response to giant fibre stimulation, but has found an excitatory input from smaller abdominal afferents, in agreement with Dagan and Parnas.

PREPARATION AND METHODS

Muscle innervation

The metathoracic coxal depressor muscles, 178, 179, 177D, E (notation of Carbonell, 1947) receive five motor axons in branch 5r1 (notation of Pipa & Cook, 1959) of metathoracic nerve 5. These axons have been classified (Pearson & Iles, 1971) as one fast axon (D_f, diam. 25 μm), one slow axon (D_s, diam. 15 μm) and three common inhibitory neurones (D_{1-3}, diam. 5 μm).

Dye injection

The technique used has been described previously (Iles & Mulloney, 1971). The right metathoracic coxa of an adult male cockroach was rotated to expose the dorsal surface which was cut away to reveal muscle 178. This muscle was removed freeing it carefully from nerve branch 5r1 which was cleared back to the main nerve 5. The connectives and all nerves other than five entering the metathoracic ganglion were cut. The ganglion and attached nerve were then excised completely, placed in one half of a two-chambered bath and perfused with saline. Nerve 5r1 was pulled through a narrow gap (sealed with petroleum jelly) into a second chamber filled with a 5% (w/v) solution of Procion Yellow in distilled water. A current of about 1 μA was passed between the chambers in the opposite direction to the injury currents flowing at the cut ends of axons in nerve 5r1 (i.e. with the anode in the saline pool). Current was passed until the dye, which is negatively charged in solution, had passed up axons in nerve five and into the ganglion (usually about 2 h). The preparation was left in saline at 4 °C for 3–6 h. to permit diffusion of the dye to occur and then treated in the conventional manner (Bentley, 1970).

Whole mounts of the ganglia were examined with a fluorescence microscope and the best preparations were embedded and serially sectioned in the transverse plane. Sections were photographed on reversal film and then projected on to sheets of paper, and the outline and fluorescent profiles were drawn in. Using the known section spacing (15 μm) a plan and side elevation of injected cells could be constructed (Pusey, 1939).

Intracellular recording

The metathoracic ganglion (T 3) and abdominal ganglia (A 1–A 6) were exposed from the ventral surface (see Fig. 1). In order to provide mechanical stability of T 3 a small platform was positioned underneath it. This platform consisted of a thin cup of brass, 3 mm in diameter and 2 mm deep, filled with wax and attached by a rod to a micro-manipulator. Fine pins were pushed into the wax around the ganglion between the nerve trunks. The sheath on the ventral surface of the ganglion was removed to facilitate microelectrode penetration.

Glass microelectrodes were filled with 1 M potassium acetate and connected to a Medistor A–35 Electrometer amplifier through a silver/silver chloride half-cell. The indifferent electrode was of saline in agar connected to a second half-cell. A bridge circuit permitted stimulation of penetrated cells through the microelectrode.

Silver wires were placed in the depressor muscles of the right coxa to record the electromyogram (EMG) and allow identification of activity in the excitatory axons D_f and D_s. Nerves 3, 4 and 6 to this leg were severed. Recording electrodes were positioned under the right connective between A 1 and A 2, and the left connective was cut, usually between A 2 and A 3. The connectives were stimulated between A 5 and A 6. An additional electrode connected through a large capacitor to ground was placed near A 5 to reduce stimulus artefact.

RESULTS

Dye injection

Fifteen preparations of metathoracic nerve branch 5r1 were made. Three were discarded during electrophoresis because dye leaked around the nerve in the petroleum-jelly seal. In the remaining 12 dye could be seen in one or two axons within the main nerve 5 moving towards the ganglion, and when examined as whole mounts ten contained one or two dye-filled cell bodies. The central parts of the three common inhibitory axons present in nerve 5r1 would not be expected to fill with dye because the axons are of small diameter and have branches in nerve 5 distal to 5r1 (Pearson & Iles, 1971) which would provide a shorter path for current flow than that into the ganglion. Thus a maximum of two filled cells (the excitatory motoneurones D_f and D_s) would be expected in each ganglion.

