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STRUCTURAL AND FUNCTIONAL  
CHARACTERISATION OF SOME  
INSECT CENTRAL NEURONES

BY

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B. Sc. (RIYAD UNIVERSITY)

A THESIS SUBMITTED FOR THE DEGREE  
OF DOCTOR OF PHILOSOPHY OF  
UNIVERSITY OF DURHAM

1982



THE GRADUATE SOCIETY

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## II.

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ABSTRACT

Histological studies of the distribution of cell bodies within the metathoracic ganglion of both the stick insect Carausius morosus and the cricket Gryllus bimaculatus were carried out using orthodox histological techniques (silver, iron haematoxylin) as well as back-filling and intracellular injection staining techniques with both Cobalt Chloride and Procion yellow as well as whole mount staining techniques. Maps of geographical groups of cell bodies of the metathoracic ganglion of both insects were reconstructed from serial sections. Salient through-tracts were described.

Neurones that innervate the dorsal longitudinal flight muscles were identified in both the mesothoracic and metathoracic ganglia of Gryllus bimaculatus, and their detailed morphological properties were described. Difficulties imposed by resistance of the neural sheath to impaling glass microelectrodes and by some of the substances used were discussed.

Neurophysiological and pharmacological experiments were carried out to identify some of the physiological properties of the neurones that innervate the dorsal longitudinal flight muscles of the cricket, Gryllus bimaculatus.

ABBREVIATIONS

Ach	Acetylcholine.
AchE	Acetylcholinesterase.
CDLM	Contralateral dorsal longitudinal motor neurone.
CNS	Central nervous system.
CoCl <sub>2</sub>	Cobalt Chloride.
d	Dorsal.
DC	Direct current.
DCMD	Descending contralateral movement detector.
DLM	Dorsal longitudinal flight muscle.
DUM	Dorsal unpaired median neurones.
DUMDL	Dorsal unpaired median neurone that innervates the dorsal longitudinal flight muscles.
epsp	Excitatory post-synaptic potential.
Epp	End-plate potential.
gm	Gram.
HZ	Hertz.
ipsp	Inhibitory post-synaptic potential.
LGI	Lateral giant interneurone
MGI	Median giant interneurone.
msec.	Millisecond
mV	Millivolt.
nA	Nano Ampère.
nm	Nanometre.
V	Ventral.

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## CHAPTER ONE

### ANATOMY AND HISTOLOGY

#### 1. Introduction:

Insect nervous systems have many advantages for studying the relationship between structure and function. In general, the muscles of insects consist of only a few motor units, each containing relatively small numbers of muscle fibres, and innervated by relatively few large neurones (Hoyle, 1970). The number of neurones in the central nervous system (CNS) is small by comparison with vertebrates and there is constancy of neuronal identity and position. This economy of innervation has encouraged anatomists and physiologists, to work on insects in the belief that it is possible to work repeatedly with single, identified neurones.

Structural studies of the main parts of the CNS of certain insects, and of some of the characteristics of the individual neurones involved have been carried out by a number of authors in an attempt to understand the physiological and behavioural events taking place within the system. These studies have led to the use of a variety of staining techniques in attempts to recognize the details of the distribution of cell bodies within the CNS and some of their axonal pathways and branches. As a result many techniques have been developed for staining, and several maps of neural organization have been produced for different species.

One of the early staining methods for the study of the CNS was methylene blue (Zawarzin, 1924a, b); but it tends to



stain certain cells completely and to leave others unstained (Hughes, 1965). Zawarzin (1924) produced detailed methylene blue studies of abdominal ganglia of nymphs of the dragonfly Aeschna. A criticism of this work is that he described the neurones on the basis of <sup>presumed</sup> physiological type (sensory, motor, association) and the anatomical relationships between the various types of neurones were not determined (Bullock and Horridge, 1965). It is, of course, one of the difficulties of histological methods that they give little information concerning function. For example, one cannot distinguish the inhibitory cells from those that are excitatory, although it is usually possible to distinguish motor and interneurones. However, in the finer endings of the neuropile, all axons and their branches appear identical (Hughes, 1965); Huber, (1974).

Silver impregnation is also a widely used technique for staining nervous tissue of insects; but it has the disadvantage of being capricious from one species to another, although many suggested improvements for its use have been produced, such as that of Rowell (1963), Blest (1976), and others (e.g. Strausfeld, 1976; 1980).

In his work on the nervous system of a winged but flightless Drosophila melanogaster, Power (1950), using a protargol (silver) method, showed that the thoracico-abdominal nervous system consists of the same main elements and does not change significantly even in mutant stocks. Pipa et al. (1959) using the Bodian activated protargol technique, described the anatomy of the thoracic

ganglia of the cockroach, Periplaneta americana, and especially the longitudinal and transverse tracts and fibres. They noted that the majority of the nerve cells were located in the ventral region of each ganglion, and used this criterion for determining the orientation of the sections. They described two types of nerve cell bodies: globular cells, characterized by their spherical shape, smaller diameters, and predominance of nuclear volume to cytoplasmic volume; and giant ovate cells with as much as or more cytoplasm than nucleoplasm. No specific axonal pathways were described.

Guthrie (1961) presented an anatomical study of the nervous system of a skating insect, Geris. He used a silver impregnation method for staining, which showed a high degree of condensation in that all the segmental neuromeres are fused; and revealed giant interneurons half a millimeter long, having dendritic branches in at least three ganglionic neuropiles.

He concentrated on one ganglion, the mesothoracic, because the three thoracic ganglia are approximately equal in size and exhibit general similarities in the internal arrangement of the nerve fibres and nerve cell bodies.

Using microelectrodes, Mill (1963) made an attempt at mapping a single ganglion of dragonfly larva, and in (1964) he presented a histological study describing the structural elements, cell body groups and axonal pathways and tracts.

Seabrook (1968) used osmium ethyl gallate in studying the simplest ganglion of the ventral nerve cord, the seventh ganglion, of the male of Schistocerca gregaria. He described the structural elements of that ganglion, i.e. cell body

groups, transverse and longitudinal tracts and axonal pathways.

Cohen and Jacklet (1965) developed a technique for recognizing motoneurone cell bodies in their ganglia. In this technique, a heavy ring of Ribonucleic acid (RNA) appears around the cell nucleus within two days of section of peripheral nerves in the cockroach, which can be stained with pyronine and malachite green. Using this technique, Cohen and Jacklet (1967) produced a map of the metathoracic ganglion of cockroach, in which they showed the distribution of the cell bodies related to the peripheral nerves through which they send their axons. Although this technique confirmed the symmetry between the two halves of the ganglion, it does not allow single axone to be followed because the nerve transection has to be made close to the ganglion.

The mesothoracic ganglion of the cockroach, Periplaneta americana, was also mapped by Young (1969) using a similar injury technique. He described the overall layout of the large cell bodies within the mesothoracic ganglion, which was found similar to that of the metathoracic ganglion.

Gregory (1974) working on the mesothoracic ganglion of Periplaneta americana, made a full anatomical study of the ganglion using silver staining and Procion yellow. He described the distribution of cell bodies but most of his work was devoted to the tracts within the ganglion and he gave detailed descriptions of them. The similarity of the three thoracic ganglia of the cockroach Periplaneta americana was confirmed by Iles (1976) when he mapped the prothoracic ganglion using toluidine blue for staining the ganglion

overall and the back filling technique of cobalt chloride through the cut ends of the ganglionic nerves. He showed the groupings of cell bodies within the ganglion as well as the axonal pathways of some individual neurones.

The giant fibres of the ventral nerve cord of Periplaneta americana, were studied by degeneration technique by Hess (1958, 1960); this technique was also used by Clark (1976a,b) in studying the morphological and physiological properties of the contralateral dorsal longitudinal motor neurone (CDLM) of the metathoracic ganglion of the cricket Teleogryllus oceanicus. The course of the giant fibres within the ventral nerve cord of the cockroach Periplaneta americana, was also studied by Farley and Milburn (1969) who described their branching in the terminal ganglion and in the thoracic ganglia; also Milburn and Bentley (1971) showed that the ramifications of two giant fibres in Periplaneta americana are different. <sup>Anatomical</sup> maps for other invertebrates than insects also have been produced such as those of Aplysia depilans (Hughes and Tauc, 1962), the lobster, Homarus americanus (Otsuka et al., 1967), and the leech, Hirudo medicinalis (Nicholls and Baylor 1968).

However, the real and more reliable link between structure and function of central neurones has been made by using the intracellular staining technique involving such dyes as Procion yellow, e.g. Stretton and Kravitz (1968; 1973) and staining through the cut ends of nerves (Iles and Mullony 1971), and the cobalt chloride technique (Pitman, Tweedle and Cohen, 1972; 1973a,b). These techniques were used in many studies that attempted to identify the individual

neurones and their branches; and also neurones in groups. Bentley (1970) used Procion yellow and silver nitrate stains in producing a map of flight neurones of the mesothoracic ganglion of the locust, Schistocerca gregaria, and in the cockroach, Periplaneta americana, the cell bodies have been described as groups in relation to the nerves through which their axons pass, after having been filled with the Procion yellow dye through the cut ends (Iles and Mullony 1971). In (1972) Iles reconstructed the fast coxal depressor motoneurone in the metathoracic ganglion of Periplaneta americana, using Procion yellow. Also a single motoneurone was demonstrated in the metathoracic ganglion of Periplaneta americana by Pitman et al. (1972) when they used their technique of cobalt chloride staining for the first time. Then followed a number of studies of individual neurones and their pattern of branching; Bentley (1973) studied the flight motoneurones in the postembryonic development of cricket, Teleogryllus oceanicus. The common inhibitory neurone of the metathoracic ganglion of the locusts, Schistocerca gregaria and Chortocetes terminifera was studied morphologically by Burrows (1973a) and the morphology of an elevator and depressor motoneurone of the hind wing of Chortocetes terminifera was also studied by Burrows (1973b) using cobalt chloride. The topography of limb motoneurones in the metathoracic ganglion of Schistocerca gregaria was also studied by Burrows and Hoyle (1973) using Procion yellow dye, and identification of the somata of common inhibitory motoneurones in the metathoracic ganglion of the cockroach Periplaneta americana was carried

out by cobalt chloride staining technique (Pearson and Fourtner 1973) although there were no detailed axon branches shown. Motor and sensory flight neurons in Chortoicetes terminifera, and their probable correlation were studied by cobalt chloride as well (Tyrer and Altman 1974). Detailed study of interneurons of the locust Schistocerca gregaria, were also presented by Siegler and Burrows (1979). On the other hand, morphology of identified neurones of other invertebrates than insects were presented by many workers, e.g. Aplysia (Winlow, 1975; Winlow and Kandel, 1976) and Lymnaea stagnalis (Benjamin et al., 1979; Haydon and Winlow, 1981).

One of the species which has received less attention is the cricket, Gryllus bimaculatus. Although the cricket is a winged insect, its wings are not used mainly for flying, but in stridulation, by rubbing them against each other, (Elsner and Popov, 1978). So, it was of interest to investigate some of the neurones that innervate some of the flight muscles.

The dorsal longitudinal flight muscles, muscles no. 112 (Snodgrass, 1929) are indirect flight muscles (Chapman 1969); and are major flight muscles <sup>in all insects</sup> where the up-stroke of the wing movement is produced ~~in all insects~~ by their contraction (Pringle 1975), and are not involved in walking, so the arborizations of their motor neurones are expected to be less complicated than those of neurones that innervate muscles which are involved in both flying and walking such as the dorso-ventral muscles (Wilson 1962). Another interest of the cricket preparation is that the muscles which are responsible

for sound production may also be used in flight, <sup>and</sup> which requires involvement of <sup>a</sup> motor pattern generating system which must involve some form of central switching process, from "flight" channel to "song" channel and vice-versa. This permits, in principle, the study of a motor tape selection mechanism (Huber, 1962; Bentley and Kutsch, 1966; Hoyle, 1970).

On the dorsal side of the thoracic and abdominal ganglia of some insects there is a group of cell bodies, group No. 2 (Figure 1.13) arranged around the dorsal longitudinal mid-line of the ganglion. The neurones of this group differ from other neurones in being unpaired and at least some of them give rise to a single short process which bifurcates into symmetrical processes that leave the ganglion through the lateral nerve trunk on each side. This group was first identified by Plotnikova (1969) who was working on Locusta migratoria, using methylene blue stain only. She described them as polyaxonal neurones with a large pear-shaped cell body <sup>ranging from</sup> (80 x 40 to 30 x 25  $\mu$ m) <sup>length x breadth</sup> located in the dorsal part of the ganglion at the exit of the dorsal connectives. She found nine neurones with symmetrical axons in the metathoracic ganglion. Among them there were no less than three neurones with axons passing into the second and the third pairs of lateral nerves. She reported that these neurones send their axons to the first, second, and third nerve pairs of the ganglion; and she suggested that these neurones have a regulatory function.

Crossman et al. (1971a) showed a similar group in the cockroach Periplaneta americana and the locust, Schistocerca gregaria. They studied it physiologically in both animals,

and they found that the group consisted of eight cell bodies arranged around the dorsal mid-line of the ganglion; they reported that the location of these cell bodies is slightly variable from one preparation to another; and that they were electrically excitable giving overshooting action potentials. They also reported that two cell bodies in the metathoracic ganglion have bifurcating axons supplying N<sub>5</sub> (nomenclature of Pringle 1939) on each side of the ganglion; and their axons were thickest where they passed on the edge of the neuropile to the side of the ganglion, and were thinnest near the cell body and its peripheral nerve root.

