# THE ORGANIZATION AND ROLE DURING LOCOMOTION OF THE PROXIMAL MUSCULATURE OF THE CRICKET FORELEG

## I. ANATOMY AND INNERVATION

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## SUMMARY

The structure of the proximal segments of the cricket (*Gryllus bimaculatus*) foreleg, together with the associated musculature and its innervation are described. The morphology of 50 motor neurones involved in the control of this musculature has been revealed using backfilling techniques with cobalt, horseradish peroxidase and Lucifer Yellow.

The 'ball and socket' pleurocoxal joint is moved by three sets of anatomical antagonists (promotor–remotor, abductor–adductor, anterior–posterior rotator muscles) inserted on each side of the three axes of rotation.

The axial coxotrochanteral joint is moved by the intrinsic levator and the depressor muscles; these depressors are composed of an intrinsic (coxotrochanteral) and a 'double' (pleurotrochanteral) subgroup.

The double depressors, and all the muscles inserting on the trochantin (promotors) or the anterior coxal rim (adductor, abductors, anterior rotators) are supplied by at least eighteen neurones, whose axons run in nerve 3.

The muscles that insert on the posterior coxal rim (remotors, posterior rotators) are innervated by at least twelve similar neurones whose axons run in nerve 4.

The intrinsic coxal muscles are supplied by branches of nerve 5 (ten motor neurones to the levators, two to the depressors).

Three presumably common inhibitors, and one Dorsal Unpaired Median (DUM) neurone have also been found.

## INTRODUCTION

The neural mechanisms underlying arthropod motor activities have mainly been investigated in decapod crustaceans (Wiersma, 1961; Clarac, 1977), arachnids (Bowerman, 1981), orthopteran (Wilson, 1961; Burrows, 1983) and stick insects

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(Bässler, 1983). These groups are of great interest because their very elaborate locomotory behaviours (such as walking, jumping, swimming and flying) involve relatively few neurones that are both easily accessible and uniquely identifiable.

In insects, walking has been examined from the point of view of behaviour (Wilson, 1966; Burns, 1973; Harris & Ghiradella, 1980), anatomy (Voss, 1905; Duporte, 1920; Snodgrass, 1929; Bräunig, 1982) and physiology (Hoyle, 1964; Hoyle & Burrows, 1973; Pearson & Iles, 1973; Bräunig & Hustert, 1985), but most of the available data on insect motor systems concern the distal joints of the ptero-thoracic legs. There are two main reasons for this: firstly, the mechanics of the axial (there is only one degree of freedom) distal joints allow a simple approach to motor control, through elementary antagonism, and secondly, the middle and hind segments also being specialized for flight, stridulation and jumping are therefore more attractive to investigators. We know, however, that the mobility of the whole leg and the amplitude of a step are mainly determined by the movements allowed by the pleurocoxal and coxotrochanteral joints while the femur–tibia and tibia–tarsus articulations allow an increase of the arc described by the tarsus: the prothoracic femur is moved horizontally, during walking, by some 50° in the anterior quadrant in the locust (Burns, 1973) and the cricket (Harris & Ghiradella, 1980); this movement therefore implies rotations of the coxa about at least two (vertical and transverse) axes and hence the involvement of the thoracocoxal musculature. Furthermore, during the swing phase, the leg is first lifted off the ground and the femur is then depressed as it is rotated towards the head of the animal. This sequence of movements contrasts with that occurring in the middle and hindlegs, where the femur is depressed only during the stance phase, i.e. when the tarsus is in contact with the substrate. During walking, turning can be achieved either by increasing the step frequency of the legs on the outside of the turn, or by changing the step length while maintaining coordination (Bässler, 1983). In the latter case, increasing the prothoracic step length will depend primarily upon the angle between the thorax and the femur, and therefore upon the activation of the coxal and trochanteral muscles. In the cricket, the forelegs, besides being involved in walking, are also used in other behavioural activities such as grooming, exploration, orientation, and eye or antennal cleaning (Honegger, Reif & Müller, 1979). During cleaning, for example, the prothoracic tarsus is brought towards the ipsilateral eye or antenna by a complex combination of movements about each of the leg joints, involving the most proximal ones in particular.

To obtain a more complete understanding of the leg movements during loco-motory activity than that given by the above observations, we felt it necessary to investigate the structure and physiology of the coxal and trochanteral joints.

In this paper we describe the exoskeleton, musculature and motor innervation of the prothoracic, thoracocoxal and coxotrochanteral articulations and the morphology of the motor neurones involved in their control. In a second paper (Laurent & Richard, 1986) we shall describe the pattern of electromyographic activity during locomotion.

## MATERIALS AND METHODS

The musculature and innervation were determined from some 60 adult males of the species *Gryllus bimaculatus* (de Geer). The nomenclature follows that used by Honegger *et al.* (1984) on neck muscles of *Gryllus campestris*, extending the systems of Misra (1946) and Shepheard (1973) for muscles of *Schistocerca gregaria* (Forskål) and that of Campbell (1961) for the innervation of *Locusta migratoria migratorioides*.

### Nerve transverse sections

Isolated nerves were fixed in 2·5 % buffered glutaraldehyde with sucrose, rinsed and postfixed in 2% osmium tetroxide; they were dehydrated and embedded in Spurr's resin, prior to cutting semithin sections (1–2 μm). The sections were counterstained with 1 % toluidine blue and photographed with a light microscope at ×250 magnification.

### Axonal filling techniques

#### Cobalt

We used the technique of backfilling *in vitro* (Altman & Tyrer, 1980) (3–10 h at room temperature) adapted from the initial method of Pitman, Tweedle & Cohen (1973) with 3–5% cobaltous chloride, nitrate and hexammine-cobaltic chloride. After silver intensification (Bacon & Altman, 1977), the ganglia were drawn as whole mounts with the aid of a *camera lucida*.

#### Lucifer Yellow

We applied the same backfilling principle for 1 h, *in vitro*, at room temperature, and photographed the preparations under a fluorescence microscope.

#### Horseradish peroxidase

After comparable filling and diffusion conditions (6–9 h at room temperature) of a 4% solution of horseradish peroxidase (HRP) in 100 mmol l$^{-1}$ KCl, we followed the histological procedure described by Nässel (1983).

The description of the neuropilar areas is derived from the data of Tyrer & Gregory (1982) and Wohlers & Huber (1985).

## RESULTS

### Proximal prothoracic exoskeleton

The proximal exoskeleton of the cricket leg, like that of other insects, comprises a coxa and trochanter. The coxa moves relative to the thorax, which comprises three main parts: the tergum dorsally, the sternum ventrally and the pleura laterally. The tergum, sternum and pleura are linked together by an internal oblique pillar formed by the union of a sternal and pleural arm. In *Gryllus bimaculatus*, the highly

desclerotized prosternum provides the coxa with an important freedom of movement about the pleura. From the furcisternum (*fs*, Fig. 1A) arises a slender but rigid sternal arm (*sa*, Fig. 1A), which joins the pleural arm above the coxal base. The tightly united episternum and epimeron (*em*, Fig. 1A) project ventrally to form the capsulated condylic pleural joint (*pj*, Fig. 1A,B); its basicoxal process shows a trochlear notch (*trn*, Fig. 1B) that might guide the coxal rotations in preferred directions. Nevertheless, rotational movements are possible around the sagittal, transverse and vertical axes (Figs 3, 5). The chitinous trochantin (Fig. 3) is articulated medioposteriorly to the anterior basicoxa, and lateroanteriorly to the basisternum (*bs*, Figs 1A, 5). Lateral to the pleural joint, the two basicoxal lobes (*a bcx l* and *p bcx l*, Fig. 5) give support to muscular insertions.

The coxotrochanteral joint is dicondylic and its axis of movement is posterolaterally orientated, the proximal end of the trochanter being posterior, the distal one anterior (Fig. 5).

*Musculature*

### The pleurocoxal joint

The pleurocoxal joint is controlled by an elaborate series of muscles comprising three pairs of anatomical antagonists (Snodgrass, 1929). Their nomenclature is here based on the location of their point of insertion: the promotor–remotors, which move the coxa around the transverse axis (Fig. 3) in a parasagittal plane; the abductor–adductors, which insert on both sides of the parasagittal axis; and the anterior–posterior rotators, which cause a horizontal movement around the vertical axis. The amplitude of each movement is limited by the elasticity of the membranous cuticle and the presence of the trochantin.

The promotors of the coxa consist of two tight bundles (70-1 and 70-2, Fig. 2A; Table 1) which originate on the medial episternum and insert on the trochantin by an apodeme, at the level of the trochantinocoxal articulation (Fig. 5).

The remotors of the coxa consist of two separate groups that both originate on the tergum: the medial one leaves the tergum near the midline (Fig. 2A) and comprises three distinct bundles (71-1a,b,c, Fig. 2A) that insert side by side on the posterior mid-basicoxa (Fig. 5); the lateral group consists of two large muscles (71-2a,b, Fig. 2B), which insert by a strong apodeme on the posterior basicoxal lobe (Fig. 5). From a strictly anatomical point of view, muscles 71-2a and 71-2b could also be named abductors, since their point of attachment is lateral to the parasagittal axis.

The abductors of the coxa consist of two differently orientated groups. The dorsal one comprises three muscles (74-1a,b,c, Fig. 2B) that originate from the episternum and converge to insert by a single apodeme on the anterior basicoxal lobe (Fig. 5); they all proceed along the episternal groove (Fig. 1A). The ventral one (74-2, Fig. 2B) originates horizontally from the inner episternum to insert on the basicoxa, at the level of the mid-trochantin (Fig. 5).