In two ganglia the fast coxal depressor motoneurone D_f was reconstructed (Text-fig. 2*a*, *b*). Identification was possible by tracing the cell to its axon in the dorsal part of nerve 5, whose diameter was *c.* 25 μm (that of D_s is only 15 μm). The cell body is located near the ventral surface of the ganglion and connected by a long neurite (diam. *c.* 15 μm) to a dilated part of the neurone nearer the dorsal surface from which dendrites and the axon arise. The terms 'link segment' and 'integrating segment' have been introduced to describe the neurite and dilated parts respectively (Cohen,

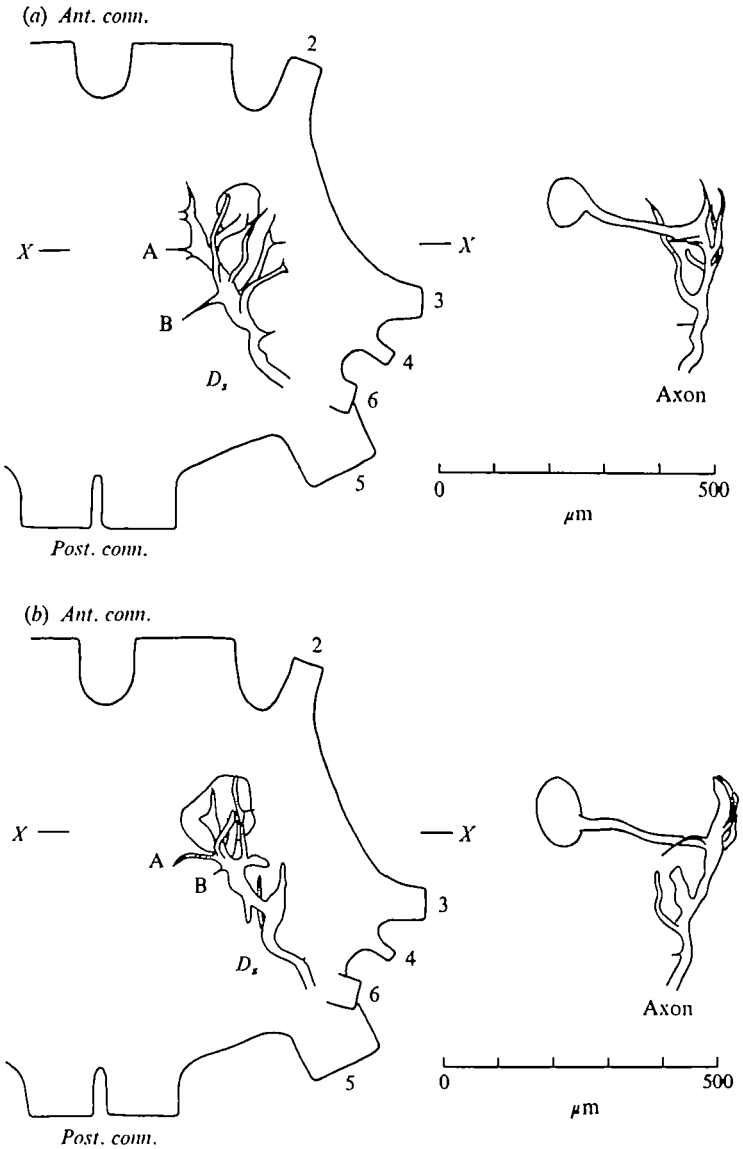

Fig. 2. Reconstructions of the fast coxal depressor motoneurone from two different preparations (*a*, *b*). Dorsal view on left with outline of T 3, medial view on right. Dye injection can cause swelling which is especially evident in the cell body of (*b*). A, B, Medially directed dendrites; D_s, position of cell body of the slow coxal depressor motoneurone; 2–6, numbering of nerve trunks; *X*–*X*, position of sections illustrated in Plate 1. *ant. conn.* Anterior connectives; *post. conn.*, posterior connectives.