Again Crossman et al. (1971b; 1972) working on the same insects reported that all the eight dorsal unpaired cell bodies send branches through all the ganglionic nerve trunks on both sides of the ganglion including the anterior and posterior connectives, but with the exception of nerve trunk N<sub>2</sub> (nomenclature of Pringle 1939). This was concluded from experiments using antidromic and orthodromic stimulation and recording the response.

However, they found that the mediodorsal nerve cells (as they called them) of the locust, Schistocerca gregaria contributed axons to nerve trunks 3-5 and the anterior connectives on both sides of the ganglion; but not to nerve trunks 1 and 2 and the posterior connectives. They were unable to get the whole neurone stained with Procion yellow and they attributed that to the failure of the dye to diffuse into the fine branches of the neurones.

The dorsal cells were studied also in Schistocerca

gregaria by Hoyle et al. (1974), who ~~they~~ called them the dorsal unpaired median (DUM) neurones; in their study they used a combination of serial sections of light and electron microscopy, methylene blue vital staining, intracellular and peripheral extracellular stimulation and recording, as well as dye injection. They found 23 neurones on the dorsal surface of the metathoracic ganglion, which ranged from 25-85  $\mu\text{m}$  in average diameter; eleven of them being large cell bodies (over 45  $\mu\text{m}$  diameter) and seven of the latter were efferent because they had one or two axons leaving the ganglion through the nerve trunks, and since reflexly-evoked impulses travel centrifugally in them. The other four were probably interneurones, with one major neurite each in either the left or the right anterior connective. The smaller neurones appear also to be interneurones, but with no major neurite leaving the ganglion. Five of the large ones send their axons to  $N_5$  (nomenclature of Campbell 1961) on both sides and give branches to  $N_4$ . The largest of them was traced to its target, the extensor tibiae muscles, hence the neurone was called DUMETI and its ending in the muscles found to contain a large number of dense core vesicles of unknown function; but Hoyle et al. suggested that DUMETI might serve a trophic function. And in (1974) Hoyle found that stimulation of DUMETI causes partial or complete inhibition of the intrinsic rhythmicity, seen in the intact animal or isolated leg (Hoyle and O'Shea 1974) of the metathoracic extensor tibiae muscle fibres. This DUMETI neurone was shown to have an octopaminergic transmitter (Hoyle 1975; Hoyle and Barker 1975; Evans and O'Shea 1977, 1978).

In the locust Schistocerca gregaria and the grasshopper Romalea microptera, Hoyle (1978) reported that the whole cluster of the DUM neurones is variable in regard to total number, relative size and location of somata. He reported that not all the mediodorsal cells are unpaired, being physically linked together indirectly by intermingling of neurites and axonal processes and by sharing of glial cells.

Another of the DUM neurones also gives rise to a bifurcating neurite; this neurone supplies the dorsal longitudinal flight muscles (DLM); Bentley (1973) in his study of the postembryonic development of insect motor systems showed this neurone in the metathoracic ganglion of the sixth instar nymph of Teleogryllus oceanicus which he had filled with cobaltous chloride through  $N_1$  (nomenclature of Campbell 1961) of both sides but he did not specify the branch through which it goes to the DLM and he found it difficult to fill it with dye in the adult stage. The same cell body also was noted in the metathoracic ganglion of the same animal by Clark (1976a,b) but without details. Hoyle (1978) reported that this neurone (the DUMDL) was located only in newly moulted insects up to five days old in the locust and grasshopper and he suggested that it may degenerate.

Davis and Alanis (1979) using extracellular stimulation and recording technique, ~~they~~ showed that the DUMDL innervates the DLM in the cricket Gryllus domesticus through  $N_1 D_1 A$  (nomenclature of Campbell, 1961) but no stained preparations were shown or intracellular recording.

In addition to innervation by the DUMDL the DLM is innervated by another neurone, whose cell body lies contralaterally on both sides of the metathoracic ganglion. Neville (1963) demonstrated physiologically that the DLM is innervated by four neurones whose cell bodies lie in the mesothoracic ganglion and a fifth one with its cell body in the metathoracic ganglion in the locust Schistocerca gregaria. Guthrie (1964) followed this neurone to the contralateral side of the metathoracic ganglion, but he thought it innervated the oblique dorsal muscle. Bentley (1970) in his map of the flight motor neurones in the mesothoracic ganglion of the locust Schistocerca gregaria, reported that he was unable to excite the DLM by stimulating its cell body, and he attributed that to the distance of the cell body from the spike initiation zone. Bentley (1973) showed this neurone stained with cobalt chloride in the cricket Teleogryllus oceanicus; and Clark (1976a,b) studied the effect of separation of the soma from its arborizations in the same animal. Tyrer and Altman (1974) investigated the flight motor neurones including this neurone in the mesothoracic and metathoracic ganglion of the locust, Chortoicetes terminifera and showed its morphology using cobalt chloride.

In the present work, a study has been made of the metathoracic ganglion of the cricket Gryllus bimaculatus, with these earlier investigations in mind, and concentrating on the neurones most studied in other insects by other authors. The purpose of the work was (a) to determine the extent to which neurones with similar functions might be grouped together, in view of

conflicting reports on this point; (b) to see how far the cricket might fit into a constant pattern of central neurone distribution among insects as a whole; (c) to investigate how far recording from <sup>the</sup> cell body might be an indicator of that cell's function, and (d) to provide some information for further anatomical and physiological studies of this insect.

A major part of the investigation, therefore, was an anatomical and histological study of the metathoracic ganglion and its neurones; and it is this that forms this first section of the present thesis.

The original intention was to carry out this investigation on the stick insect, Carausius morosus Br. which although much studied from a variety of viewpoints, had not been systematically investigated as far as its neuronal content was concerned (see for examples: Wood, 1957; 1963; Treherne and Maddrell, 1967a,b; Huddart and Oates, 1970; Finlayson and Orchard, 1977; Fifield and Finlayson, 1978; Cruse, 1981; Cruse and Pfluger, 1981). A considerable amount of anatomical and histological work was performed on this animal before it was found to be impossible to penetrate the sheath surrounding its ganglia with intracellular microelectrode (see section II for details). Thus, the account that follows in this chapter relates to both the stick insect Carausius morosus and the cricket Gryllus bimaculatus, it being necessary to substitute the latter for the former.

## II. MATERIALS AND METHODS

### Maintenance of the insects

#### (i) Insectary :

The insectary was maintained at a temperature of  $30 \pm 0.5^{\circ}\text{C}$  and  $60 \pm 5\%$  relative humidity. Circulation of air was maintained by three large electric fans and a slight continuous air exchange was effected by means of an 'Xpelair' ventilator. A constant photoperiod, 12 hours light and 12 hours dark, was maintained.

### Animals

#### (ii) Gryllus bimaculatus :

These were maintained in bulk cultures supplied with apple, lettuce and dry animal food. Experiments were performed mostly on adult males at least one week after the final moult, for the males are easier to dissect than females, which contain egg masses.

#### (iii) Carausius morosus :

These were maintained in large glass and plastic containers at room temperature (approximately  $22^{\circ}\text{C}$ ) and were fed on Ivy.

### Histological Methods

#### (i) Direct observations on the peripheral ganglionic nerves :

These were carried out under a dissection microscope as follows:

After dissecting the animal and exposing the nerve cord and the peripheral nerves the dissection was flooded overall with 70% alcohol and left for 30-60 minutes after which the nerve cord and the peripheral nerves became white and fairly visible and could be followed to their muscles.

On some other occasions, about twenty minutes before dissection, the specimen which was to be examined was injected with a solution composed of 50 per cent 0.1 per cent methylene blue and 50 per cent saline solution. The specimens were fixed in Bouin's <sup>fixative</sup> and afterwards preserved in 70% alcohol (Campbell 1961) until examined.

#### Methods for staining serial sections

##### (ii) Heidenhain's Iron Haematoxylin

Ganglia were fixed in Bouin's fixative for 24 hours to 3 days, dehydrated through alcohol series, cleared in Xylene, embedded in paraffin, serially sectioned at 10  $\mu$ m and mounted.

The sections were stained in Heidenhain's haematoxylin using 4% iron Alum for mordant and differentiation. Then the slides were dipped into ammoniated water for 2-5 minutes, washed in tap water, dehydrated in an ascending alcohol series and dipped in alcoholic eosin for 2 minutes before going into the absolute alcohol. They were cleared in Xylene and mounted in DPX.

##### (iii) Silver nitrate staining method :

Best results were obtained in both the stick insect and the cricket by using the Holmes-Blest technique as described by Strausfeld (1976) and according to which the following steps were undertaken:

Freshly dissected ganglia were fixed in Bouin or Carnoy's or AAF (Formol-acetic acid-ethanol), for 2-12 hours, washed in 70% alcohol, dehydrated and embedded in paraffin. Paraffin was removed from 10  $\mu$ m sections using Xylene, and the sections hydrated and then placed in 20% silver nitrate solution for 2-3 hours, in the dark.

Sections were rinsed in distilled water, then incubated at 37°C in the following solution:

27.5 ml M/5 boric acid; 22.5 ml M/20 borax; 1% silver nitrate, 5-10 ml; 2-6 ml pyridine; 250 ml distilled water. Best results were found to be obtained after incubation for 8 hours compared to the original schedule of 10-20 hours.

Sections were reduced without washing, in a solution containing 3 grams hydroquinone and 30 grams sodium sulphite in 300 ml distilled water, for 3-7 minutes at 55°C. They were washed in tap water and rinsed in distilled water.

For toning, contrasting and fixation, sections were treated as for the Bodian-Power procedure as follows:

The sections were placed in a 1% solution of gold chloride, containing 1 ml of citric acid or acetic acid/100 ml solution, for 10-15 minutes at 25°C in bright light and then rinsed quickly in distilled water. Sections were next placed in a 2% solution of oxalic acid for 10-30 minutes until the sections appeared bluish red (under the microscope), and then rinsed very quickly in distilled water and fixed in a 5% solution of sodium thiosulphate (Blest, 1976) for 15 minutes, dehydrated and mounted.

#### Methods for staining the ganglion in bulk

##### (iv) Toluidine blue method :

This was carried out according to the technique of Altman and Bell (1973). The freshly dissected ganglion was immersed in a solution of toluidine blue in a solid watch glass for 10-15 minutes at 50°C; from the stain, specimens were transferred directly to Bodian's No. 2 fixative (Bodian 1937), which acts as a differentiator as well as a fixative; it was changed

every minute for the first five minutes, until it remained almost colourless. At this stage the ganglia appear dark blue with a metallic sheen. Differentiation was continued until cell bodies were clearly visible under a dissecting microscope and the nerve roots and neuropile areas were almost white. As a little stain is lost during dehydration, differentiation is stopped just before the desired appearance is reached. The ganglia were dehydrated in 90% ethanol and two changes of absolute ethanol, and the ganglia then were cleared in methyl benzoate according to the method of Pantin (1946).

The specimens were examined either immersed in methyl benzoate in a cavity slide, or mounted in DPX and examined after drying.

(v) Cobalt chloride solution for staining whole mount

In view of the ease with which individual neurons seem to stain with cobalt chloride, it was decided to see if this substance could be used for staining ganglia in bulk.

The freshly dissected ganglion was immersed in a diluted solution of  $\text{CoCl}_2$  (50 mM) for 1-5 minutes; then in a 2% (v/v) solution of ammonium sulphide to precipitate the cobalt, for 5 minutes; then fixed in alcoholic Bouin's fixative, dehydrated, cleared in methyl benzoate and examined as a whole mount. All steps were carried out at room temperature.

Although the method was not always completely successful, because it proved easy both to overstain or understain the ganglia, it was a useful method for demonstrating the general distribution of cell bodies, and had the great advantage of

speed. Figures 1.13C, 1.17B show two of these preparations.

(vi) Cobalt chloride method for staining individual neurons through cut ends of their nerve trunks:

This staining technique has been used according to the procedure described by Pitman et al. (1972; 1973b).

A range of different concentrations of  $\text{CoCl}_2$  was tried. Good fillings were obtained by using 10% (w/v) through relatively short nerves; but as the nerves get longer and thinner dilution is needed down to 1-2% (approximately 50-100 mM)  $\text{CoCl}_2$  solution. The required weight of  $\text{CoCl}_2$  salt was weighed and dissolved in the corresponding volume of distilled water, or 50% insect saline if the preparation was intended to be left overnight, for this was found to give better results, probably by keeping the cut end in good condition for a longer time.

The apparatus used for filling was of two types;

As can be seen in Figure 1.1 one type (Fig. 1.1A) is for single filling, similar to that described by Iles and Mulloney (1971) and which consisted of a 'Perspex' bath with two chambers joined by a narrow channel, that allows a nerve trunk to pass through. In some baths, the two chambers were joined by up to three narrow channels, so three preparations can be filled in the same bath.