The adductor of the coxa muscle (75-3, Fig. 2C) is a single thin muscle that arises from the sternal arm, and inserts on the ventral membranous cuticle (Fig. 5). The
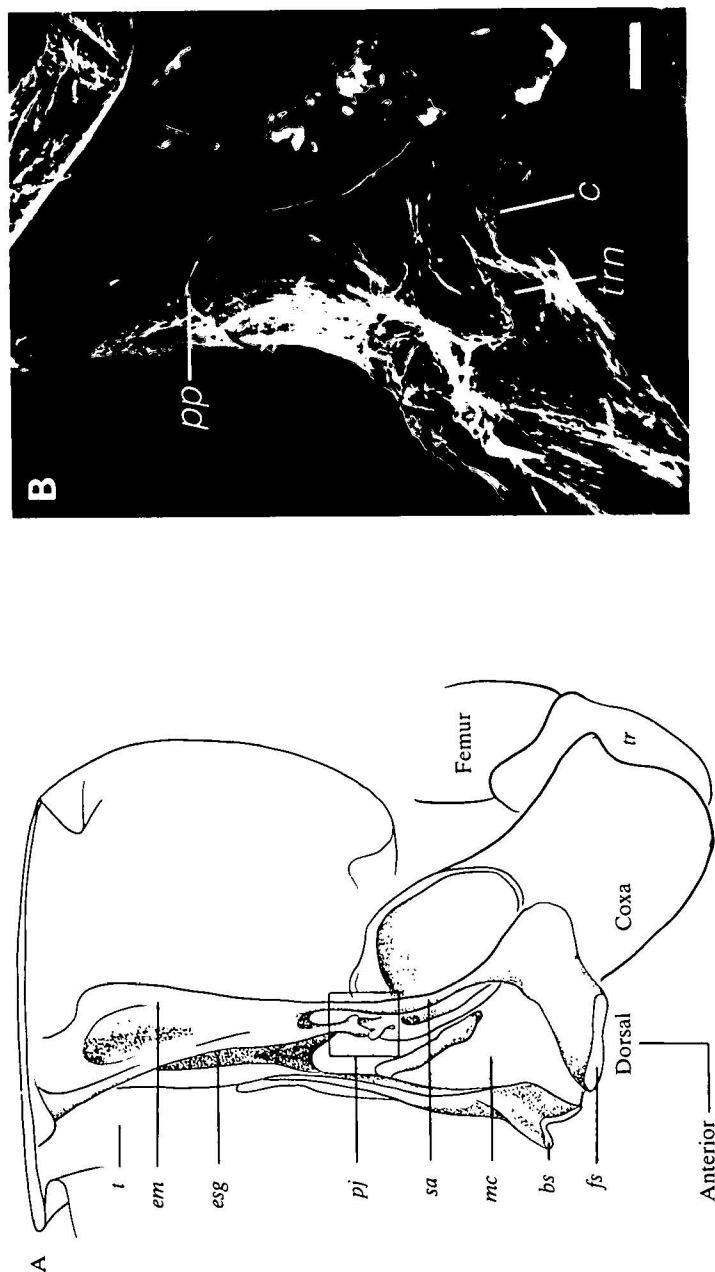
Fig. 1. Proximal limb skeleton. (A) Schematic representation of the right hemi-prothorax, as seen from the inside of the animal. *bs*, basisternum; *em*, epimeron; *esg*, episternal groove; *fs*, furcisternum; *mc*, membranous cuticle; *pj*, pleural joint; *sa*, sternal arm; *t*, tergum; *tr*, trochanter. Magnification, ×12. (B) High magnification of the pleural joint with scanning electron microscopy; *c*, condyle; *pp*, pleural process; *trn*, trochlear notch. Scale bar, 100 μm.

Table 1. *Muscular nomenclature adapted for* Gryllus bimaculatus *from the works of Misra (1946), Campbell (1961), Shepheard (1973) and Honegger et al. (1984)*

| | | | |
|---|---|---|---|
| Promotors: | 70-1,2 | Posterior rotators: | 73<br>75-1a,b |
| Medial remotors: | 71-1a,b,c | Anterior rotators: | 61<br>75-2 |
| Lateral remotors: | 71-2a,b | Levators: | 76-1a,b,c,d<br>76-2 |
| Abductors: | 74-1a,b,c<br>74-2 | Double depressors: | 77-1a,b,c,d<br>77-2 |
| Adductor: | 75-3 | Intrinsic depressors: | 77-3a,b,c |

effective and functional importance of this muscle is difficult to appreciate, since its small size is compensated by a long lever-arm distance to the joint (Fig. 5). It should be noticed that all the muscles that insert medially to the parasagittal axis going through the pleural joint (see Fig. 5) are also indirect adductors.

The rotators that insert on the anterior basicoxa are consequently named anterior rotators. Muscle 61 (Fig. 2A) originates from a contralateral neck sclerite and is considered as a neck muscle (Honegger *et al.* 1984), but, because it inserts on the coxa medially to the coxatrochantin joint (Figs 2A, 5), it is also a leg muscle that moves the right coxa clockwise. Muscle 75-2 (Fig. 2A) is a stout bundle of fibres that proceeds from the anterior rim of the sternal arm, to the inner basicoxa (Fig. 2A); it moves the right coxa anticlockwise.

The posterior rotators insert on the posterior basicoxa; muscle 73 originates from the spinasternite (Fig. 2A) and inserts at the level of the medial remotors (Fig. 5): mimicking its contraction by pulling on its apodeme towards its insertion point moves the right coxa anticlockwise. Muscles 75-1a and 75-1b originate from the ventral proximal sternal arm (Fig. 2C) and insert on the coxa (which they rotate clockwise) between the lateral and medial remotors (Fig. 5).

The resulting coxal movements are schematically represented in Fig. 3; the coxa is drawn in the same orientation as in Fig. 1A, and the vertical, sagittal and transverse axes cross at the pleurocoxal articulation. The straight arrows indicate the direction and mean point of origin of the forces exerted on the coxa; black ones correspond to a movement about the vertical axis (rotation), whereas open and stippled ones indicate movement about the sagittal (abduction/adduction) and transverse (promotion/remotion) axes, respectively. It can easily be seen that the three degrees of freedom provided by this 'ball and socket' pleural joint give the coxa the great spatial mobility that determines the large angular amplitude of the whole limb movements.

*The coxotrochanteral joint*

The coxotrochanteral joint is controlled by a single pair of antagonists, the depressor and the levator of the trochanter: since no movement is allowed about the trochanter–femur articulation, these muscles also depress and elevate the femur.

Fig. 2. Prothoracic thoracocoxal musculature. The prothorax (inset) is sectioned along the midline, and the musculature of the right leg is represented, seen from the inside of the animal, as in Fig. 1A.
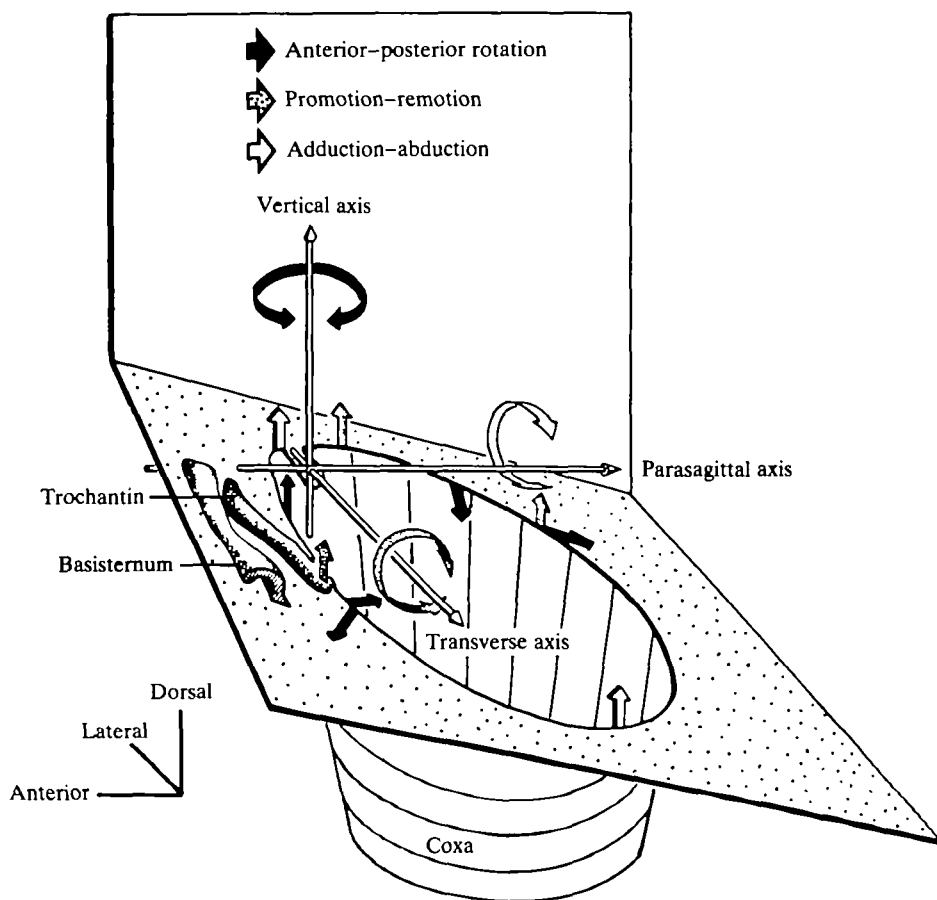
Fig. 3. Diagrammatic representation of the dynamics allowed by the pleural joint: movements are allowed about the three axes of rotation. (See text for further explanation.) Magnification, ×19.

The elaborate depressor group comprises eight muscles, which are divided into three groups, tergal, pleural and intrinsic, according to their origin. The tergal group consists of four muscles (77-1a,b,c,d, Fig. 2D) that run either anterior (77-1b,c) or posterior (77-1a,d) to the sternal arm. The pleural element consists of only one muscle (77-2, Fig. 2D) that originates from the junction of the pleural and sternal arms. Both tergal and pleural groups are named 'double depressors' since they span the pleural and the trochanteral joints. The intrinsic coxal group is made up of three short, stout muscles (77-3a,b,c, Fig. 4) that originate from the inner proximal wall of the coxa. These eight bundles all insert on a single strong apodeme (Fig. 5), which is itself articulated to the trochanter by means of a condylar surface and two lateral ligaments.

The intrinsic levators of the trochanter consist of four strong (76-1a,b,c,d, Fig. 4) and one accessory (76-2, Fig. 4) muscles that originate on the inner distal wall of the coxa.

In brief, this makes a total of 30 individual muscles (Table 1), shared out in four pairs of anatomical antagonists, that directly determine the movements of the femur relative to the body, and indirectly those of the tibia and tarsus.

### Motor innervation

These proximal leg muscles are supplied by nerves 3, 4 and 5 of the prothoracic ganglion (Fig. 6).