1970; Sandeman, 1969; Rowe, Moberly, Howard & Cohen, 1969). Dendrites could be traced down to diameters of less than 4 μm (Plate 1) but finer ramifications may well exist. The volume of ganglion enclosing the dendritic tree is at least 340 μm long by 230 μm broad and 160 μm deep. No processes were found to cross the midline, but dendrite 'A' (Text-fig. 2; Plate 1*a*) could be tenuously traced to the region of the ventral intermediate tract (Pipa *et al.* 1959) and dendrite 'B' to the dorsal inter-

mediate tract. These two tracts contain fibres ascending from the abdominal cord and it is therefore possible that synaptic contacts are formed with the motoneurone (cf. Davis, 1970). Although the detailed structure of the two reconstructed cells is slightly different, the number and direction of the main branches is the same. Part of the dendritic tree was reconstructed in a third ganglion confirming the above observations, and soma position was checked in a further seven.

In five preparations a second, smaller cell (cell body 50 μm diam.) was also filled with dye. This was most probably the slow depressor motoneurone D_s, but its small size prevented reliable reconstruction. The cell body was located in a posterior and somewhat dorsal position (Text-fig. 2*a*, *b*).

Recording from motoneurones

Activity of the two different excitatory motoneurones D_f and D_s could be distinguished in the EMG from the coxal depressor muscles. The electrical response of the muscle to an impulse in axon D_s was smaller than that from D_f and showed facilitation at high frequencies. Only axon D_s normally fired spontaneously.

Selective activation of abdominal giant fibres (G.F., conduction velocity 5–7 m/sec.) could be achieved by threshold stimulation at A 6–A 5 with the response monitored at A 2–A 1. Increasing the stimulus strength then excited smaller fibres with slower conduction velocities (Dagan & Parnas, 1970).

Stimulation at G.F. threshold (at 5 /sec) had no effect on the spontaneous firing of D_s (recorded by means of the EMG). When the stimulus strength was increased to more than 5 times threshold some driving of the D_s discharge with a latency of 12 msec (measured from the first G.F. response recorded at A 2–A 1) was observed. This result is in agreement with Dagan & Parnas (1970) who found that stimulation of abdominal afferents smaller than the giant fibres was necessary to cause excitation of nerve 5 motoneurones. No maintained intracellular recordings were made from D_s.

Single shocks to A 6–A 5 even at high strength did not usually excite D_f, although the high-frequency discharge in abdominal afferents of all types produced by pinching the ipsilateral cercus did. When the central part of D_f was penetrated with a microelectrode the cell generally fired spontaneously, presumably as a result of mild injury. These action potentials recorded intracellularly were of 2–5 mV in amplitude and followed by an after-hyperpolarization (cf. Rowe, 1969). Resting potentials were from 20–50 mV. Records were deliberately made from the dorsal part of the ganglion (and hence from the integrating segment or dendrites) because cell bodies are generally electrotonically remote from synaptic inputs (Bentley, 1969). Action potentials tended to arise from varying levels of depolarization, suggesting that the recording site was still remote from the spike-initiating zone (assuming a constant voltage threshold). Stimulation of A 6–A 5 with single shocks at G.F. threshold did not affect the steady discharge of D_f and no post-synaptic response could be observed. High-frequency stimulation was similarly without effect. Increasing the stimulus strength to 10 times threshold produced a tendency for D_f to fire with a latency of 7–8 msec after each stimulus (Text-fig. 3). On some occasions when the stimulus did not elicit an action potential a small excitatory post-synaptic potential (EPSP) could be observed (Text-fig. 3, row *b*). These results suggest that there is no direct input from giant fibres to motoneurone D_f but that an excitatory input from smaller fibres exists.