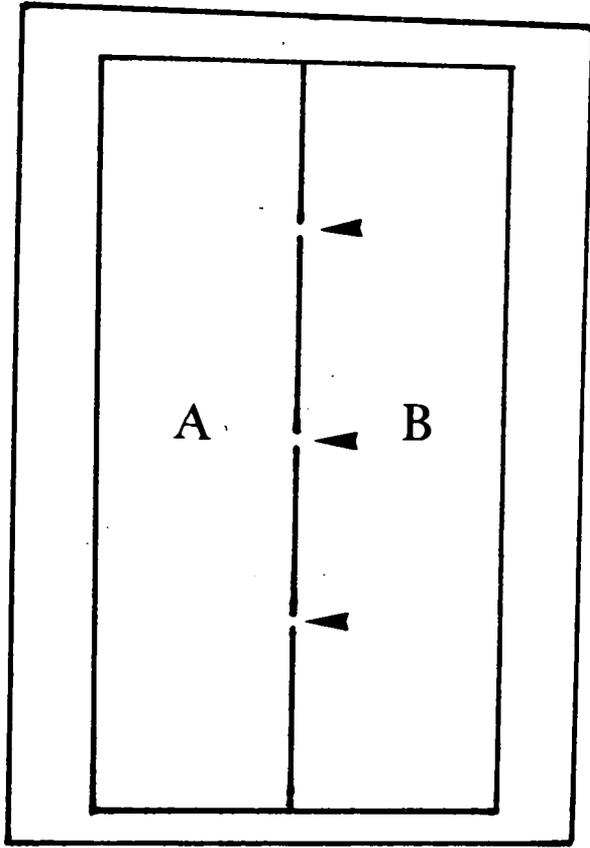
The other type of bath is for double filling, and consisted of three chambers (Fig. 1.1B), the middle of which is narrow, only around 2 mm in width, to accommodate the ganglion, and is joined to another bigger chamber on each side by a narrow channel, so enabling each nerve of the same pair to be put through a channel in the corresponding side of the

Figure 1.1

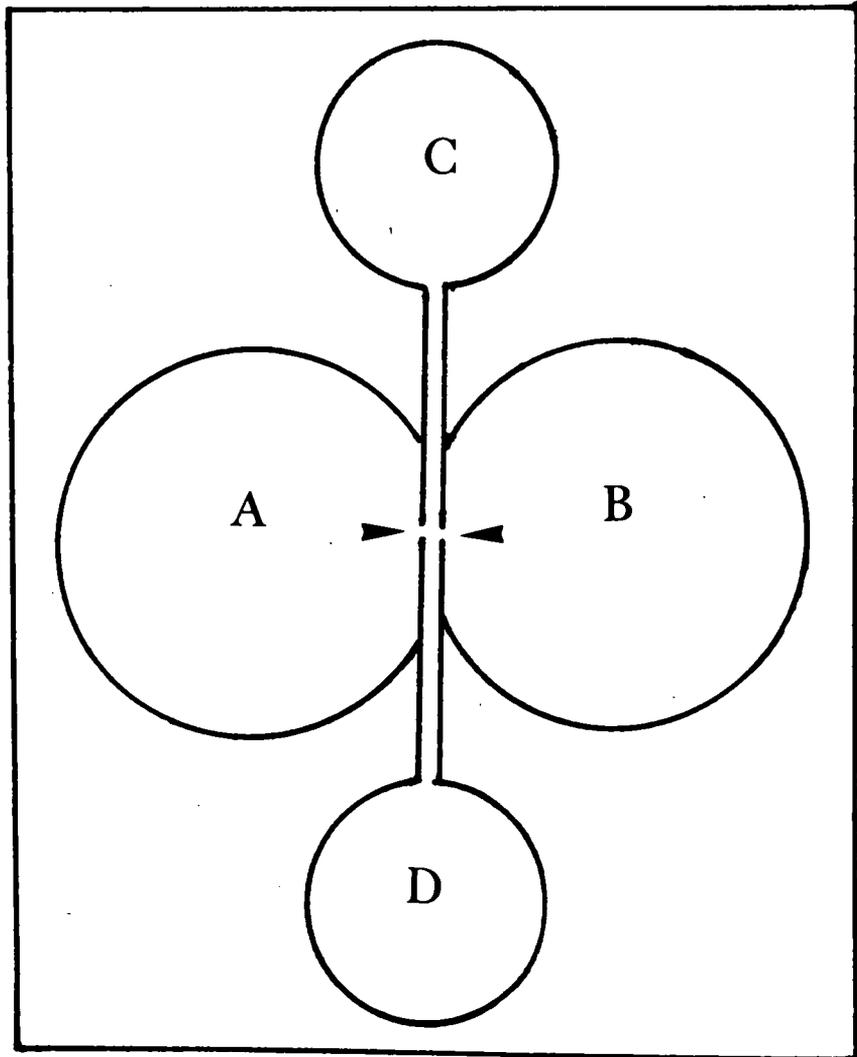
A drawing of the apparatus used for back-filling with  $\text{CoCl}_2$  solution. (A) is a double chamber bath consisting of two chambers A and B joined by narrow channels (arrows) through which a nerve trunk can pass. This kind of bath was used for single fillings (unilateral fillings) and up to three preparations can be made at the same time. (B) is a triple chamber bath, the middle chamber being narrow to accommodate the ganglion and joined to another bigger chamber on either side, A and B, by narrow channels (arrows) for the passage of a pair nerves, one on each side of the ganglion. Chambers C and D are extensions to the narrow chamber to ease adding or sucking solution. This kind of bath was used for double fillings (bilateral fillings).

1-1

(A)



(B)



middle chamber.

(vii) Preparing the ganglion for back-filling :

The animal was laid in a waxed bottom petri dish in the natural position (the dorsal side was uppermost) and fixed by small pieces of plasticine put around its legs; the wings and tergum were removed (in <sup>the</sup> case of Gryllus), then a longitudinal cut was made along the dorsal midline of the body starting from the posterior end of the abdomen right through to the neck, the two flaps of the body were pinned down. The viscera, fat bodies and connective tissues were removed, the arms of the mesofurca and metafurca were cut to free the nerves that lie beneath them and the muscles that cover the thoracic ganglia were also cut and removed until the ganglia and their nerves were quite visible. This dissection was carried out carefully under a dissecting binocular microscope. The preparation was moistened with Fielden's (1960) insect saline in <sup>the</sup> case of Gryllus and with Wood's (1957) <sup>Saline</sup> in <sup>the</sup> case of Carausius from time to time during the dissecting process to prevent dryness.

Motor nerves were followed to the muscle they innervated and cut close to it; other nerves issuing from or running to the ganglion were cut close to it; then the branches of the nerve of interest were cut at about 0.5-1.0 cm length and the nerve was then dealt with as mentioned later.

The anterior connectives were cut between the mesothoracic and the metathoracic ganglia when neurones in the metathoracic ganglion only were to be filled; but when filling through N<sub>1</sub> was carried out the prothoracic/mesothoracic connectives were cut and in some cases the three thoracic ganglia were left

intact; the posterior connectives were cut a little further behind the first abdominal ganglion. The tracheæ were cut as distal as possible from the ganglion; and the ganglion (or ganglia) was finally lifted and transferred to the filling bath.

When a filling of a single nerve was to be made, the preparation was transferred to a double chamber bath and the ganglion was put in one of the two chambers, containing saline solution, close to the narrow channel. A little "Vaseline" (petroleum jelly) was usually placed beneath the ganglion to help to stabilize it against the motion of the saline solution; the nerve to be filled was led through the narrow channel to the second chamber which also contains saline solution as well to keep the nerve moistened; the narrow channel was then sealed carefully with "Vaseline", thus leaving the ganglion in one chamber and its nerve in the other. If the nerve had more than one branch emerging from its trunk, as is the situation in  $N_1D$  (Fig. 1.9), all the ends of the branches, except that to be investigated, were carefully sealed with small amounts of "Vaseline" using fine forceps under a dissecting microscope. When the nerve was ready for filling, the saline in the chamber was sucked off and replaced immediately with the cobalt solution, and the whole bath was put into a small plastic box with a lid containing a moistened filter paper to prevent evaporation of the saline and cobalt solution. The cobalt solution was allowed to diffuse through the nerve to fill neurones with axons in that nerve. The diffusion time was 10-24 hours and was kept at room temperature ( $18-22^{\circ}C$ ) following Tyrer and

Altman (1974) and Goodman (1974). At first preparations were kept in a cold room at 4°C, following Iles (1976), but it was found that this merely causes the nerve to take a longer time to fill and permits less diffusion, and this procedure was therefore abandoned. Also the effect of passing small amounts of current was tried according to the method used by Iles and Mulloney (1971), originally for procion yellow, but reversing the direction of current by making the  $\text{CoCl}_2$  pool positive. This latter procedure was found unnecessary as it was by Tyrer and Altman (1974) and Iles (1976).

When a filling of a pair of nerves (double filling) was to be made, the nerves of interest were followed to their muscles on either side of the ganglion and cut close to them. The unwanted nerves and the anterior and posterior connectives were cut as previously described for the single filling. The ganglion was lifted and placed in the narrow middle chamber of a triple chamber bath (Fig.1.1B); each nerve of the two nerves of interest on either side of the ganglion was treated in the way described for the single filling; and each of them was led through the corresponding narrow channel to the ipsilateral chamber that contains  $\text{CoCl}_2$  solution, and the process was completed as described above.

(viii) Developing and processing :

After the period of diffusion was over, the precipitation of the cobalt that had diffused into the ganglion was undertaken by immersing the filled ganglion in a freshly prepared 2% solution of ammonium sulphide in the suitable insect saline (Fielden's 1960; Wood's 1957) for 5-10 minutes; then

the ganglion was briefly washed in insect saline and fixed following Pitman et al. (1973b) or in Carnoy's fixative, or in alcoholic Bouin's <sup>fixative</sup>. Most of the work was carried out using alcoholic Bouin's <sup>fixative</sup> for it was found that this gives better tissue preservation. This is also in accordance with previous work. Tyrer and Altman (1974) found that Carnoy's fixative causes some distortion, and Bacon and Altman (1977) found that an aldehyde fixative may cause problems during the intensification process, for aldehydes reduce silver nitrate, causing too much precipitation outside the ganglion.

Specimens were then dehydrated in a series of ethanol and cleared in methyl benzoate, and examined as a whole mount in a cavity slide in methyl benzoate. After being photographed or drawn, the ganglion was intensified following the method of Bacon and Altman (1977) and examined again.

(ix) Intensification of the Cobalt dye :

The ganglion (or ganglia) was brought through a descending alcohol series to distilled water.

Intensification was carried out in the dark in an oven maintained at 50°C. All solutions, except silver nitrate, and all glassware were brought to oven temperature to ensure a constant temperature and rate of reaction. The ganglion was transferred to warm distilled water in the oven for 5 minutes before being pre-soaked for one hour in warm developer base solution (3 grams of gum acacia, 0.8 gram of citric acid, 0.17 gram of hydroquinone and 10 grams of sucrose in 100 ml. distilled water). The constituents were added to the water at 50°C and electrically stirred until all were dissolved.

The ganglion was then transferred to freshly made up

developer solution containing 10 parts developer base to 1 part 1% silver nitrate, for twenty to thirty minutes, washed well in warm water, and left to cool. It was then dehydrated and cleared, examined as a whole mount, and photographed or drawn. After being washed well in absolute alcohol, it was embedded in paraffin and sectioned at 10  $\mu$ m, the sections being mounted in DPX.

In some cases the ganglion was over-intensified over prolonged periods to reveal the fine processes. It was then found that the nerves and the ganglion were too dark. In such cases, the preparation was de-stained to the required degree by treating it under observation with a binocular microscope using Farmer's reducer:

A. potassium ferricyanide 7.5 gm; water 1000 c.c.

B. sodium thiosulphate 200 gm; water 1000 c.c.

(equal quantities of A and B mixed just before use).

(x) Staining with Procion Yellow :

At the beginning of this histological work, I used Procion Yellow dye according to the method of Iles and Mulloney (1971) and Mulloney (1973), in which the dye was allowed to diffuse through the cut ends of the nerves, but unsatisfactory results were obtained, probably because the neuronal pathways in insects are too narrow for the dye to diffuse properly. So thereafter the dye was used for intracellular staining by an iontophoretic method (Stretton and Kravitz 1968).

A stock of the powdered dye was thoroughly washed in acetone to remove contaminants soluble in acetone (the dye itself being insoluble in acetone), then it was filtered through a Buchner funnel and dried.

The required weight was weighed out and dissolved in distilled water to make a 4% (w/v) solution of Procion yellow.

(xi) Electrophoretic intracellular dye injection :

Attempts have been made to stain neurones individually through electrophoretic intracellular staining technique with Procion Yellow, M4RS, (Stretton and Kravitz, 1968; 1973) and Cobaltous chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , (Pitman et al. 1972; 1973).