Nerve 3 divides close to the ganglion and gives three main roots (N3A, N3B and N3C). The first one (N3A) supplies neck and tergocoxal muscles, and neck and prothorax sensory structures. It innervates the coxal abductors through N3A5 and the promotors through N3A4. N3A5 contains six main axons (diameters from 15 to 20 $\mu$m) whereas N3A4 contains five (Fig. 7G,I; Table 3). N3B is a sensory nerve and passes around the anterior side of the promotor apodeme on the trochantin. N3C, which is purely a motor nerve, innervates eight different muscles: the anterior rotator muscles 61 and 75-2 through N3C1 and N3C2, respectively; the former nerve contains at least six medium axons (diameter less than 10 $\mu$m) (Fig. 7F) and the latter
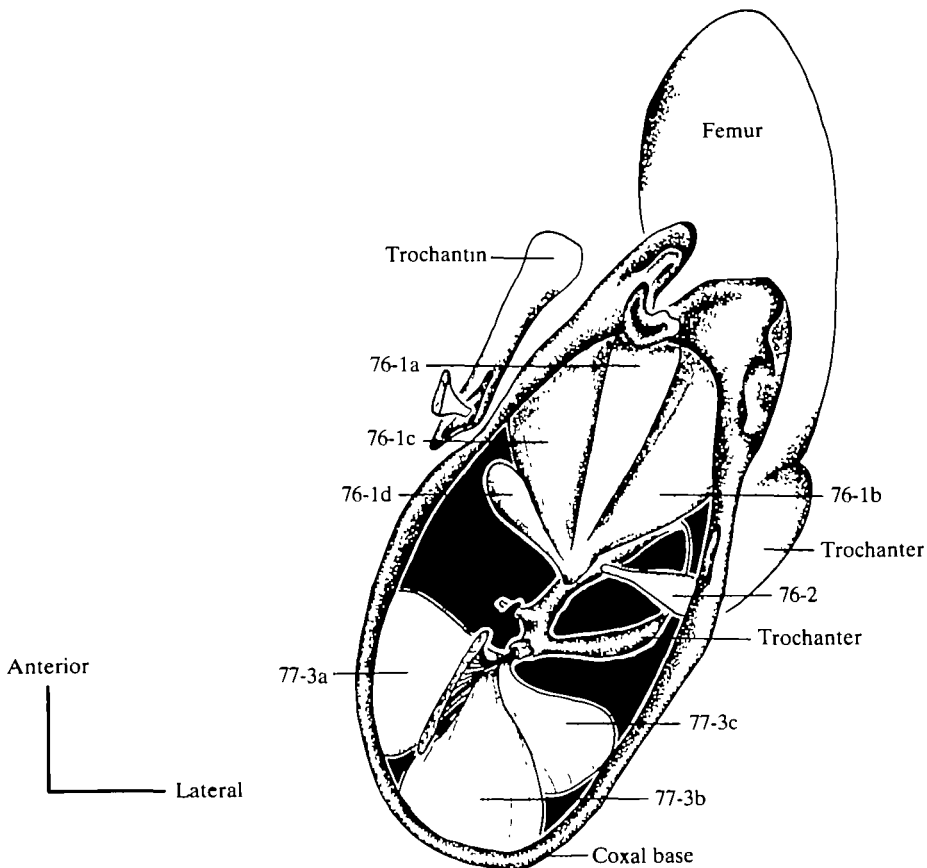


Fig. 4. Coxotrochanteral (intrinsic coxal) musculature, as seen from above, through the coxal base. Magnification, ×35.
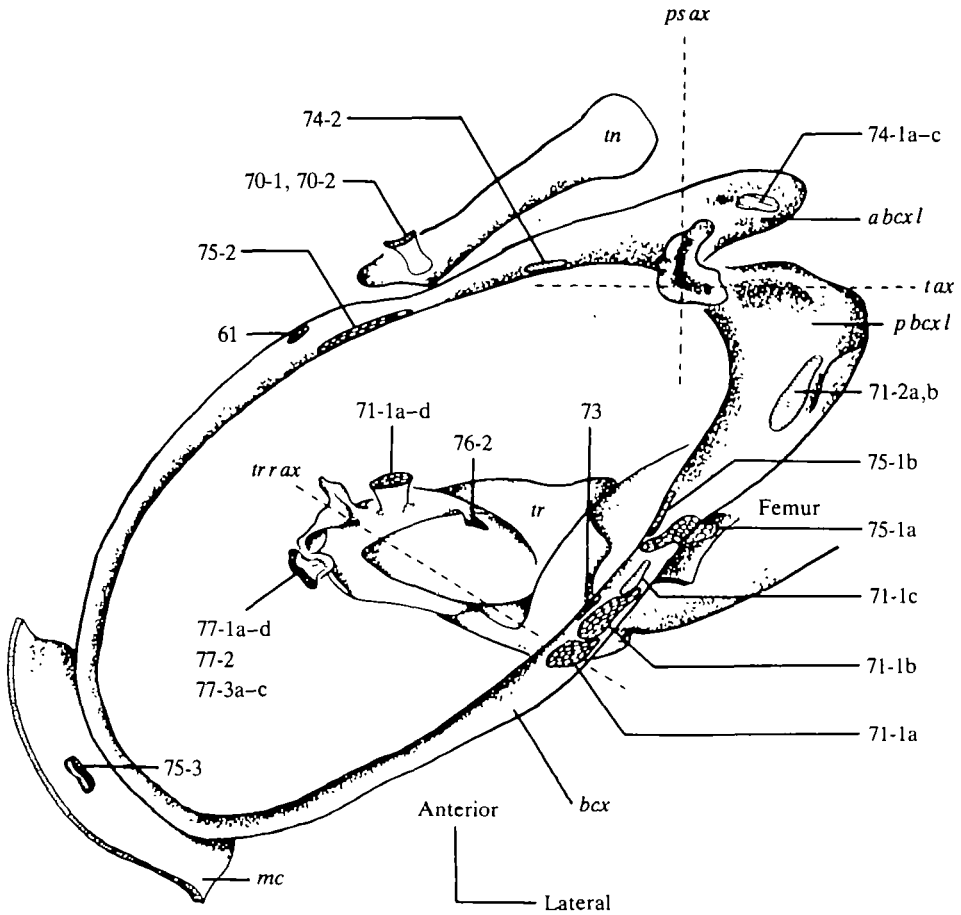
Fig. 5. Location of the insertion of the 30 proximal leg muscles, on the coxal and trochanteral bases, viewed dorsally. *a bcx l*, anterior basicoxal lobe; *bcx*, basicoxa; *mc*, membranous cuticle; *p bcx l*, posterior basicoxal lobe; *ps ax*, parasagittal axis; *t ax*, transverse axis; *tn*, trochantin; *tr*, trochanter; *trr ax*, trochanteral rotation axis. Magnification, ×47.

contains two (Fig. 7E); the adductor (M75-3) innervated through N3C4, which has only one axon (Fig. 7B); the five double depressors, innervated through N3C3 (to muscles 77-1a,b) and N3C5 (to muscles 77-1c,d and 77-2, Fig. 6); N3C3 contains two large (20 µm diameter) axons (Fig. 7B,D), whereas N3C5 contains three large (20 µm diameter) and two small (5 µm diameter) axons (Fig. 7B).

Nerve 5 gives off two branches (N5A and N5B) before entering the coxa, and becoming the 'leg nerve'. N5A supplies the intrinsic depressors of the trochanter (muscles 77-3a,b,c) and has more than 150, presumably sensory axons (<5 µm diameter), three small (5 µm diameter), one medium (8–10 µm diameter) and one large (>20 µm diameter) axon (Fig. 7H). N5B supplies the levators of the trochanter and has some nine large (15–20 µm diameter) axons (Fig. 7A). This pattern of innervation differs from that of the locust, in that in *Schistocerca* the levators are supplied by a branch of nerve 4.

Nerve 4 supplies the posterior rotators through branches 4A and 4B (Figs 6, 7C), and the medial and lateral remotors through branches 4C and 4E, respectively; N4D is a sensory nerve that runs towards the pleural joint.

Between 40 and 45 larger axons ($\geqslant 10\,\mu$m diameter) supply the 30 proximal leg muscles.

### Neural morphology

Backfills have been carried out on branches of nerves 3, 4 and 5 supplying 28 of the 30 muscles, in more than 150 animals. Fifty different efferent neurones have been observed, among which one is of the Dorsal Unpaired Median (DUM) type
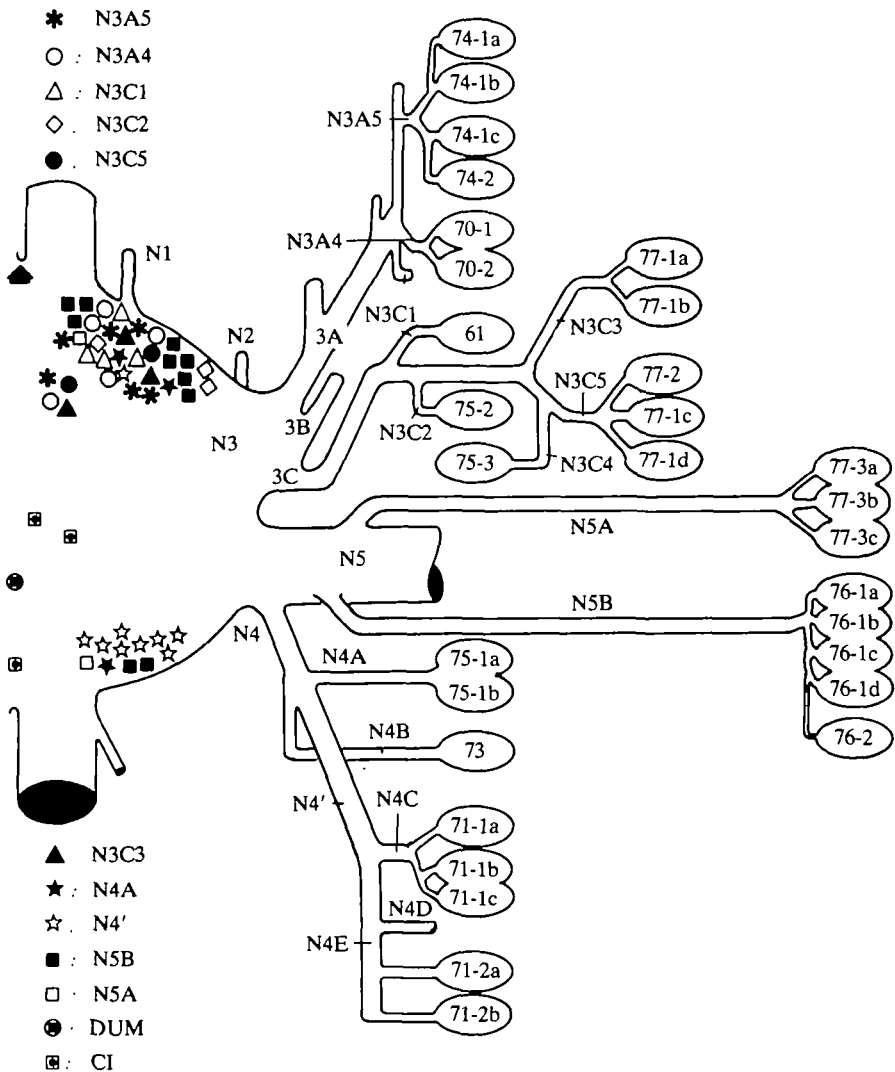


Fig. 6. Peripheral innervation of the proximal leg musculature, and the location of the 50 stained motor neurone somata, in the prothoracic ganglion. (Arrow indicates anterior.) CI, common inhibitor; DUM, dorsal unpaired median neurone.