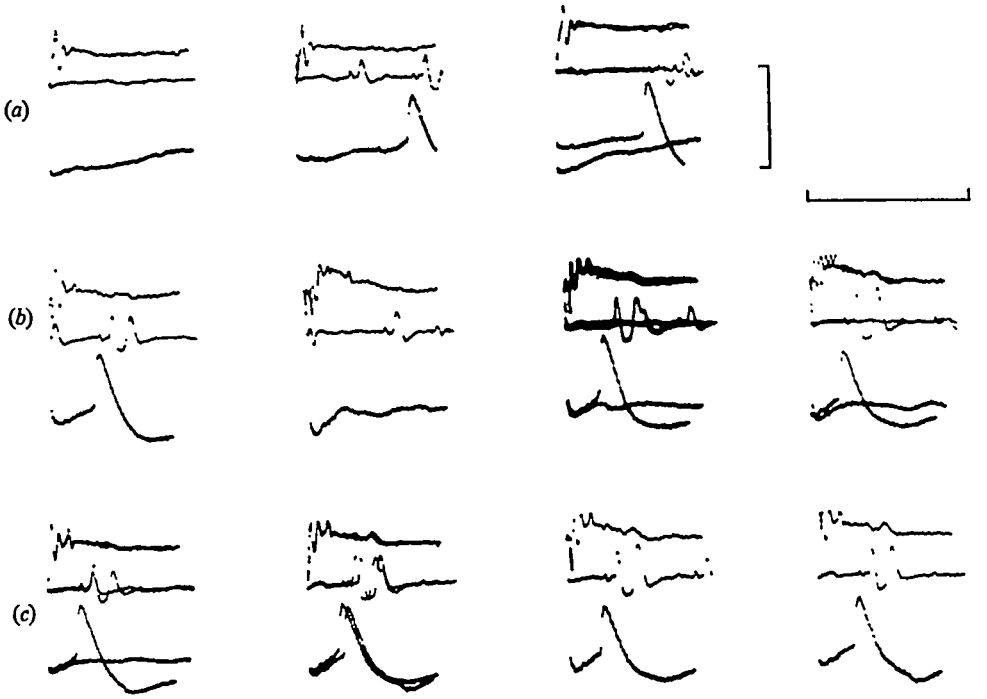

Fig. 3. Response of motoneurone D_f to stimulation of abdominal afferents at A5–A6. In each photograph the top record is the afferent volley recorded at A1–A2, the second is an EMG from the coxal depressor muscles and the third an intracellular record from motoneurone D_f. The stimulus triggers each sweep (in some cases two sweeps are superimposed). The action potentials recorded intracellularly in D_f correspond to the large response in the EMG (the smaller uncorrelated response in the EMG results from activity of the slow motoneurone D_s). Row (*a*) stimulation of A5–A6 at G.F. threshold, note the absence of post-synaptic response in D_f; rows (*b*, *c*) stimulation at 10 times threshold, note the tendency for D_f to fire with a fixed latency and the small EPSP visible when an action potential is not elicited (row *b*). Calibration 2 mV (intracellular record), 25 msec.

Fig. 4 shows intracellular records from an unidentified cell recorded near D_f in the ganglion (probably a motoneurone). This cell had no response to G.F. stimulation but showed a small EPSP when the stimulus strength was increased 3 times. Stronger stimuli produced a graded increase in EPSP size, and then cell firing suggesting spatial summation from several small afferent fibres.

Although no evidence of direct connexions between giant fibres and motoneurones was observed there remained the possibility of indirect input via interneurones. Penetrated motoneurones showed a background of post-synaptic potentials which probably result from interneuronal activity but it was not clear whether stimulation of abdominal afferents influenced this input (cf. Bentley, 1969). Interneuronal pathways may have become habituated completely even with the low frequency (5/sec) of giant-fibre stimulation used in these experiments.