Glass microelectrodes were pulled from 2 mm diameter glass tubing with capillary insert, on a horizontal puller (Palmer). The microelectrodes had a DC resistance of 40-60 M $\Omega$  when filled with the dye solution (1-2%  $\text{CoCl}_2$  or 4% Procion yellow, both w/v in distilled water). The glass microelectrodes were filled with the dye solution through a hypodermic needle after passing the dye through a 0.22  $\mu\text{m}$  pore filter. A stock of dye-filled microelectrodes was prepared, and stored in a suitable covered plastic container containing moistened filter paper to prevent evaporation of the dye solution and kept in a refrigerator. The cell body of interest was detected intracellularly, then the dye was electrophoretically injected by passing pulses of negatively charged (in <sup>the</sup> case of Procion yellow) constant current (15 nA in 500 m sec. pulses repeated every second) delivered from a stimulator for 30-60 minutes (description of the electrical system used will be given in chapter two). On some occasions a continuous current of the same strength was used with Procion yellow in particular. When cobalt chloride was injected, the same pulses of current were used but with reversed charge. After the dye was injected, it was allowed

to diffuse at room temperature for about 8 hours; on some occasions it was allowed to stay in a cold room (4°C) for 24 hours. Fixation and post treatment of ganglia injected with  $\text{CoCl}_2$  was carried out as previously described for back-filling through cut ends of nerve trunks. Ganglia injected with Procion yellow were fixed in Glutaraldehyde/formaldehyde  $\text{pH}_{4.0}$  (stretton and Kravitz, 1973), dehydrated in alcohol series, cleared in methyl benzoate and viewed under a Zeiss microscope using colour filter. After being examined as a whole mount and photographed or drawn, the ganglion (or ganglia) was washed thoroughly in absolute alcohol, embedded in paraffin, sectioned at 10  $\mu\text{m}$ , mounted in fluoromount and examined as serial sections.

B. METHOD OF PRODUCING A GEOGRAPHICAL MAP  
OF THE CELL BODY DISTRIBUTION WITHIN  
THE METATHORACIC GANGLION :

This was prepared by reconstruction of serial sections of the ganglia stained by one of the two staining methods for staining serial sections mentioned above; though the silver nitrate stained sections were found more reliable and were more extensively used in the reconstruction.

(i) Drawing of the serial sections :

Serial sections of the ganglion were drawn using, principally, the methods of reconstruction of Pusey (1939) and Pantin (1946). In this method, the stained sections were drawn, each on a separate paper sheet using a camera lucida or by projecting them from a Zeiss microprojector onto a white sheet of paper which was attached to a board at a suitable fixed distance from the microprojector. The position of the first paper sheet on the board was marked by

means of a pencil at its four corners and each successive sheet of paper was fastened in the same position. The outline of each section was drawn first, then the outline of each cell body. Each drawing was given a code number to denote (a) its position in the series, (b) the position of the section on the slide, (c) the position of the slide among all the slides.

(ii) Reconstruction of the map from the drawings :

After all the serial sections of a ganglion were drawn in the way mentioned above, the reconstruction of a geographical map of the distribution of cell bodies within the ganglion was undertaken as follows:

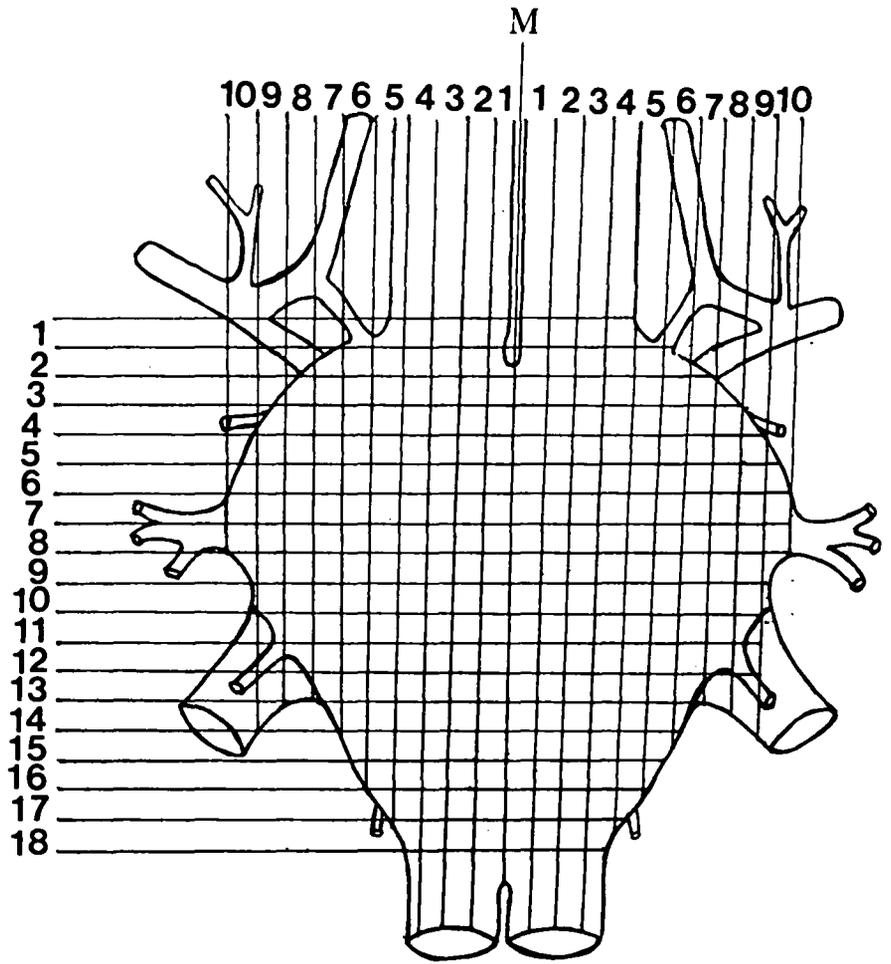
*idealized* A grid was ruled on a previously prepared outline of an ganglion (Fig.1.2A); the transverse divisions of the grid corresponded in number with the number of serial sections of the ganglion, so every transverse division represents one section. The longitudinal divisions represent another grid drawn on the drawing of the section (Fig.1.2B). Using these grids, the outlines of the cell bodies for each serial section were transferred to the ganglionic outline. In this way, a map was produced showing the distribution of the cell bodies throughout the ganglion. Dorsal and ventral maps were produced in each case. Although this method does not give a three-dimensional picture of the distribution of the cell bodies, it gives a reliable idea of their peripheral distribution.

Figure 1.2

A drawing showing the reconstruction process. (A) a grid was ruled on an outline of the ganglion. The transverse divisions correspond with the actual number of sections of the ganglion to be reconstructed. The longitudinal divisions on the outline of the ganglion correspond with a similar number of divisions on the drawing of each section.

(B) A drawing of a transverse section with the longitudinal divisions. M, the mid-line between the two lateral halves. H, a horizontal line <sup>mid-way</sup> between the dorsal and ventral halves of the ganglion.

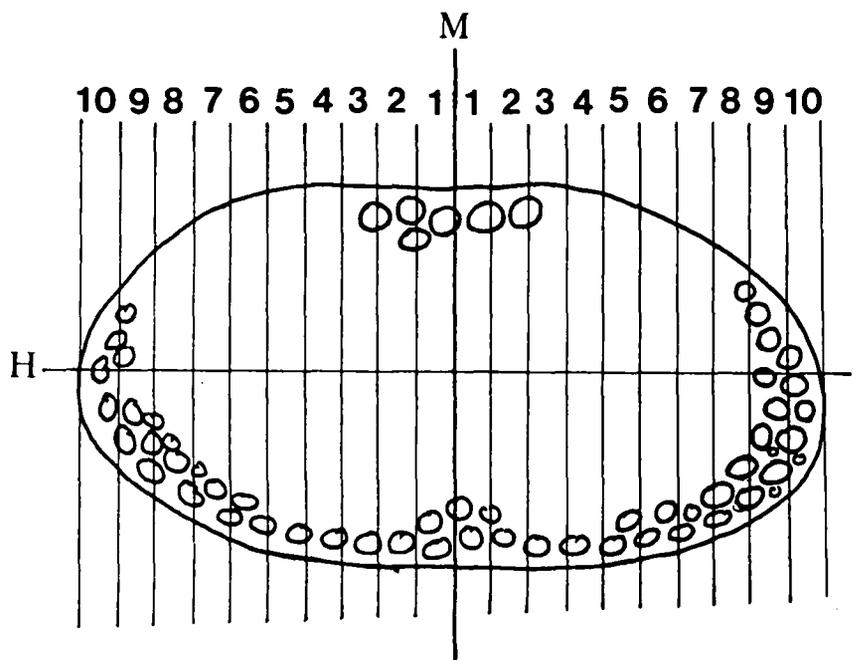
1.2



(A)

$\overline{100\mu\text{m}}$

(B)



$\overline{100\mu\text{m}}$

(iii) Reconstruction of individual neurones :

Serial sections of ganglia contained individual stained neurones or a group of neurones stained through a particular nerve trunk with back-filling technique were drawn separately using a camera lucida; then reconstruction of them was undertaken. Whenever possible, the drawings were superimposed on each other on one paper sheet.

Problems and difficulties :

Detailed pictures of individual neurones were obtained from preparations in which the neurones were filled with cobalt chloride solution that finally precipitated as cobalt sulphide; or filled intracellularly with the dye Procion yellow through a microelectrode; or through the cut end of the nerve trunk through which the neurone of interest sends its axon. However, injection of cobalt chloride solution into the cell body electrophoretically was found difficult and success using this method was limited. This difficulty could have originated from the tendency of cobalt chloride solution to coagulate with some of the contents of the cytoplasm with the result that the microelectrode became blocked. The solution itself was always filtered before filling the microelectrode to make sure that it was free from any solid contaminants. Varying concentrations of solution were first tried and both types of current, continuous and pulsed, with different strength and durations, but the problem remained that the microelectrode became blocked after a few minutes from the start of applying the current. This is why cobalt staining was carried out largely by back-filling technique. But parallel with back-filling, I continued to try electro-

phoretic injection of cobalt. Eventually, it was decided to investigate whether the saline vehicle used in the microelectrode itself might be causing precipitation in the tip of the electrode, and by varying the composition of the saline, it was found that by elimination of the phosphate, carbonate, and bicarbonate from the saline solution, acceptable electrophoretic injection of cobalt could be obtained. It was concluded that the difficulty encountered had been due to precipitation of cobalt as a complex (or complexes) of the above-mentioned chemical groups in the tip of the microelectrode under the influence of the electric current.

Similar problems were encountered with the Procion yellow on some occasions, but this was overcome by washing the dye powder with acetone prior to preparing the dye solution (see earlier in this chapter). However, although the injection of Procion yellow electrophoretically was found much easier than that of cobalt chloride, its diffusion was confined to the main large branches of the neurone, and it was impossible to resolve the fine branches by its use, a disadvantage that cobalt chloride solution does not possess. However, Procion yellow was used as a cell marker on some occasions.

Another major problem encountered was the resistance of the neural sheath surrounding the ganglion, especially in the stick insect. It was necessary to penetrate the neural sheath first in order to impale the neuronal cell bodies beneath, but the electrode invariably broke or bent rather than pass through the sheath. Attempts were made to overcome

the problem as follows:

(1) By using softening enzymes such as proteases (sigma type III) and collagenase (Sigma type II), to soften the neural sheath. Although this method worked on a few occasions, it was found that such substances not only caused softening of the neural sheath, but also produced a rapid decline in the physiological state of the neurones beneath. With use of such reagents, resting and action potentials declined rapidly. Different durations of application and different concentrations were used in an attempt to avoid this neuronal decline, but without success. Table 1.1 shows the different concentrations and durations tried.

Apart from this, although the use of the enzyme did result in the softening of the sheath, the latter became sticky and rubbery and it was found to block the micro-electrodes.

(2) An alternative approach was to use a platform-like device similar to that described by Hoyle and Burrows (1973). This device was used to avoid the use of enzyme to ease impaling the neural sheath. It provided a solid support from underneath the ganglion, and by lifting the ganglion gently, by the micromanipulator to which the device was attached, it caused the sheath to be stretched so it became somewhat easier to be penetrated. There was no evidence that this stretching caused damage to the neurones. This method required the use of microelectrodes with fine, short tips, produced by adjusting the puller heat.

TABLE 1.1

Concentration % (weight in gm/v)	0.5	1	2
Time after which the effect on sheath starts (min)	8 - 20	6 - 15	3 - 8

The effect of protease and collagenase on the ganglionic sheath. On some occasions, the enzyme was sprinkled directly on the sheath and left for 5 - 10 minutes.

However, neither method of dealing with the neural sheath helped in impaling the neural sheath of the stick insect. This proved far too tough to impale without enzyme treatment, yet enzyme treatment produced a highly sticky sheath that consistently blocked the microelectrode. It was for this reason that a switch had to be made to the cricket and much of the histological work already done on the stick insect had to be repeated on the cricket.

III: RESULTS

A. GENERAL OBSERVATION ON THE METATHORACIC GANGLION OF THE  
STICK INSECT AND DISTRIBUTION OF CELL BODIES WITHIN IT.

The shape of the metathoracic ganglion of Carausius morosus appears somewhat spherical and it is not distinguished from the other thoracic ganglia by a unique shape as is the metathoracic ganglion of the cricket. Internally, the neuropilar mass occupies most of the ganglion and the cell bodies form an interrupted ring around it (Figure 1.3). However, the number of cell bodies, in general, appeared much less than in the cricket where the number of the cell bodies whose diameter was 20  $\mu\text{m}$  or larger was found <sup>to be</sup> 94; and estimation of cell bodies whose diameter was less than 20  $\mu\text{m}$  was found to be 1540. The size (diameter) of the cell bodies does not exceed 50  $\mu\text{m}$ .