(Plotnikova, 1969; Hoyle, Dagan, Moberly & Colquhoun, 1974). Three are likely to be common inhibitors, since their axons split in the outer border of the ganglion into several axonic branches (Fig. 8), and their somata lie in the ventral–median cortex, where all known insect common inhibitors are located (Iles, 1976). The DUM neurone was found after backfilling nerve 3C5, and the location of its cell body is indicated in Fig. 6. Filling nerve 5A or 5B always revealed the three common inhibitors; in Fig. 8 a ganglion is photographed where nerve 5A has been stained with HRP. Three axons can be followed in N5B (to levator muscles) and N5 (to femorotibial muscles), whereas only one is present in N4 and N3.

The remaining 46 neurones were presumably (according to their morphology) all excitatory motor neurones; two (aDTr and pDTr, Fig. 15) appeared to have a unique morphology, whilst the other 44 were impossible to identify simply on the basis of their central projections. They could, however, be arranged in eight groups that contained from two to 11 motor neurones of similar morphology; the different members of each morphological group can supply either the same or different muscles. We shall give an anatomical description of the 10 types of neurones which can definitely be morphologically distinguished (Fig. 9), and give the pattern of peripheral distribution of all 46 excitors to the four sets of antagonistic muscles. Each of the motor neurones described has been stained at least five times.

The soma and central projections of all motor neurones described here are ipsilateral to their muscle(s) and have the same basic morphology; their soma lies in the outer cellular cortex and the arborizations are restricted to the dorsal (Zawarzin, 1924; Tyrer & Gregory, 1982) and lateral neuropiles, except for aDTr which also projects into the dorsal part of the intermediate neuropile (see below).

Table 2. *Comparison between the proximal musculature of the cricket, locust, grasshopper and cockroach prothoracic leg*

| | Locusta migratoria (Albrecht, 1953) | Dissosteira (Snodgrass, 1929) | Periplaneta americana (Carbonell, 1947) | Gryllus bimaculatus (this paper) |
|---|---|---|---|---|
| Promotors | 2 (one is divided in 2 sections) | 1 (stout) | 3 | 2 |
| Remotors: | | | | |
| 　medial | 0 | 0 | 2 | 3 |
| 　intermediate | 3 | 3 | 0 | 0 |
| 　lateral | 0 | 0 | 3 | 2 |
| Abductors | 2 | 2 | 3 | 4 |
| Adductors | 2 | 1 | 1 | 1 |
| Anterior rotators | 0 | 0 | 1 | 2 |
| Posterior rotators | 2 | 2 | 1 | 3 |
| Double depressors | 3 | 3 | 4 | 5 |
| Intrinsic depressors | 2 | 2 | 6 | 3 |
| Intrinsic levators | 1 | 1 | 3 | 5 |
| Total | 17 | 15 | 27 | 30 |

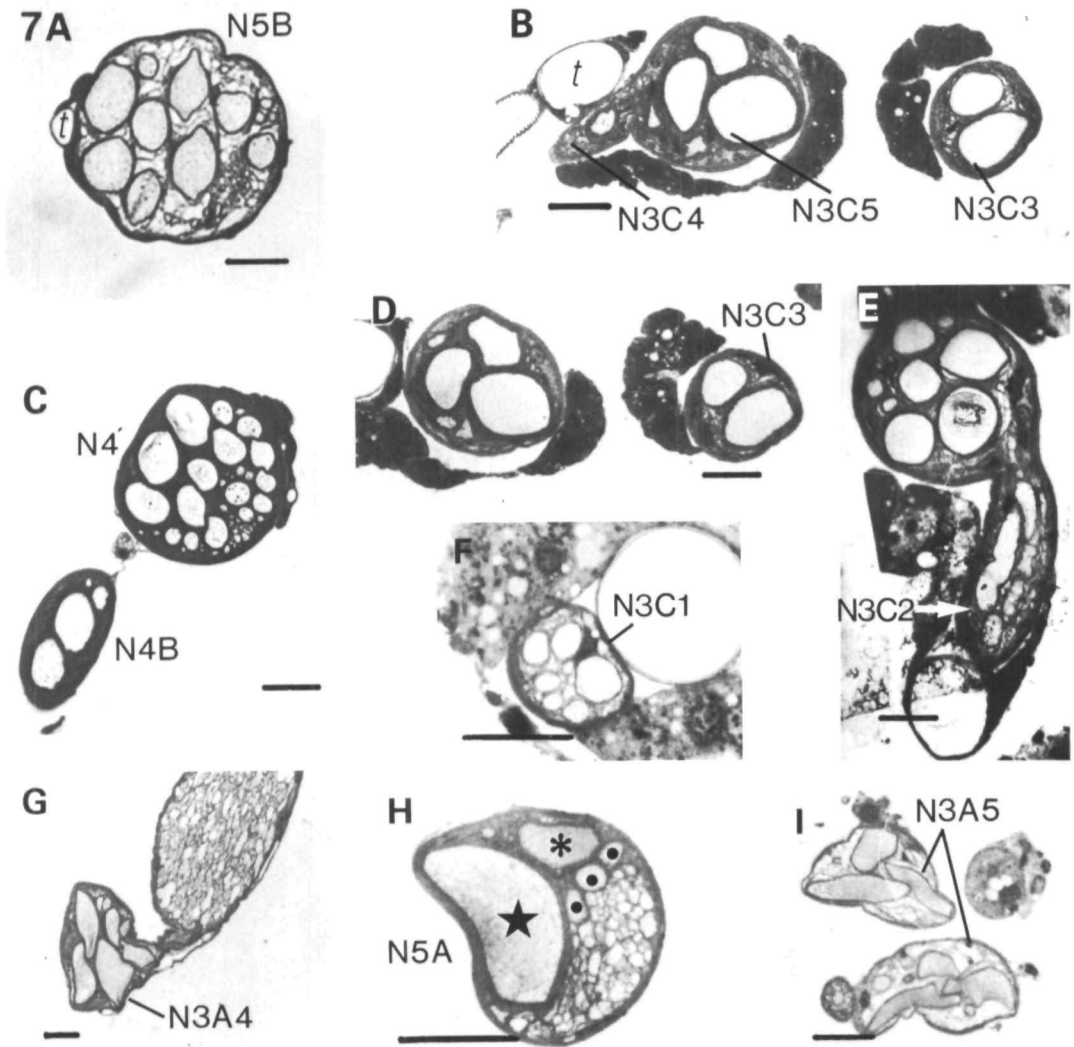Each number refers to the number of muscles described by the corresponding authors.

Fig. 7. Transverse semi-thin sections of the peripheral nerves supplying the proximal leg musculature. In Fig. 7H, the star and asterisk indicate the axons of the anterior and posterior depressor trochanteris motor neurones, respectively, whereas the three dots probably indicate those of the three common inhibitors (see Fig. 8); *t*, trachea. Scale bars, A–I, 20 μm.

For ease of description, the cells which supply more than one muscle will be named referring to the shape of their primary neurite.

*U-shaped cells* (Fig. 10)

Eleven such cells have been found, innervating at least four different anatomical groups of muscles (Fig. 16), through nerves 3A4,5 and 3C2,3,5; this therefore constitutes the most frequently detected morphological type. Their large somata
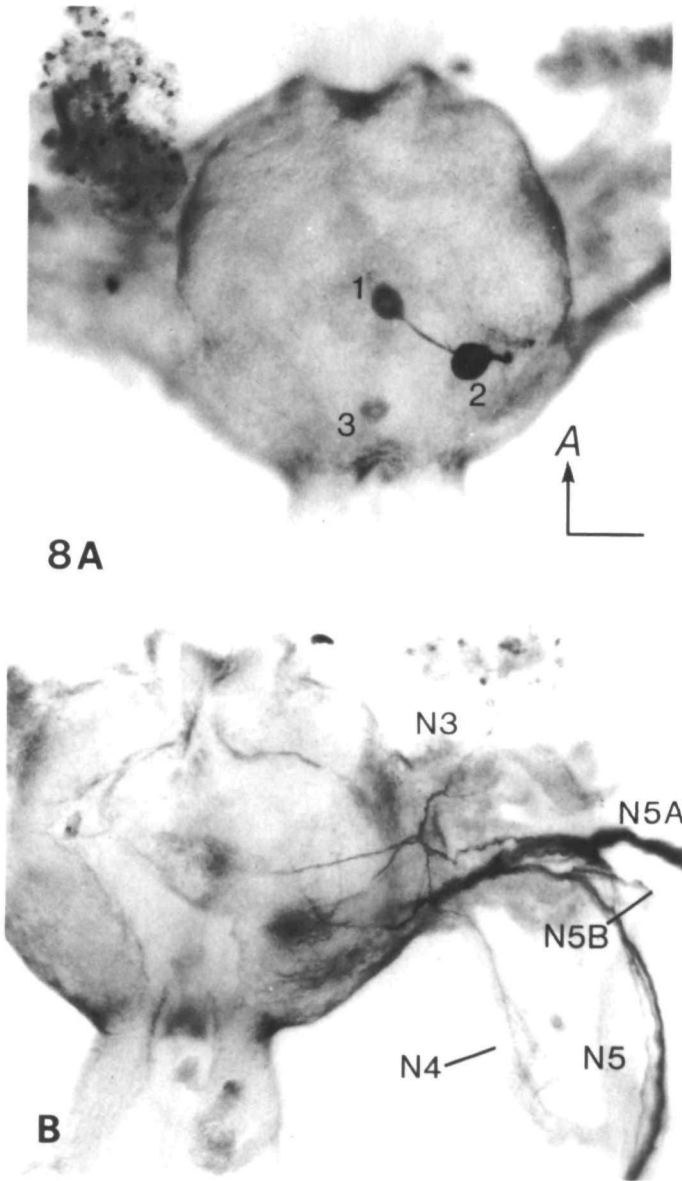
Fig. 8. Micrographs of the prothoracic ganglion and leg nerves, showing the somata (1–3 in A) and the peripheral and central distribution of the axons of the three common inhibitors (B), after backfilling of N5A with horseradish peroxidase. The plane of focus is ventral in A and dorsal in B. *A*, anterior. Scale bar, 100 μm.