DISCUSSION

The reconstructions of the metathoracic fast coxal depressor motoneurone show that it is a typical insect unipolar neurone (Cohen, 1970). The cell body is in the position expected for nerve 5 motoneurones from the map of Cohen & Jacklet (1967), probably

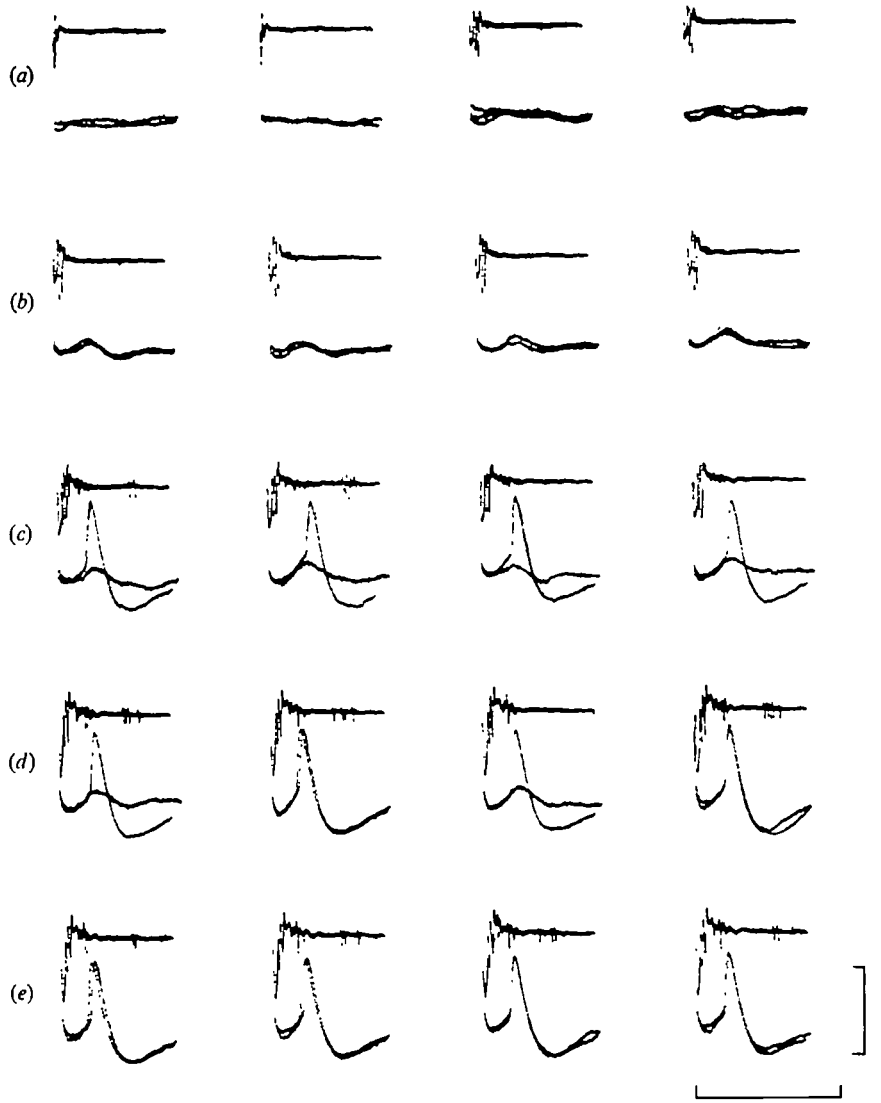

Fig. 4. Response of an unidentified cell (presumed motoneurone) to abdominal cord stimulation. In each photograph the upper record is from A 1–A 2 and the lower is intracellular. At least two sweeps triggered by the response at A 1–A 2 have been superimposed in each record. Row (*a*), stimulation of A 5–A 6 at G.F. threshold and at twice G.F. threshold; row (*b*), 3 times threshold; rows (*c*, *d*, *e*) successively stronger stimulation. Calibration 2 mV, 50 msec.

corresponding with the one numbered 28 by these authors. This is one of the largest motoneurones in the ganglion. The two dendritic branches from the integrating segment terminating near the longitudinal fibre tracts which pass through the ganglion may be the anatomical basis for excitatory input from small abdominal afferents. Harris & Smyth (1971) have recently shown that some of the ventral group of giant fibres ascending from the abdomen have branches in the metathoracic ganglion at about the same level as dendrite A (Text-fig. 2) of the motoneurone, but there is no evidence available for smaller afferent fibres. The fast coxal depressor motoneurone

must also receive other inputs, e.g. from interneurones involved in the generation of the walking rhythm.