The groupings of cell bodies in the dorsal region of the ganglion look similar to those of the cricket; but in the ventral region there are two extra small groups of cell bodies which can be recognized and were designated No. 4. Analogous groups No. 4 were not detected in the ventral region of the metathoracic ganglion of the cricket. Table 1.2 and Figures 1.4, 1.5 show the distribution of the cell bodies within ranges of sizes. It can be seen that the pattern of the whole ganglion is similar to that of the cricket with the exception of groups 2 and group 4. Also Figure 1.6 shows the reconstructed maps of cell body groupings on the dorsal and ventral regions of the ganglion. Back-filling through the peripheral nerves of the ganglion revealed a rather small number of cell bodies, 31, on the ventral region which were filled mostly through the nerve that innervates

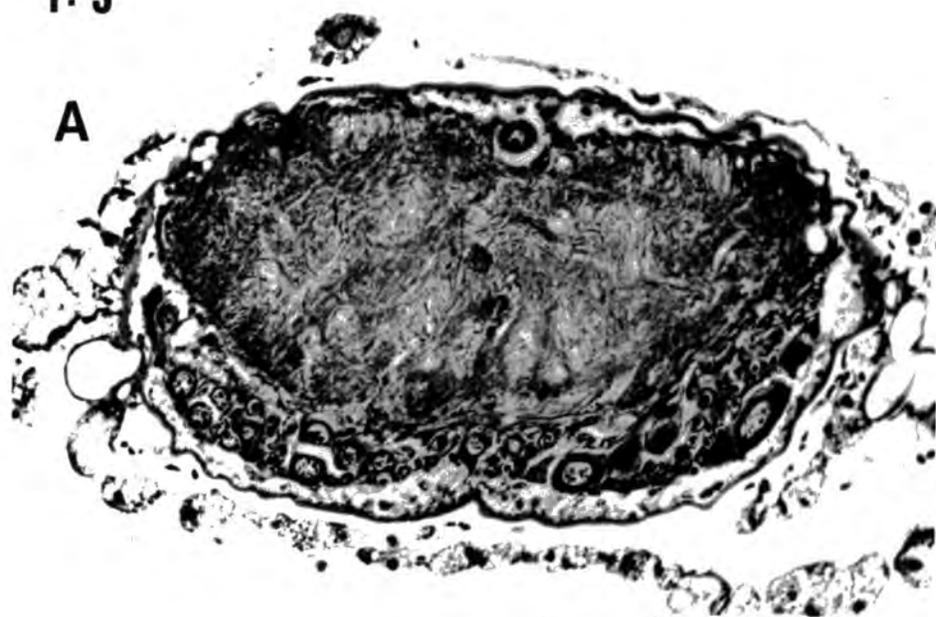
Figure 1.3

Three cross sections of the metathoracic ganglion of the stick insect. (A) and (B) are silver stained. (C) is Haematoxylin-stained. In all three sections the neuropile is occupying most of the ganglion and the cell bodies rind is interrupted and shallow. Group 4 is shown in (C) (arrows), Group No. 2 (dorsal) is also shown in (C): (arrow).

Calibration : Bar represents 100  $\mu$ m.

1.3

A



B



C

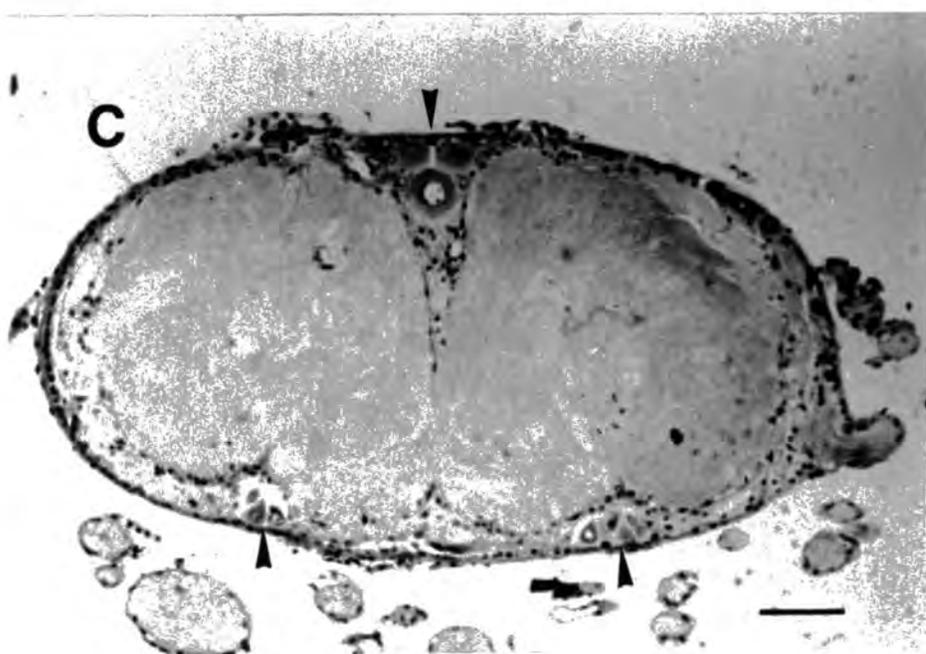


TABLE 1.2

Cell body diameter μm	Group 1v		Group 1d		Group 2v		Group 2d		Group 3v		Group 3d		Group 4v	
	m	S.D.												
20 - 30	19	3.2	10	1.4	-	-	-	-	12	1.7	8	1.7	3	0
31 - 40	8	1.4	4	0.8	1	0	2	0	6	0.8	4	0.8	4	0.8
41 - 50	2	0	-	-	3	0.6	6	0.6	2	0	-	-	-	-

Distribution of cell bodies over 20 μm diameter in the different groups of the metathoracic ganglion of the stick insect. (Figures from 5 ganglia).

d = dorsal

v = ventral

m = mean

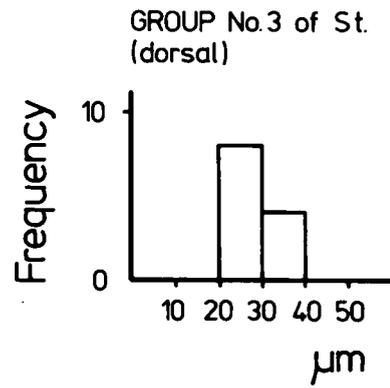
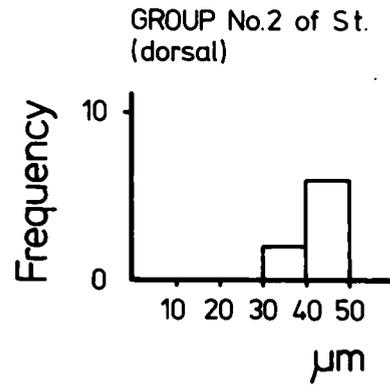
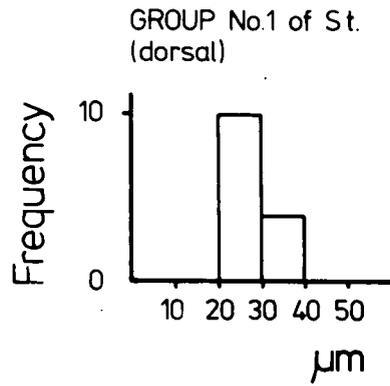
S.D. = standard deviation

Figure 1.4

Histograms showing the distribution of cell bodies of the metathoracic ganglion of the stick insect within ranges of cell sizes. It can be seen that the pattern of distribution is similar in groups one and three of both dorsal and ventral regions, i.e. the number of cell bodies decreases as the size increases. In groups 2 and 4 which contain much less cell bodies, the number of the large cell bodies is greater than that of the smaller ones.

St. = stick insect.

1.4



GROUP No.4 of St.

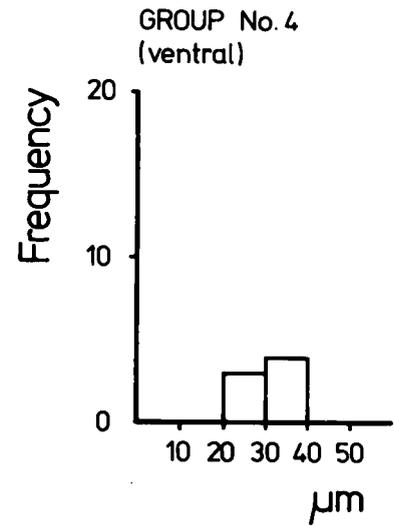
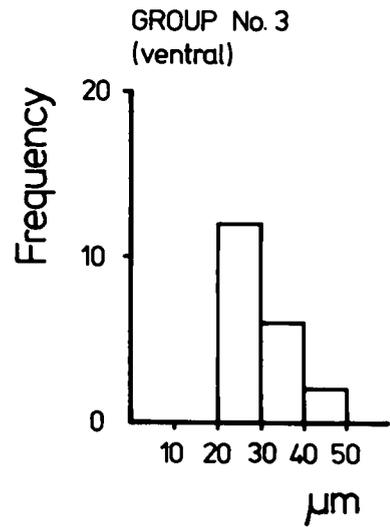
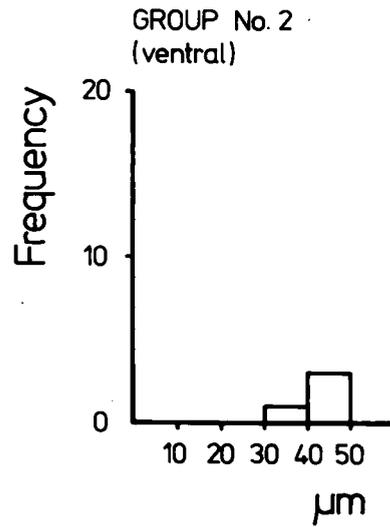
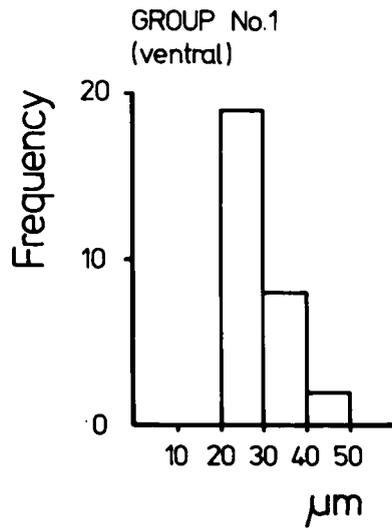


Figure 1.5

Histograms of the distribution of cell bodies within the ganglion as a whole. It can be seen that the pattern of distribution within ranges of sizes is similar in both the dorsal and ventral regions of the ganglion; though the number of cell bodies on the dorsal region is greater than in the ventral region.

St. = stick insect.

The whole of the ganglion of S t.

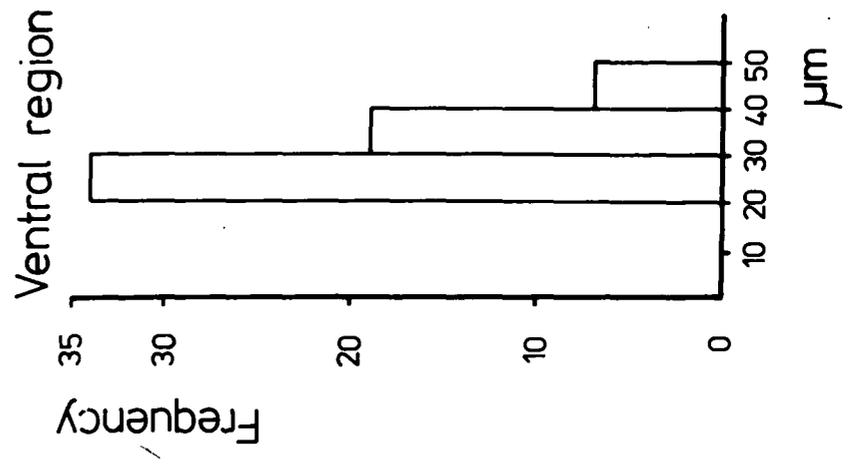
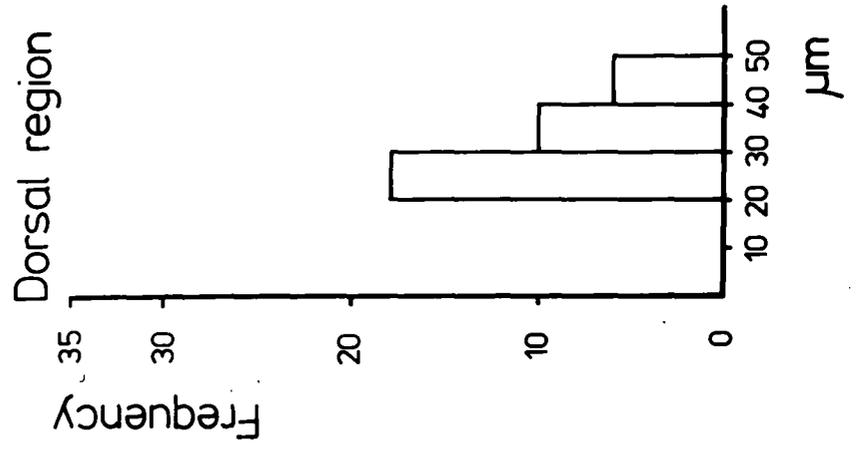