(more than 50 μm in diameter) lie in the anterior dorsal cortex, at the approximate level of nerve 1 (Fig. 6). Usually four secondary neurites emerge from their U-shaped primary neurite. Secondary neurite 1 proceeds dorsally towards the midline (Fig. 10A), terminating near the dorsal longitudinal tracts, in a zone limited dorsally by the Median Dorsal Tract (MDT, Tyrer & Gregory, 1982) and ventrally by the

Dorsal Intermediate Tract (DIT) (Fig. 10E). Secondary neurites 2–4 project in the dorsal neuropile, laterally to a line joining the two lateral edges of the anterior and the posterior connectives (Fig. 10A–D). It can be seen from Fig. 10B–E, where two simultaneously filled U-shaped neurones have been sectioned and drawn, that all neurites proceed in close contiguity through the neuropile, justifying the observation that none of these neurones, if stained individually, could be distinguished from the others.
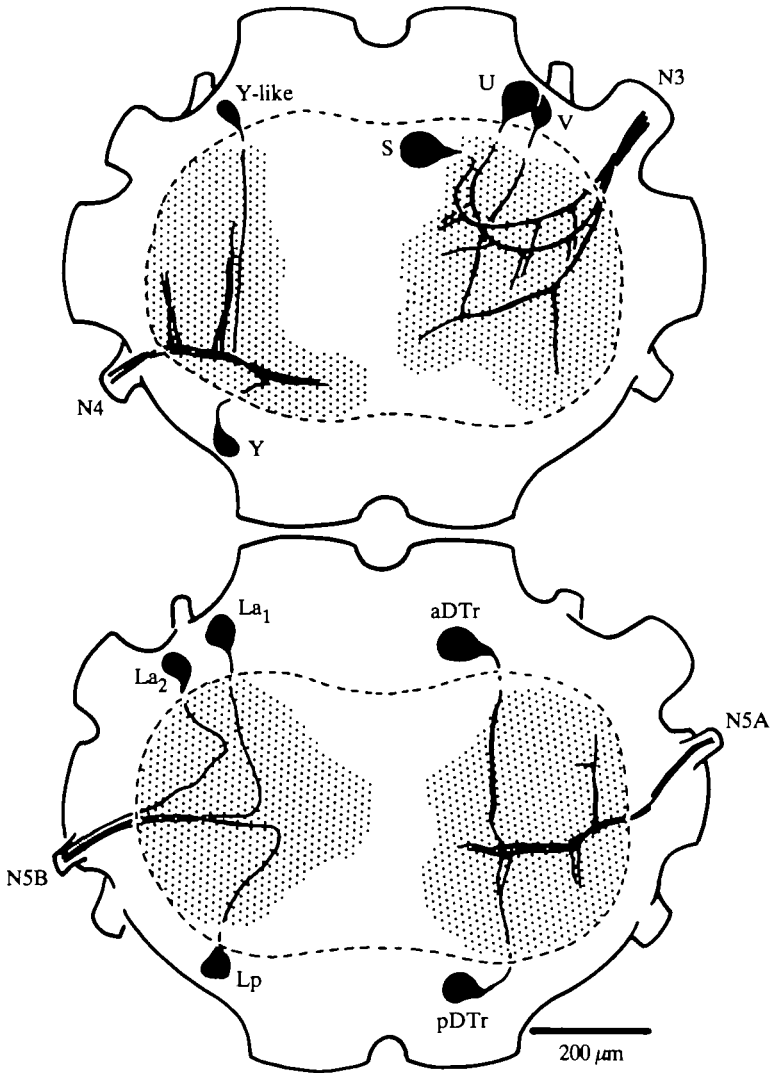


Fig. 9. Schematic structure of the 10 morphological types of leg motor neurones and their main branching areas. The two motor neurones coming out in N5A are unique, whereas all other eight have from 1 to 10 'twin' cells, which innervate the same or a different muscle (see Fig. 16). The boundaries of the neuropile are indicated by stippling.
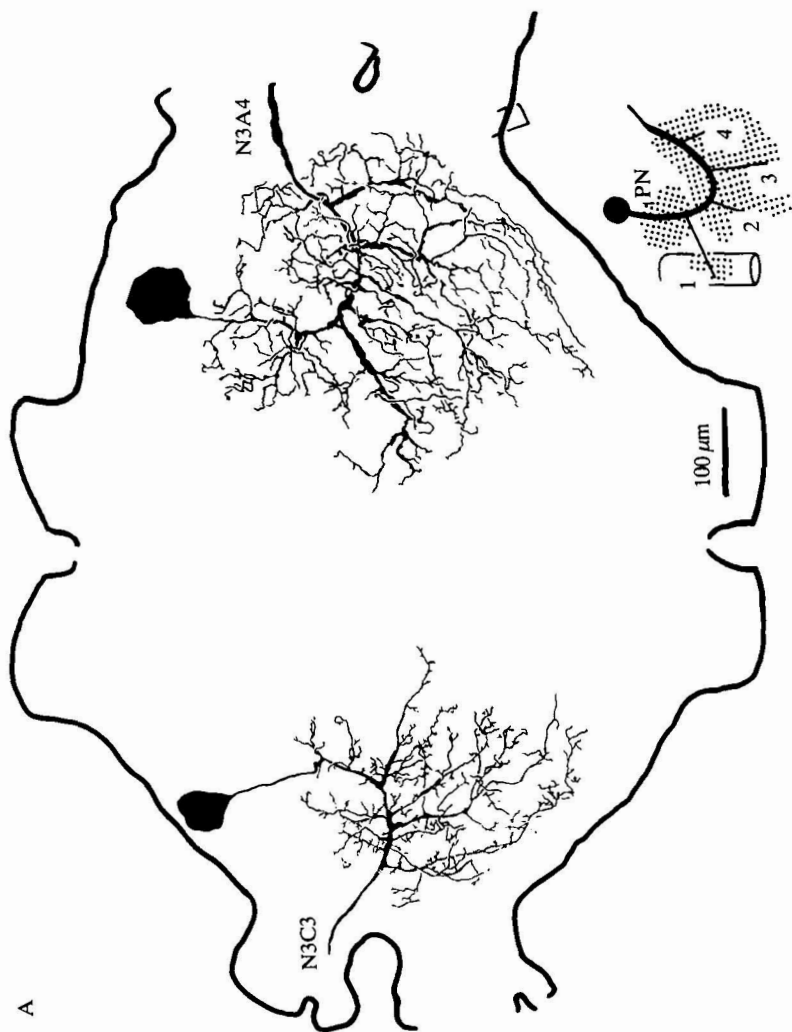
Fig. 10. Structure of the U-shaped motor neurones. (A) Two cells have been stained in two different nerves, in different animals; note the similarity of their structure. The primary (PN) and secondary (1–4) neurites are schematically represented in the inset. (B)–(E) Parasagittal sections (40 μm) of a ganglion where two U-shaped cells (a and b) have been stained from N3C; the levels of sections are indicated in the inset, also showing the three-dimensional arrangement of the longitudinal tracts (DIT, Dorsal Intermediate Tract; LDT, Lateral Dorsal Tract; MDT, Median Dorsal Tract; VIT, Ventral Intermediate Tract; VMT, Ventral Median Tract; Tyrer & Gregory, 1982) through the neuropile. (B) Arborizations of their primary neurites (PN) and fourth secondary neurite (SN4) in the dorsal lateral neuropile. (C) Arborizations of SN3 in the dorsal neuropile. (D) Location of their soma in the dorsal anterior cortex, and some tertiary neurites (TN) coming off SN2; *t*, trachea. (E) Arborizations of SN1, branching in the dorsal median neuropile, between MDT and DIT. Note that most of the arborizations of the two cells lie close together.
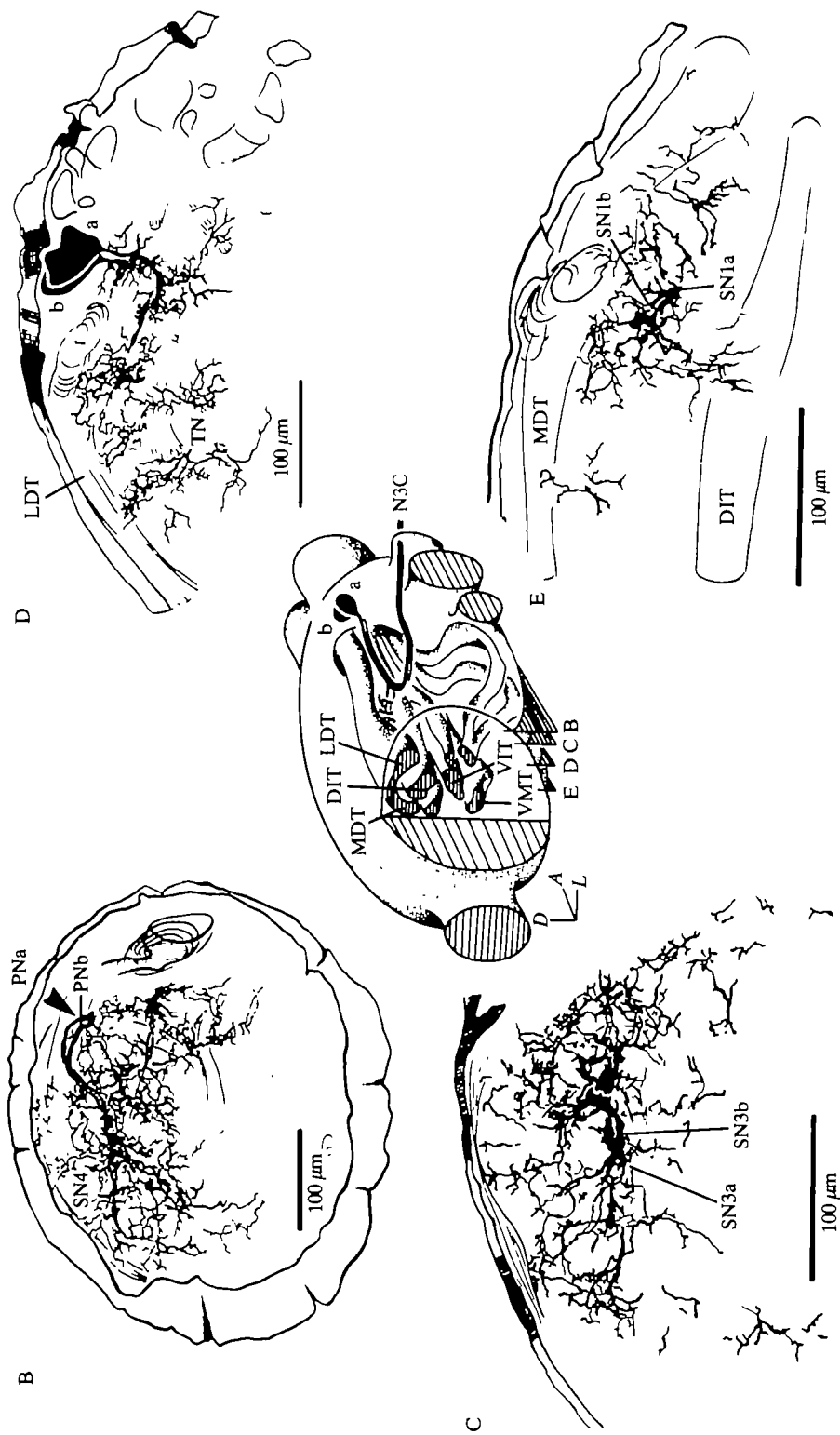
Fig. 10B–E