The cell body of the slow coxal depressor motoneurone is located in the posterior part of the ganglion well outside the region expected from Cohen and Jacklet's map. However, a small cell body has now been observed in this posterior region using the same technique as these authors (D. Young, personal communication). Thus fast and slow motoneurones which may innervate the same muscle fibres in the periphery (Pearson & Iles, 1971) are not necessarily adjacent within the ganglion.

The demonstration of an excitatory input to some thoracic motoneurones from small, high-threshold fibres in the abdominal cord is in agreement with Dagan & Parnas (1970). The absence of any response to giant-fibre stimulation does not, however, necessarily imply that giant fibres cannot influence the activity of thoracic motoneurones. It has been suggested that the synaptic pathway to motoneurones is very labile (Roeder, 1962, 1963) or could be under inhibitory control. Alternatively the giant fibres might act only upon interneurones, possibly those involved in running subsequent to a jump. In the experiments described here a pure small-fibre input has not been achieved and it could be argued that the observed excitatory effects result from the combined input of the giant and small fibres to the motoneurones. However, Dagan & Parnas (1970) have performed experiments on cockroaches in which the giant fibres have been caused to degenerate and show conclusively that small fibres have an excitatory effect on nerve 5 motoneurones.

The smallest members of the two groups of fibres which are histologically recognizable as giant fibres in the abdominal connectives are no larger (diam. *c.* 15 μm) than some of the other fibres in the connectives (Spira, Parnas & Bergmann, 1969). It is therefore interesting to consider whether the smallest giant fibres might contribute to the observed excitatory input to motoneurones from small fibres, especially since Harris & Smyth (1971) have shown that the smallest member of the ventral group of giant fibres has branches in the metathoracic ganglion. Dagan & Parnas (1970) stimulated individual giant fibres with a microelectrode and failed to influence the discharge of nerve 5 motoneurones. However, this stimulating technique would tend to select the largest giant fibres, and a single afferent fibre might not produce a noticeable effect on motoneurone discharge. The diameters of the small afferent fibres responsible for the excitation observed in the present paper can be indirectly estimated from their conduction velocity (C.V.). Assuming a single synaptic delay of 1·5 msec at the motoneurone the latency of the intracellular response gives a maximum C.V. for the afferents of 2·4 m/sec. This is within the range given by Dagan & Parnas (1970). The C.V. of the largest giant fibres (diam. *c.* 50 μm) is 7 m/sec. If C.V. varies with the 0·78 power of axon diameter (Pearson, Stein & Malhotra, 1970) the maximum diameter of the small fibres can be estimated as 13 μm. Given the errors likely in the above calculation, the result is sufficiently close to the diameter of the smallest giant fibres for there to be some doubt as to whether they can be safely excluded from contributing to the direct excitatory input to motoneurones.

The simplest conclusion is that whereas no direct influence of the largest giant fibres was observed on the motoneurones studied, an excitatory input from smaller fibres could be demonstrated. In at least one cockroach species, *Macropanesthia rhinoceros*, conspicuously large giant fibres are absent (Day, 1950).

SUMMARY

1. Using Procion Yellow dye injection the structure of the fast coxal depressor motoneurone was determined.

2. The cell body of the slow depressor motoneurone was located within the metathoracic ganglion.

3. Intracellular records from the fast motoneurone failed to reveal any post-synaptic response when the largest abdominal giant fibres were stimulated.