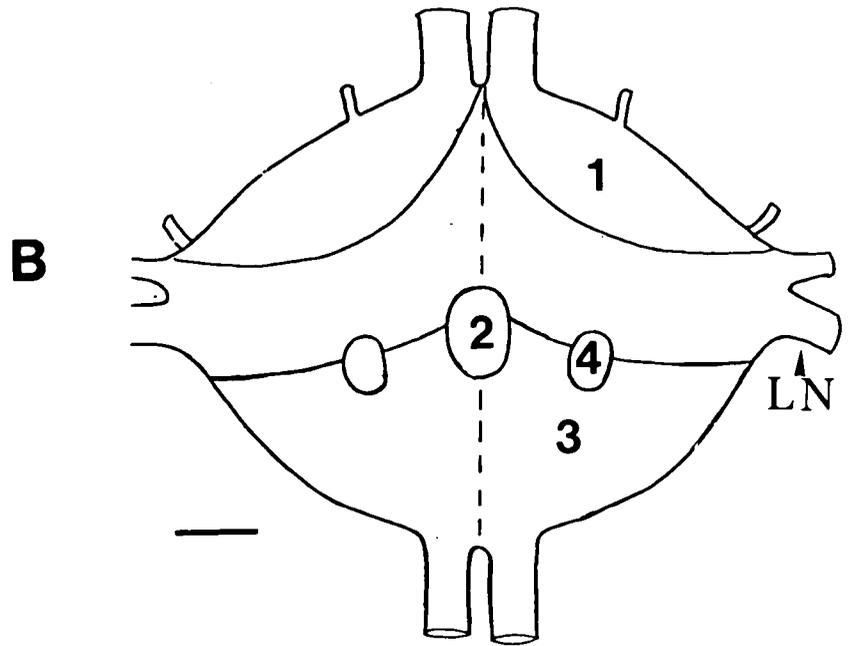
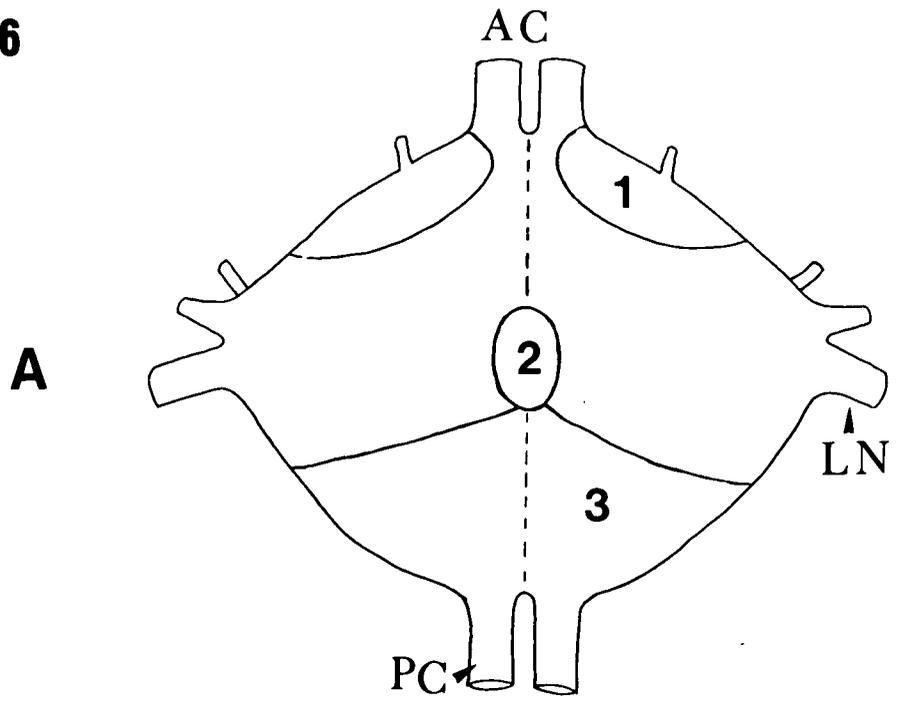
Figure 1.6

Reconstructed maps showing the geographical distributions of cell bodies within the metathoracic ganglion of the stick insect. (A) represents a dorsal view and (B) represents a ventral view. It can be seen that the cell bodies are found more in the ventral region than in the dorsal region. The groups were given numbers (see text) AC, anterior connectives. Pc, posterior connectives. LN, the hind leg nerve.

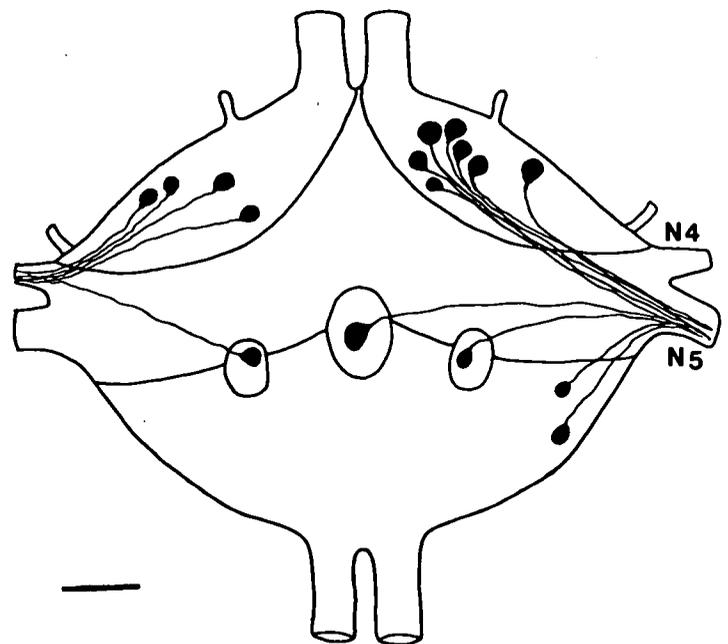
Figure 1.7

A drawing of efferent cell bodies that were back-filled through peripheral nerves of the metathoracic ganglion. All the stained cell bodies are ventral and mostly belong to N5 (the hind leg nerve) and N4. Most of the filled cell bodies belong to group lv (ventral). It can be seen that N5 contributes neurones to all the ventral groups. (ventral view). (Different groups of neurones shown on each side).

1.6



1.7



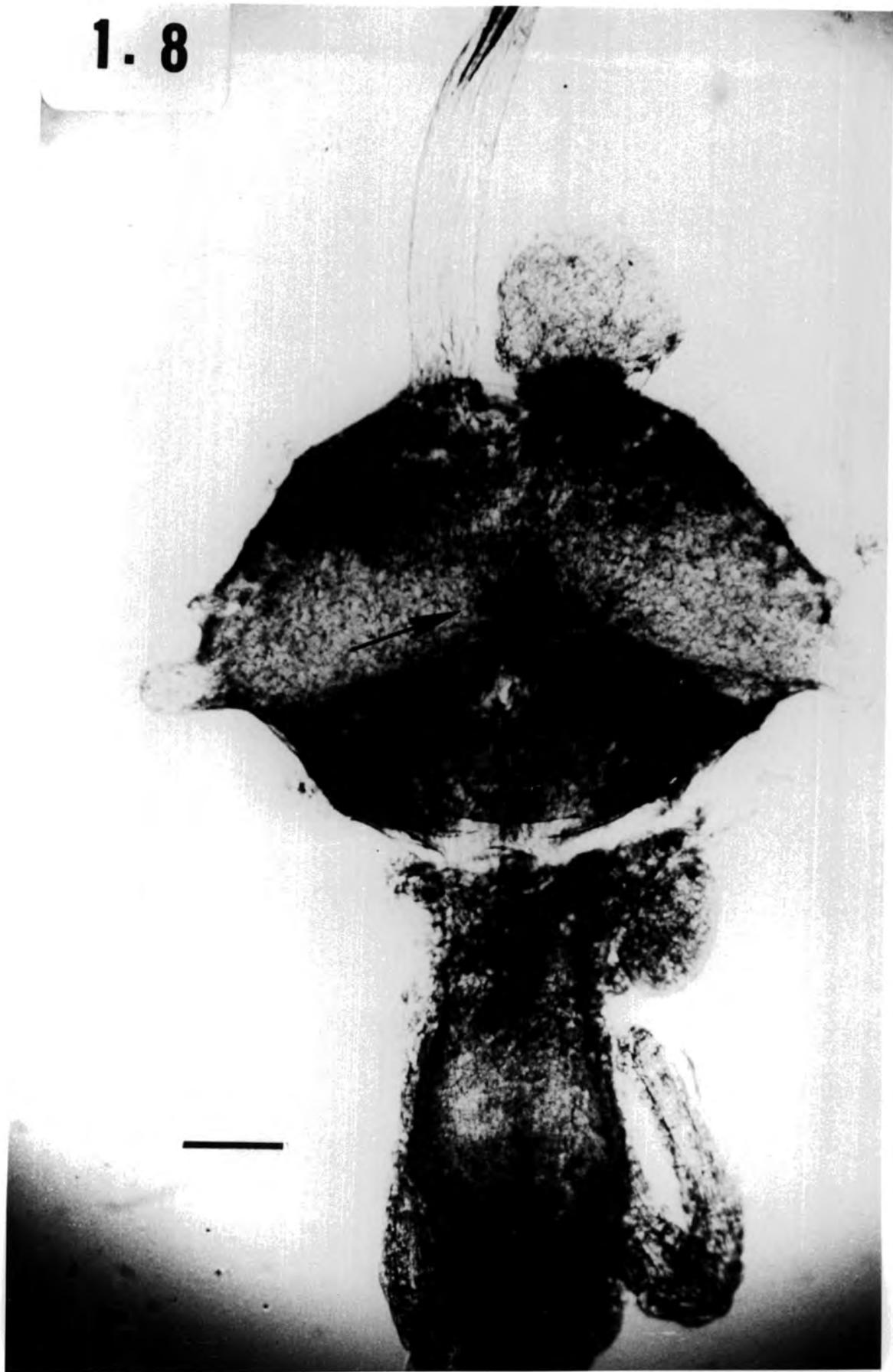
the hind leg (Figure 1.7). No cell bodies were filled on the dorsal region. Attempts have been made to back-fill through nerve 1 to see if any of the dorsal unpaired median (DUM) neurones, group 2, could be stained but none was found to be filled of this group, whose number of cell bodies is eight only (Figure 1.8).

Figure 1.8

A photograph of the metathoracic ganglion (dorsal view) of the stick insect stained with toluidene blue. The most obvious group is group 2d (2 dorsal, arrow) (DUM Cells).

Calibration : 200 $\mu$ m.

1.8



B. GENERAL OBSERVATIONS ON THE METATHORACIC GANGLION OF THE CRICKET.

The metathoracic ganglion of the cricket, Gryllus bimaculatus, is heart-shaped (Figure 1.9), resembling the corresponding ganglion of the locust; this peculiar shape, which results from the fusion between the third thoracic ganglion and some of the abdominal ganglia during the early stages of development (Johannsen and Butt, 1941), gives it a distinctive appearance compared with the other thoracic ganglia.

The ganglion is connected anteriorly to the mesothoracic ganglion via the anterior connectives (Figure 1.9) and posteriorly to the first abdominal ganglion, via the posterior connectives which are thinner than the anterior ones. As in the whole CNS and peripheral nerves of the insect, the metathoracic ganglion is well protected by a neural sheath.

The supply of oxygen to the ganglion is achieved through small tracheae, which originate from a single trachea on each side that branches from the middle of the large tracheal trunk (Burrows, 1980).

(i) The peripheral nerves :

Five main pairs of nerve trunks emerge from the ganglion on both sides. Although the proximal part of the first nerve of the cricket is different from that of the locust, Campbell's nomenclature (1961) has been adapted to crickets by a variety of authors, e.g. Clark (1976a, b) and Davis and Alanis (1979) proximal to the ganglion; the distal part

Figure 1.9

A drawing showing a dorsal view of the metathoracic ganglion (Mt) and the mesothoracic ganglion (Ms) of Gryllus bimaculatus. Peripheral nerves are shown. The topography of N1 is shown to the dorsal longitudinal flight muscle (DLM).

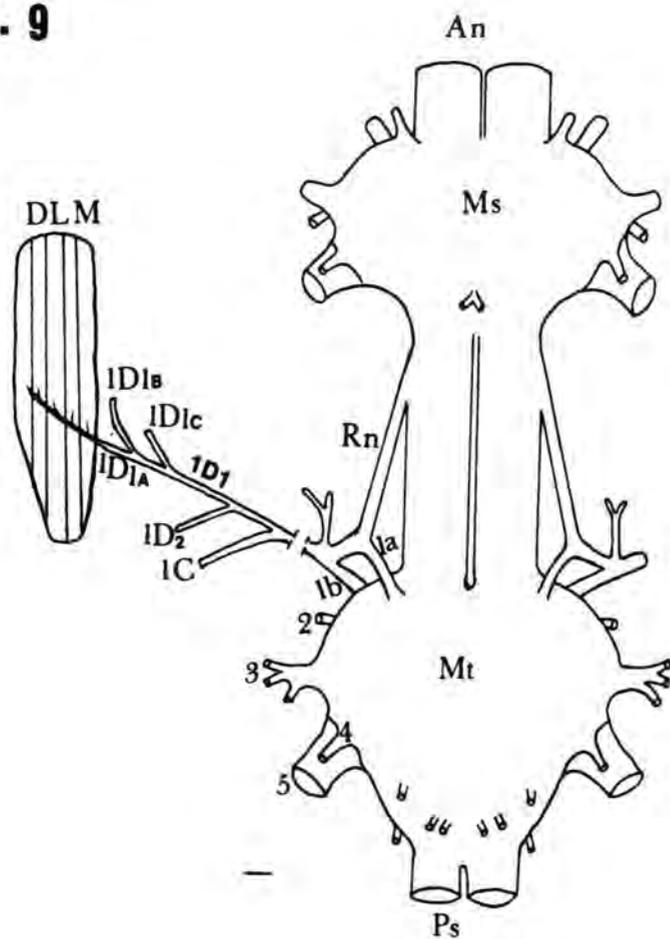
An, anterior; Rn, recurrent nerve; Ps, posterior. Bar represents 100  $\mu$ m. For other abbreviations, see text.