*S-shaped cells* (Fig. 11)

These cells (four have been found) innervate at least four individual muscles, of three anatomical groups (Fig. 16), through nerves 3A4,5 and 3C3,5 (Fig. 6). Their large somata (60 μm in diameter) lie dorsally by the midline, above the MDT, and are easily identified in transverse sections at the level of N3. Only three secondary neurites usually branch from the primary one, but their main branching area is similar to that of the U-shaped cells (Figs 9, 10, 11).

*V-shaped cells* (Fig. 12)

Three cells have been identified supplying the promotors and abductors, through nerves 3A4,5. Their small somata (20–30 μm in diameter) lie in the anterior cortex, ventral and lateral to the connective. The distal anterior branching of their SN1 neurite (Fig. 12) overlaps that of U- and S-shaped cells at the same dorsal–median level. Secondary neurite 2 gives rise to five to six tertiary branches that arborize profusely. Viewed dorsally, the outline of the branching area of V-shaped cells cannot be distinguished from that of U- and S-shaped cells (Figs 9, 12).

To summarize, U-, S- and V-shaped cells have significantly different (in shape, course and number, see Fig. 9) primary and secondary neurites, but have their branches in a similar region of neuropile, with one common medial projection towards MDT. They all leave the ganglion through N3, and supply muscles that either insert on the anterior basicoxa, or depress the trochanter (Fig. 16): the
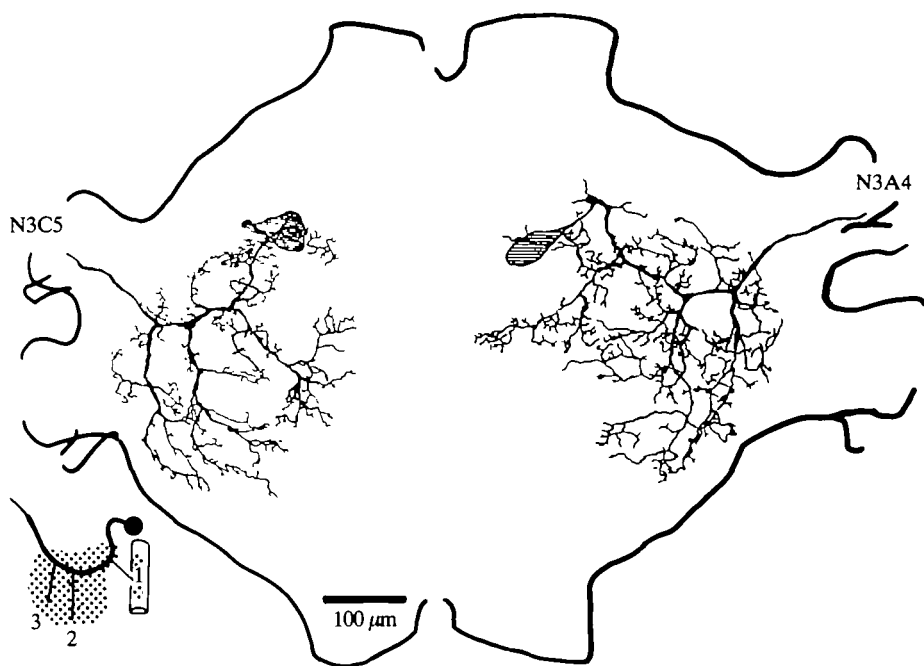


Fig. 11. Morphology of two S-shaped cells, backfilled from different nerves in different animals.
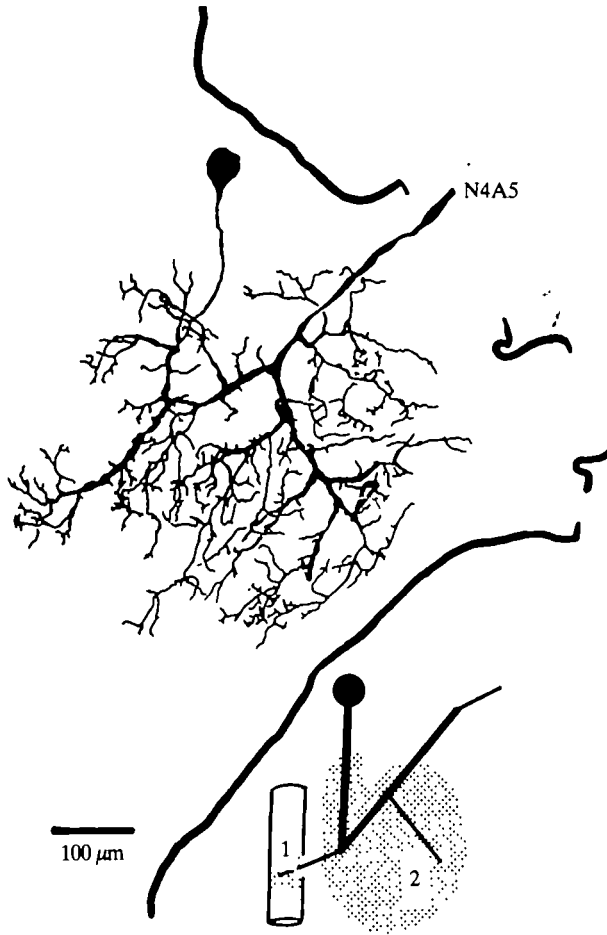
Fig. 12. Morphology of a V-shaped cell, backfilled from nerve 4A5, supplying the abductors. SN1 (inset) projects mediodorsally in the same region as the SN1 of U- and S-shaped cells do. Note that the main branching areas of V-, S- and U-shaped cells are very similar.

abductors are supplied by three U-shaped, one S-shaped and two V-shaped cells; the promotors are supplied by three U-shaped, one S-shaped and one V-shaped cells; the anterior rotator 75-2 is supplied by two U-shaped cells; the five double depressors are innervated by three U-shaped and two S-shaped cells.

*Y-shaped and Y-like cells* (Fig. 13)

Nine Y-shaped cells have been identified. They all leave the ganglion in nerve 4 and supply two of the posterior rotators (muscles 75-1a,b) and the two groups of remotors (i.e. seven muscles in all). Their small somata lie in the ventral posterior cortex. Their first secondary neurite is the only one to reach to the midline, in the MDT area and in the posterior quadrant of the neuropile. Secondary neurites 2–3 project laterally in the dorsal and lateral neuropiles.

Three Y-like cells have been obtained from the staining of N4A and N4C. Their branches follow the course of the primary and secondary neurites of Y-shaped cells, in close proximity, intermingling their finer processes; their primary neurite travels posteroanteriorly almost parallel to the sagittal axis, towards the ventral anterior cortex, where their small somata lie. Hence, Y-shaped and Y-like cells, which all show the same basic morphology, supply the remotors (eight Y-shaped, one Y-like) and posterior rotators (one Y-shaped, two Y-like), which all insert on the posterior basicoxa (Fig. 16).

*Levator (L) motor neurones* (Fig. 14)

Ten different cells were identified from staining of N5B, but none was obtained individually. Consequently, no precise description of their patterns of arborization can be given, though a certain degree of symmetry exists between their projections in the anterior and posterior quadrants. The 10 cells can, however, be separated into
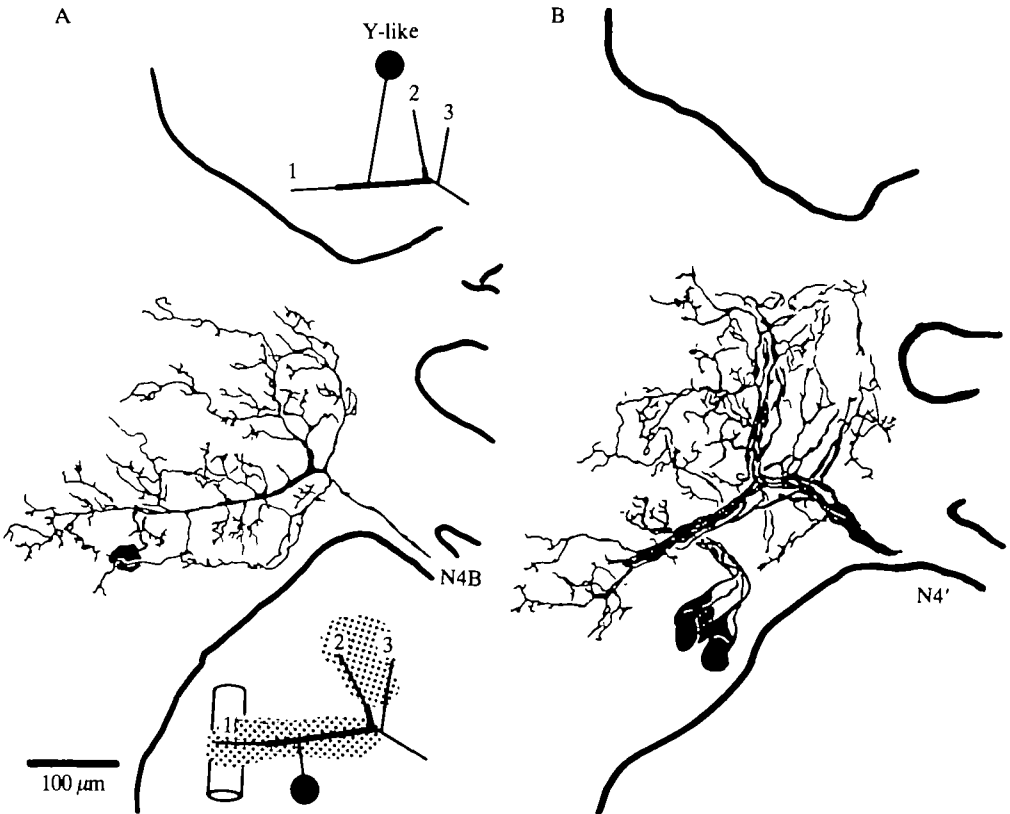