4. Smaller abdominal afferent fibres gave an excitatory input.

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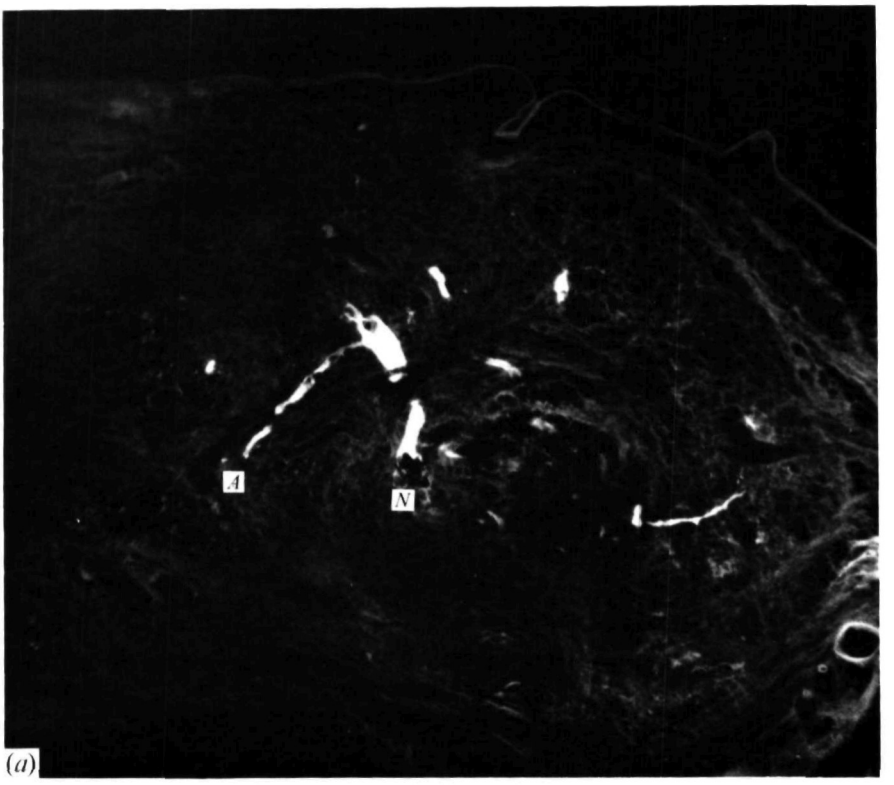
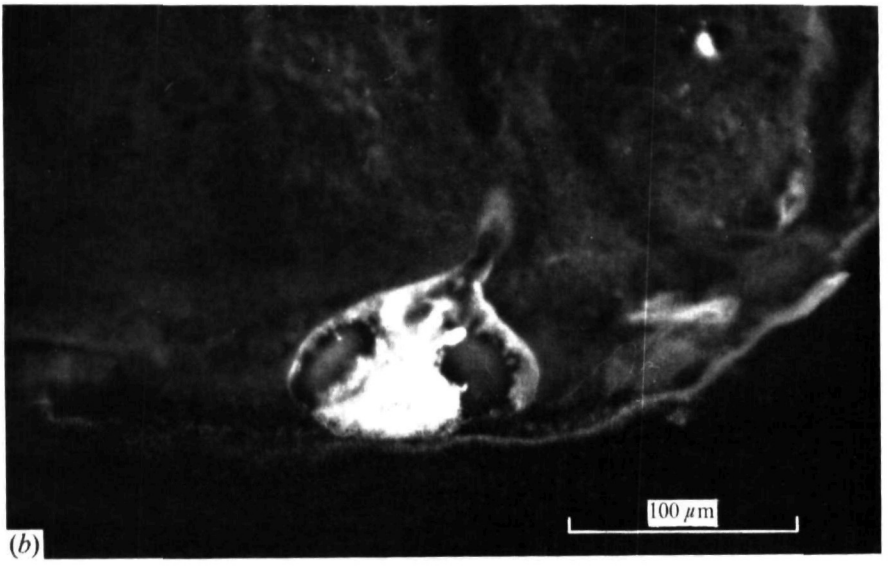
EXPLANATION OF PLATE

Fig. (*a*). Section *X–X* of the ganglion reconstructed in Text-fig. 2(*a*) photographed under fluorescence. The ventromedially directed dendrite *A* can be seen branching off from the integrating segment. Part of the neurite *N* is visible.

Fig. (*b*). Section *X–X* of Text-fig. 2(*b*), showing the ventral cell body of motoneurone D_t. Calibration 100 μm.



(*a*)



(*b*)