Figure 1.10

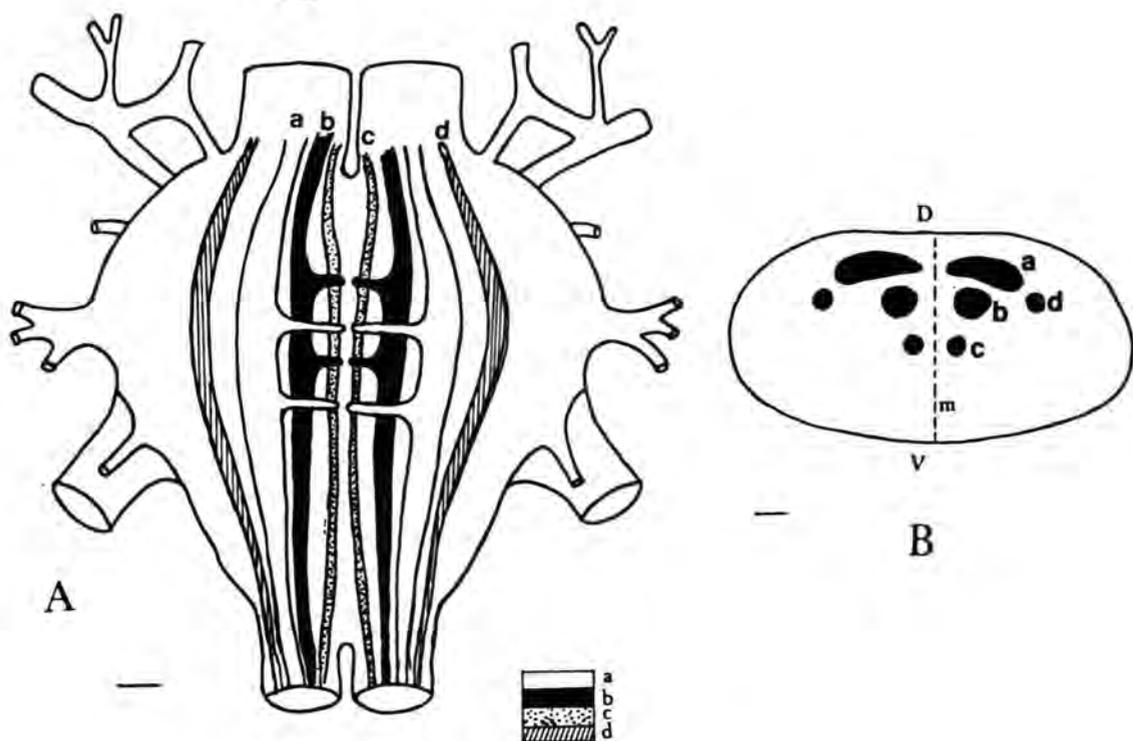
(A) A dorsal view of the metathoracic ganglion of Gryllus showing the position of some of the through-tracts.

(B) The positions of the same through-tracts are shown in a cross-section. Bar represents 100  $\mu$ m.

1.9



1.10



of  $N_1$  conforms to that of the locust. In Locusta, the topography of  $N_1$  is different in that it originates from the ganglion (as in many other insects) as one branch only.

(ii) The pattern of branching of  $N_1$ :

Preliminary investigation was carried out to follow the branches of  $N_1$  to the dorsal longitudinal flight muscles using methylene blue and 70% alcohol; it showed that the pattern of  $N_1$  branching of Gryllus bimaculatus, is similar to the pattern of  $N_1$  of Gryllus domesticus described by Davis and Alanis (1979).

Unlike some other orthopteran insects (such as the locusts), the first nerve ( $N_1$ ) of the metathoracic ganglion of Gryllus bimaculatus, originates as two separate branches on each side of the ganglion (Figure 1.9); they are  $N_{1a}$  which is smaller and more anterior, and  $N_{1b}$  which is more latero-ventral in joining the ganglion.  $N_{1a}$  is joined by the recurrent nerve ( $N_6$ ) of the mesothoracic ganglion to form a joint nerve, which in turn is joined by  $N_{1b}$  forming the main compound trunk, that is  $N_1$  itself (Figure 1.9). This compound nerve,  $N_1$ , later splits into two branches:  $N_{1C}$  and  $N_{1D}$ .  $N_{1C}$  runs to the sensory receptors on the anterior base of the metawing (Altman et al., 1978; Davis and Alanis 1979).  $N_{1D}$  divides into  $N_{1D_1}$  and  $N_{1D_2}$ .  $N_{1D_2}$  runs to the stretch receptors on the posterior base of the wing.  $N_{1D_1}$  divides into three branches:  $N_{1D_1A}$  innervates the dorsal longitudinal flight muscle;  $N_{1D_1B}$  innervates a small dorsal oblique flight muscle (almost an oblique segment of the dorsal longitudinal flight muscle; Guthrie 1964), extending from the lateral

post-notum to a posterior lateral attachment on the scutum; N<sub>1</sub>D<sub>1</sub>C innervates the integument of the posterior lateral surface of the scutum.

(iii) Salient structures within the ganglion :

Like other central ganglia of other insects, most of the metathoracic ganglion is occupied by a complex fibrous mass, the neuropile. It is in this area of the ganglion that contacts between the different neural elements take place (Smith, 1967; Huber, 1974). However, some of the fibres that pass through the ganglion or terminate in it collect together to form distinct bundles of fibres that could be recognized. These are called "through tracts" by Tyrer and Altman (1974) and are regarded as land marks within the ganglion; only the most obvious ones are mentioned here.

Four pairs of longitudinal through-tracts can be distinguished in both left and right halves of the ganglion, and they have been designated a, b, c, and d (Figures 1.10A,B). These tracts can be distinguished in haematoxylin and silver-stained preparations as well as cobalt chloride preparations. From the cobalt chloride preparations it can be seen that some fibres of these tracts give off some branches into the ipsilateral half of the ganglion, and on some occasions branches to the contralateral half were also given off (Figure 1.11).

Figures 1.10A,B show the arrangement of these tracts in which tract a lies dorsally in the ganglion; it extends from a tapering point at the mid-dorsal line of the ganglion to a further lateral point at which it becomes thicker and extends more lateral to tracts b and c. Tracts b lie in the

Figure 1.11

Photographs of whole mounts of the metathoracic ganglion of Gryllus. (A) Back-filling through the anterior connectives showing bundles of fibres (through tracts).

(B) Back-filling through one anterior connective showing branches given off in the ipsilateral half of the ganglion.

(C) On some occasions a few branches cross the middle of the ganglion to the contralateral half (arrows). (D) and (E) show some of the giant fibres that pass through the ganglion.

Calibration : (A) (B) (C) 100  $\mu$ m; (D) (E) 50  $\mu$ m.

1.11

A



B



C

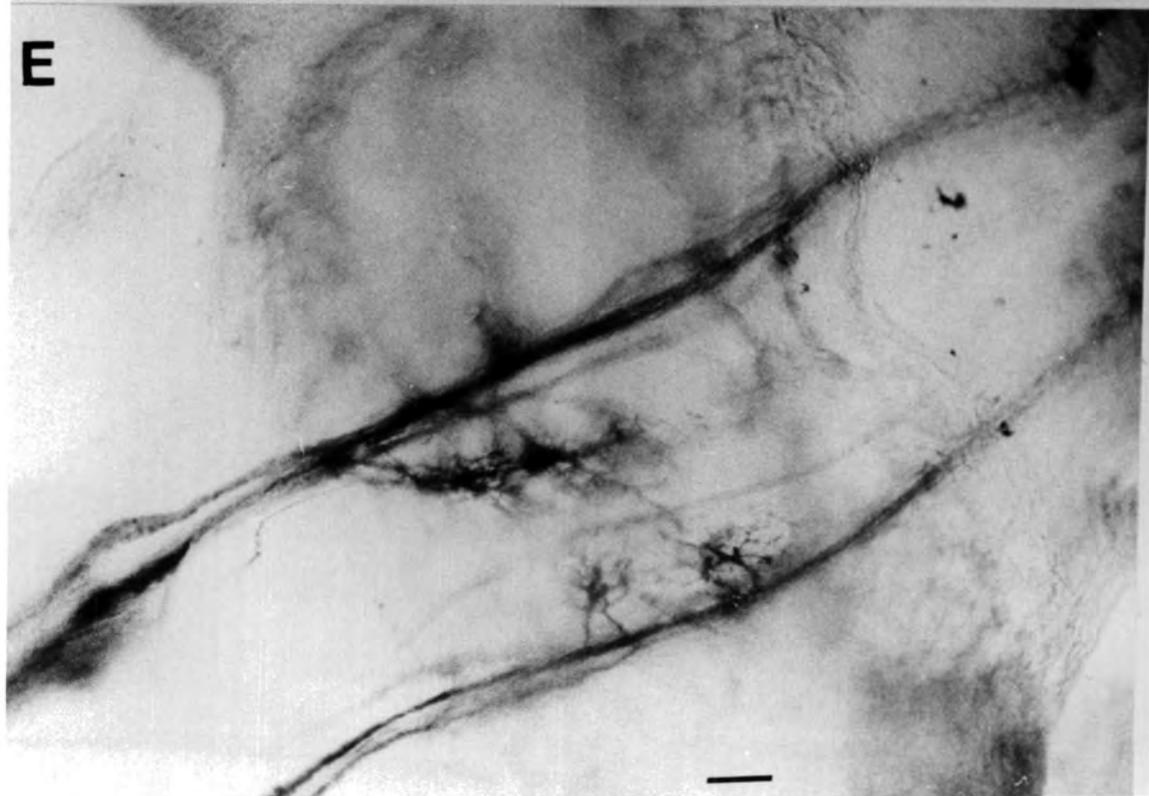


1.11

D



E



upper dorsal half of the ganglion close to the longitudinal mid-line of the ganglion and are less thick than tracts a. Tracts c lie almost centrally and they are closer to each other than tracts b. Tracts d lie lateral to tracts a, b, and c and almost level with tracts b.

Other small diameter tracts, longitudinal, transverse, (commissures), circular and oblique as well as the nerve roots (e.g. Figure 1.12) can be seen, but those mentioned above are the most obvious and presumably the most important in terms of the number of neurones they involve.

(iv) General distribution of cell bodies in the metathoracic ganglion of the cricket :

Reconstruction of serial sections of the metathoracic ganglion, stained with silver nitrate or iron haematoxylin, provided a general, fairly reliable picture of the cell body distribution within the ganglion as geographical groups. This picture was confirmed with whole-mount staining techniques with toluidine blue and cobalt chloride, (see Materials and Methods section).

It was found that the cell bodies were distributed peripherally in the cortex of the ganglion in a bilaterally symmetrical way with the exception of the dorsal and ventral median groups, group No. 2, immediately under the neural sheath and around the neuropile. They are more numerous in some areas and less in others, being more concentrated in the anterior and posterior parts of the ganglion whereas a few are found in the middle areas, which are almost entirely occupied by the neuropile (Figure 1.13). Also they are much more numerous in the ventral region

Figure 1.12

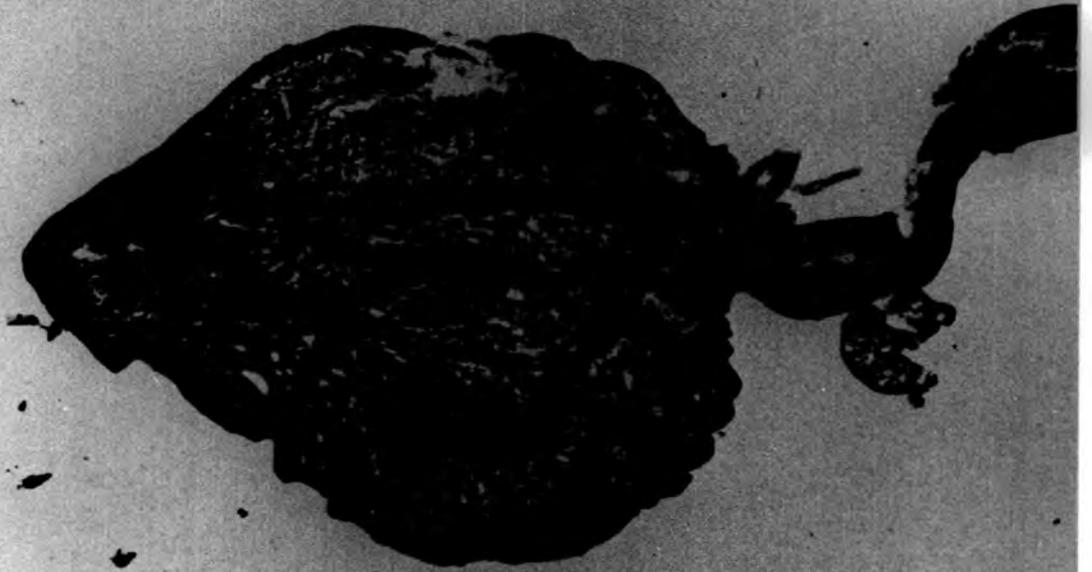
(A) A sagittal section pass through an anterior connective showing the longitudinal through-tracts, a,b,c. Groups No. 1 and 3 of the ventral region are shown.