Fig. 13. Morphology of Y-shaped and Y-like cells, which all come out through N4. (A) Arborizations of a Y-shaped cell backfilled from N4B; the branching pattern of Y-like cells is identical, but their soma is located anteriorly (above inset). (B) A group of four Y-shaped cells has been stained from N4' (see Fig. 6) in the same preparation to show the similarity of their arborizations.
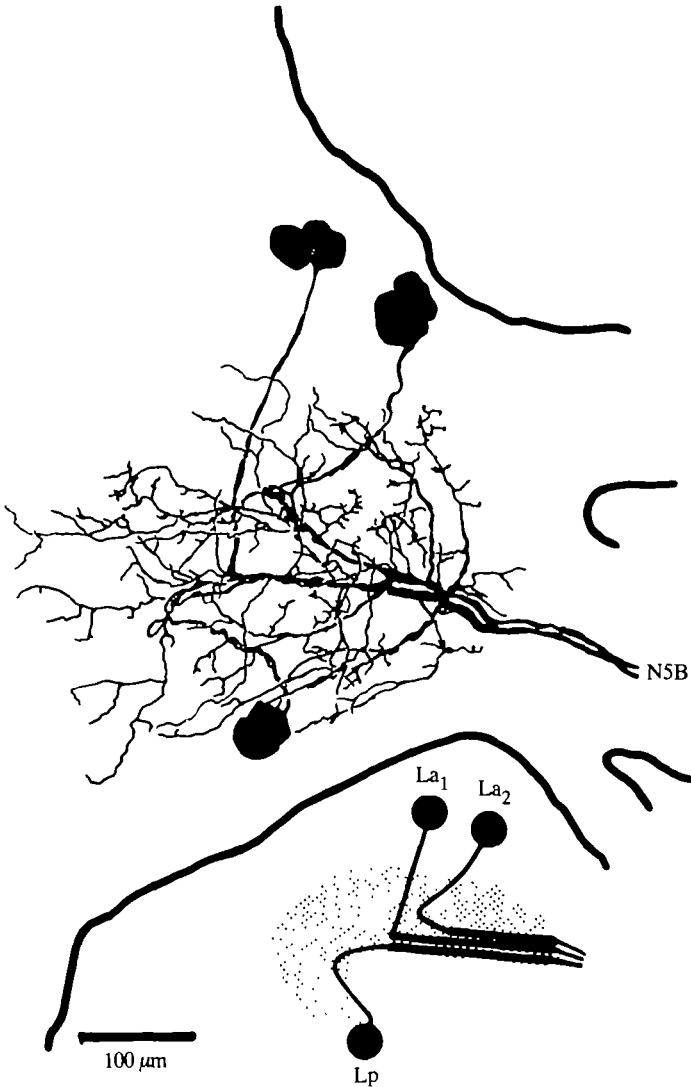
Fig. 14. Central projections of the motor neurones supplying the intrinsic levators, back-filled from N5B. La$_1$, anterior–medial; La$_2$, anterior–lateral; Lp, posterior clusters.

three groups, according to the position of their cell bodies [anterior–medial (La$_1$), anterior–lateral (La$_2$) and posterior (Lp) clusters] (Figs 14, 16).

*Intrinsic depressor of the trochanter* (Fig. 15)

Only two excitatory cells (aDTr and pDTr: anterior and posterior Depressor Trochanteris) supply these muscles. Their axons are clearly visible in transverse sections of nerve 5A (Fig. 7H). The morphology and ultrastructure of aDTr is described in detail elsewhere (G. Laurent, C. Palévody & D. Richard, in preparation), but three features should be noted. (1) The aDTr primary neurite has a loop-like form (Fig. 15A, inset). (2) This neurone has three secondary neurites
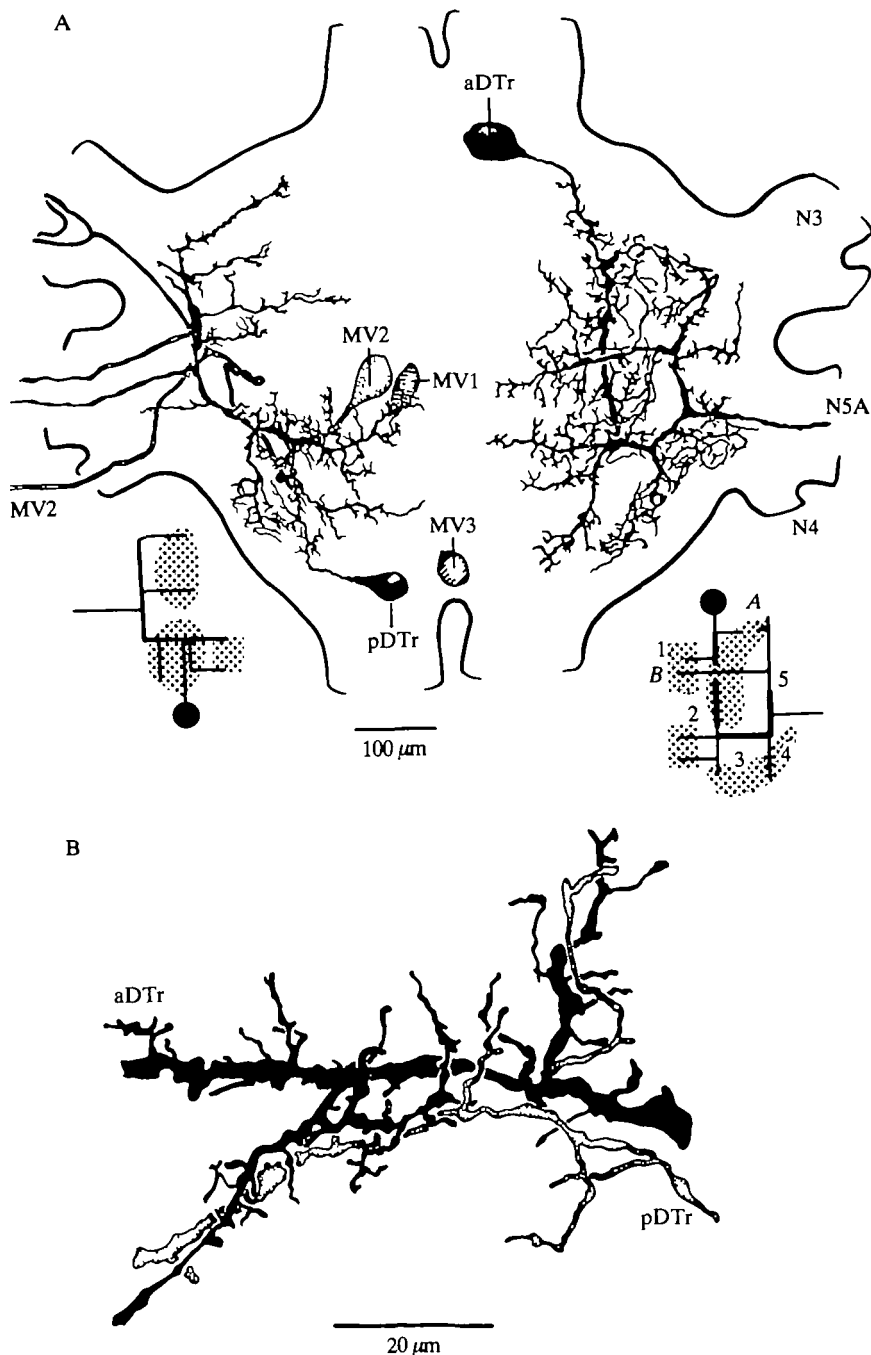
Fig. 15. Morphology of the two excitatory motor neurones supplying the intrinsic depressors through N5A, and location of the medioventral somata of the putative common inhibitors (MV1,2,3). (A) Arborizations of the two excitatory cells; note the loop-like organization of aDTr, closed in regions A and B (indicated in the right inset). (B) High magnification of the arborizations of both excitors in the dorsal neuropile, showing the blebbed structure of their fine branches, and the close proximity of their small neurites.
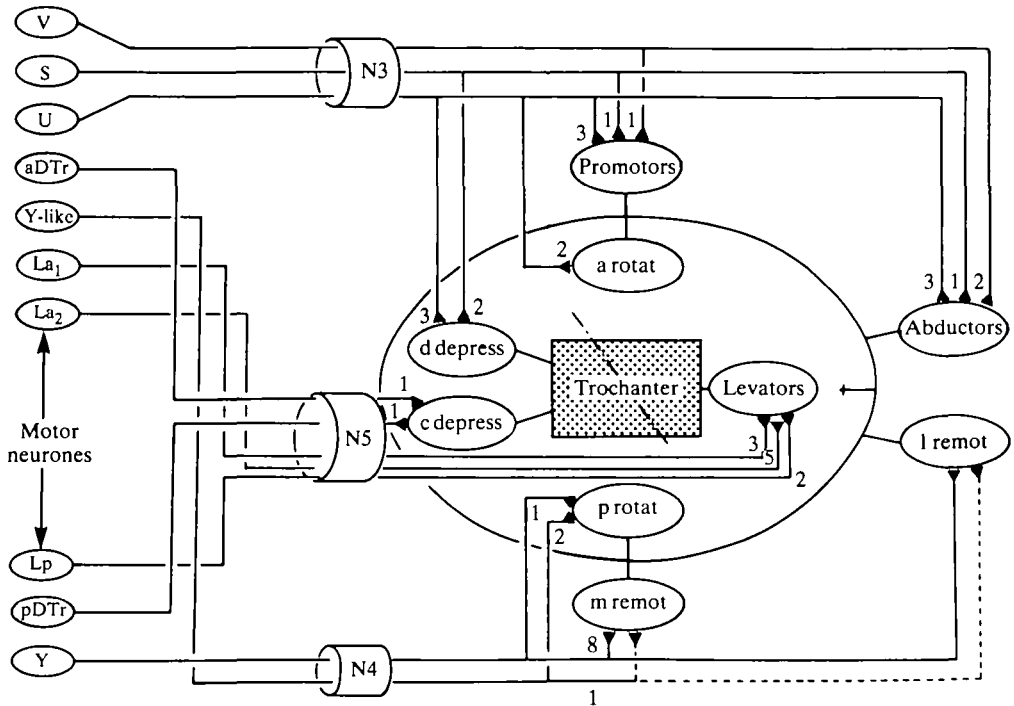
Fig. 16. Peripheral distribution of the 46 excitatory motor neurones to the main muscular groups. The numbers of identified cells of each morphological type are indicated near the ending symbols. The dotted line towards the medial (m remot) and lateral (l remot) remotors indicates that it was not possible to determine which of either group is innervated by a Y-like cell. p, posterior; a, anterior; d, dorsal; l, lateral, c, coxal; rotat, rotators; depress, depressors.

(SN2, SN3 and SN5) that project mediodorsally in the posterior quadrant of the ganglion. (3) The proximal part of the primary neurite of aDTr crosses the whole dorsal neuropile from the anterior ventral cortex, sending several branches into the intermediate neuropile.

Both aDTr and pDTr show a dense pattern of arborization; their axons and distal primary neurites run in parallel and most of their branches intermingle in the posterior half of the ganglion (Fig. 15B), making them likely to share central inputs and outputs.

## DISCUSSION

### *Anatomy of the cricket musculature and innervation: comparison with other Orthoptera and Dictyoptera*

The structure of the exoskeleton and musculature of the prothoracic leg accords with the ground plan given by Snodgrass (1929) for a grasshopper. The origins and insertions of muscular groups are located roughly at equivalent places on homologous sclerites, and there are only slight differences in the path that certain muscles take from the tergum to the coxa or the trochanter. For example, the cricket *Gryllus* double depressor 77-1a, which originates anteriorly, passes along a skeletal groove to the posterior thoracic cavity before entering the coxa (see Fig. 2A,D), whereas its

course is straight and linear in the grasshopper *Dissosteira*. This is probably due to differences in the structure of their thoracic exoskeleton. The prothorax is bilaterally flattened in the grasshopper, but dorsoventrally flattened in the cricket. This flattening probably causes the muscle proximal attachment sites to be different and their paths to twist. This seems to be confirmed by the muscular organization of the ·foreleg of the dorsoventrally flattened cockroach (Carbonell, 1947) whose muscle attachments are even more rotated than those of the cricket.

The numbers of individual muscles in the proximal prothoracic segments of the locust, grasshopper, cockroach and cricket are different. For example, there are twice as many in the cricket as in the grasshopper (see Table 2). However, the significance of such differences is difficult to explain in terms of function and motor abilities, since the criteria chosen by different investigators to define a muscle might have been slightly different (Alsop, 1978). Another reason for these differences may be metabolic. Big muscles require a high rate of detoxification and must thus be bathed in a large volume of haemolymph (Alsop, 1978). As the volume of the prothoracic cavity is fixed by the external skeleton, it is necessary to increase the surface area presented to the haemolymph by dividing the muscle into separate bundles. This could explain why running or walking insects (the cockroach and the cricket) have more prothoracic muscles than flying ones (the locust and grass-hopper), because the latter mainly use their pterothoracic segments during this mode of locomotion. However, insect muscles generally work in the aerobic mode, so that the only limitation is the elaboration of tracheal supply. In the case of the prothoracic proximal musculature this seems to be good.

The most important difference between the muscles of *Locusta/Dissosteira* and *Periplaneta/Gryllus* lies in the remotor and anterior rotator groups. There is no anterior rotator in the locust and grasshopper foreleg, and, furthermore, the two sets of lateral and medial remotors in *Gryllus* are fused in only one group that inserts in an intermediate location on the coxal rim. This absence and fusion of muscular groups might lead to a decreased control of foreleg movement compared to that of the cockroach and cricket and can also be correlated with the differences between their usual modes of locomotion.

Another difference between the locust and the cricket lies in the nervous supply of the intrinsic levators. These are innervated by N5B in *Gryllus,* but are supplied by a branch of N4 in *Locusta.* As the levator motor neurones of *Locusta* are morpho-logically similar to those of the cricket (personal observation), this suggests that some differences in the fusion or formation of nerves might have occurred during the process of evolution of both species from a putative common ancestor, rather than that the homologous muscles in *Gryllus* and *Locusta* are supplied by non-homologous motor neurones.

### The depressor system

The cricket depressor system, like that of the locust, grasshopper and cockroach, comprises two main sets of muscles: the long and slender double depressors, and the short, fan-shaped intrinsic depressors. The nervous supplies of these two sets differ:

the double depressors are innervated by U- and S-shaped cells through N3, whereas the intrinsic ones are supplied by aDTr and pDTr through N5A. Previous investigations on the cockroach *Periplaneta* (Pearson & Iles, 1973) showed that the fast and slow depressors (Df and Ds) – the cockroach homologues of aDTr and pDTr – are active during the stance phase of the middle and hindleg step cycle, whereas Burns & Usherwood (1979) demonstrated that the locust foreleg goes through an extension (depression of the femur) during the swing phase. Long muscles are generally considered to cause large displacements by a major shortening, while short ones generate force with little movement (Hoyle, 1983). It would therefore be interesting to determine whether the two muscular subsystems (double and intrinsic depressors) are functionally distinct, although they insert on the same apodeme and hence both cause movements in the same direction. Their pattern of innervation would tend to show them to be functionally distinct, which would support their involvement in different parts of the step cycle.

### Motor neuronal architecture and peripheral distribution

Gregory (1974) and Iles (1976) suggested that the pro- and mesothoracic ganglia of the cockroach each contain some 250 motor neurones. As the muscular organization of the cricket prothorax is very similar to that of the cockroach (see Table 2), one can suppose that the motor neuronal supply of *Gryllus* muscles is similar to that of *Periplaneta* (Iles, 1976). Our finding of one unpaired and 49 paired neurones for the proximal joint's musculature (which makes a total of 99 cells for the entire ganglion), would then represent more than one-third of the putative total of prothoracic motor neurones (250). As shown in Table 3, the number of 50 stained motor neurones represents more than 70 % of the total of putative motor axons, and more than the total number of larger axons ($\geqslant 15\,\mu$m in diameter).

All the motor neurones conform to the basic description of insect (Gwilliam & Burrows, 1980), arachnid (Bowerman & Burrows, 1980) and crustacean (Bevengut, Simmers & Clarac, 1983) excitatory motor neurones. Unlike some of the cricket neck muscle motor neurones that lie in the prothoracic ganglion (Honegger *et al.* 1984), all the cricket cells project exclusively in the ipsilateral half of the ganglion, as other leg motor neurones do in the cockroach (Iles, 1976) and the locust (Wilson, 1979). Many of them could not be distinguished from one another, and were consequently described as members of a population. It is likely that fine, individual, morphological differences exist between such neurones which could not be revealed by the use of backfilling techniques. In N3 and N4, the packing of cell bodies corresponds to the nerve in which the axons lie (except for Y-like cells), and hence also corresponds more or less to the function the cells supply. However, this correlation between soma position and function is not valid for any of the more distal leg segments (all supplied by collaterals of N5), as shown in this paper (coxotrochanteral muscles) and in other available leg motor neuronal maps of arthropods (Iles, 1976; Wilson, 1979; Bowerman & Burrows, 1980). Furthermore, the muscles supplied by N3, are innervated by at least 18 cells (U,V,S) that display very similar central projections, whereas N4 contains at least 12 other cells (Y, Y-like) that are hardly distinguishable

Table 3. *Comparison between the number of identified (stained) cells, and the numbers of axons, in the corresponding peripheral nerves (Fig. 7)*

| | N3A5 Abductors | N3A4 Promotors | N3C1 M61 | N3C2 Anterior rotator | N3C3 Double depressors | N3C4 Adductor | N3C5 Double depressors | N5A Intrinsic depressors | N5B Intrinsic levators | N4A Remotors | N4B Posterior rotators |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of axons | 9 | 8 | 10 | 3 | 2 | 1 | 5 | 5 +sensory axons | 13 +sensory axons | 26 +sensory axons | 4 |
| Number of large axons (≥15 μm diameter) | 6 | 5 | 0 | 0 | 2 | 0 | 3 | 1 | 9 | 15 | 2 |
| Number of neurones revealed by backfilling | 6 | 5 | 4* | 2 | 2 | 1* | 3 +1 DUM +1 CI | 2 +3 CIs | 10 +3 CIs | 9 +1 CI | 3 |

*The precise morphology of these cells has not been revealed. However, four somata, whose location in the ganglion is indicated in Fig. 6, were stained by backfilling N3C1, and one by backfilling N3C4.

When the nerve was also known to supply sensory structures, the smaller axons (<5 μm diameter) were assumed to belong to sensory neurones. These were not counted and are referred to here as sensory axons.

CI, common inhibitor; DUM, dorsal unpaired median neurone.

from one another except for three somata. One explanation for these morphological resemblances might be developmental and phylogenetic: the cricket, together with other Orthoptera and the Dictyoptera, are evolved Pterygota, whose coxal musculature is much more elaborate than that of Collembola and Diplura (Manton, 1979), for instance. Therefore, it can be supposed that the muscles inserting on the trochantin and anterior coxal rim (the promotors, abductors and anterior rotators) were originally (like their antagonists) part of the same and unique bundle, supplied by a common 'pool' of motor neurones through a single nerve. The similarities in the pattern of innervation of different anatomical bundles in the modern Orthoptera would then only be a consequence of peripheral neuromuscular refinements during the process of evolution. This hypothesis would at least apply to the remotor system, which comprises two sets in the cricket (this paper), but only one, in a median position, in the locust and grasshopper (see Table 2).

It is, however, important to determine the pattern of activity of these groups of motor neurones during a step cycle, to determine whether this pattern corresponds to what can be deduced from the present anatomical data. This will be the object of the following paper.

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