(B) Oblique cross section showing group No. 1 dorsal and nerve root (N3) terminates in the neuropile. Two cell bodies of group No. 2 ventral are also shown.

Calibration : 100  $\mu$ m.

1-12

A



B

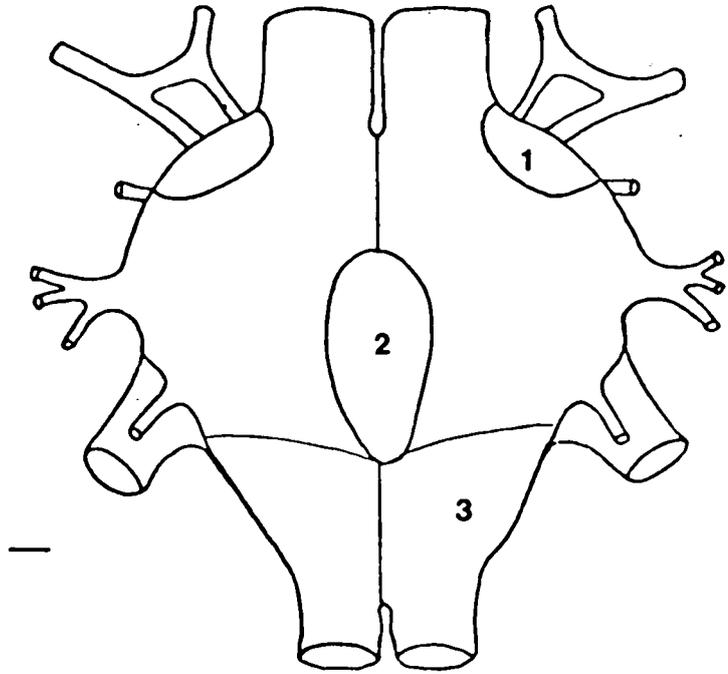


Figure 1.13

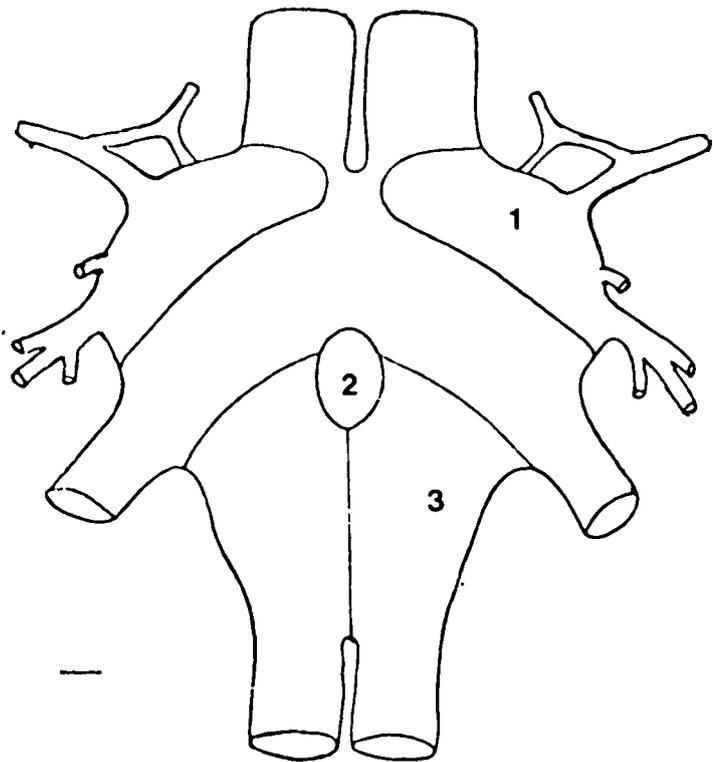
Reconstructed maps of the metathoracic ganglion of Gryllus showing the distribution of cell bodies as geographical groups designated by numbers in the ganglion. (A) Dorsal view, (B) ventral view. (C) a whole mount (ventral view) stained with cobalt chloride.

Calibration : 100  $\mu$ m.

**A**

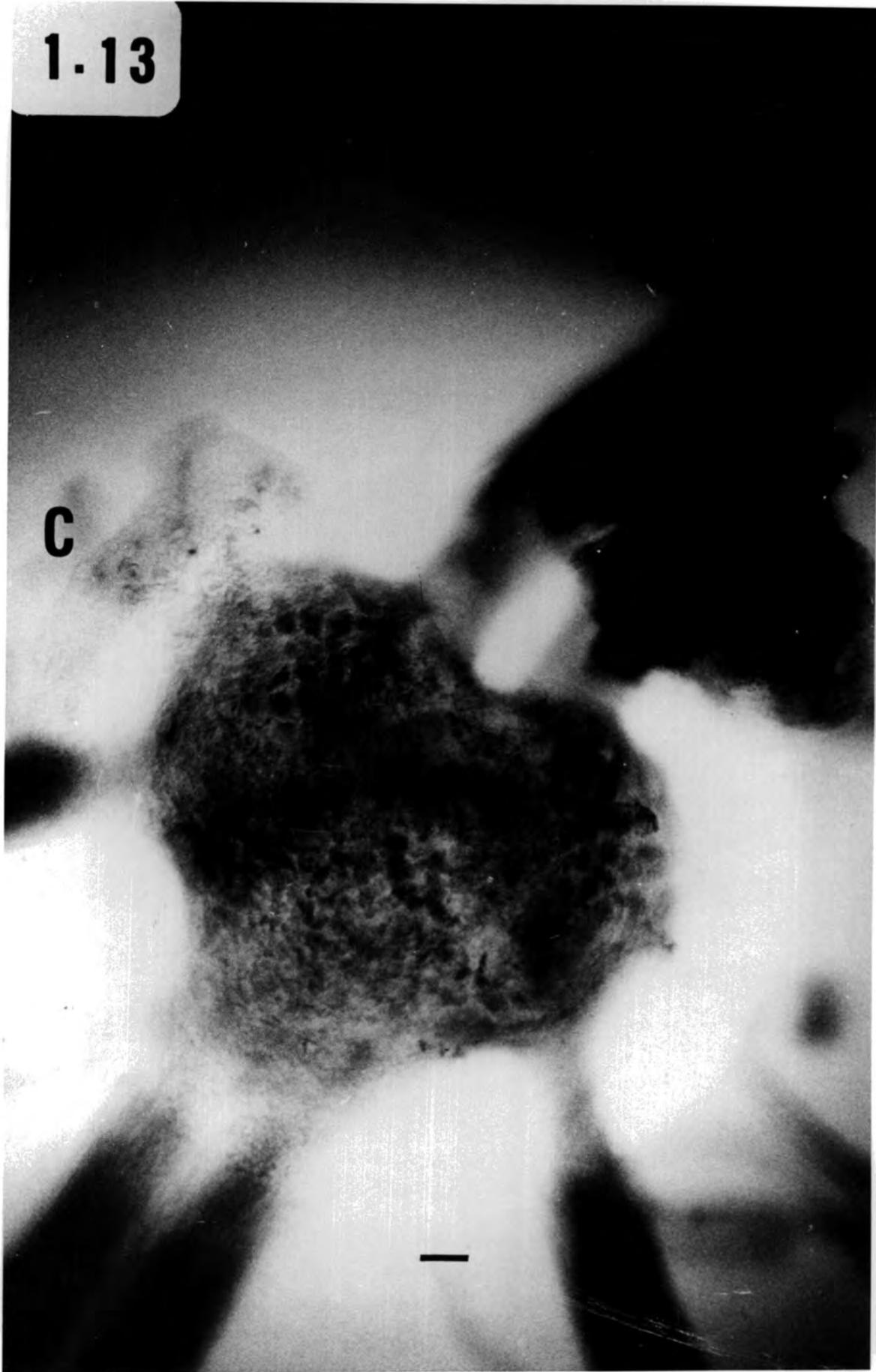


**B**



1.13

C



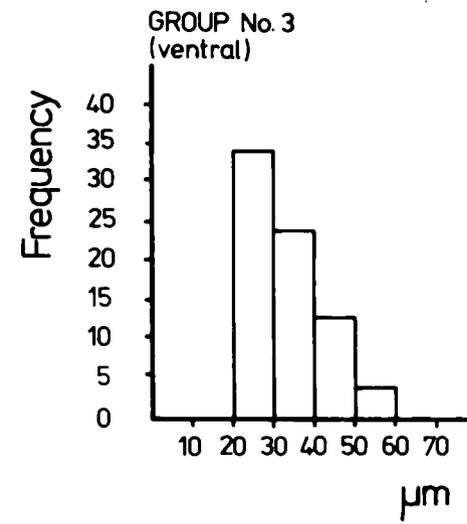
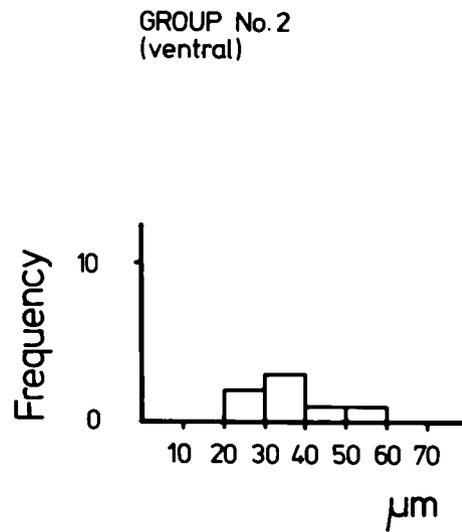
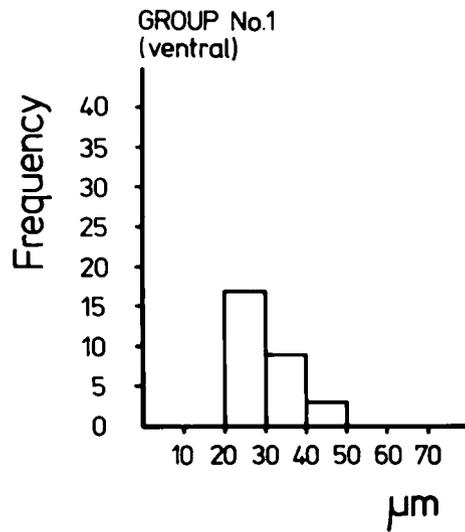
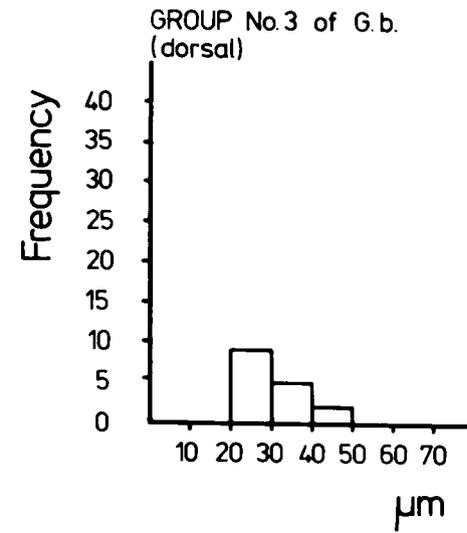
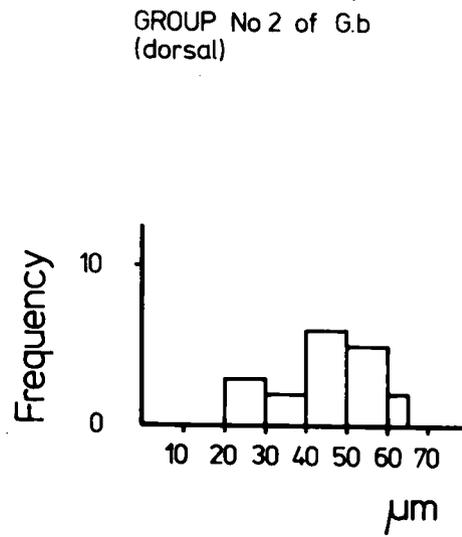
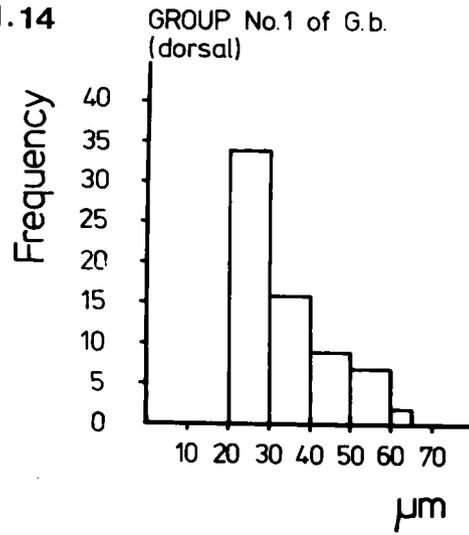
than the dorsal region (Figures 1.14, 1.15, table 1.3).

The classification of cell bodies by Pipa et al., (1959) can be applied to this ganglion. Some cells are large ( $\sim 60 \mu\text{m}$ ) and ovate and their ratio of cytoplasm/nucleus is high, approximately 1.5; others are globular, and the ratio of the cytoplasm/nucleus is low, approximately 1.15, and may even approach unity in some cases. There are some large ovate cells that contain obvious granules and these may possibly be neurosecretory; however, there is no specific type of cell confined to a particular region of the ganglion.

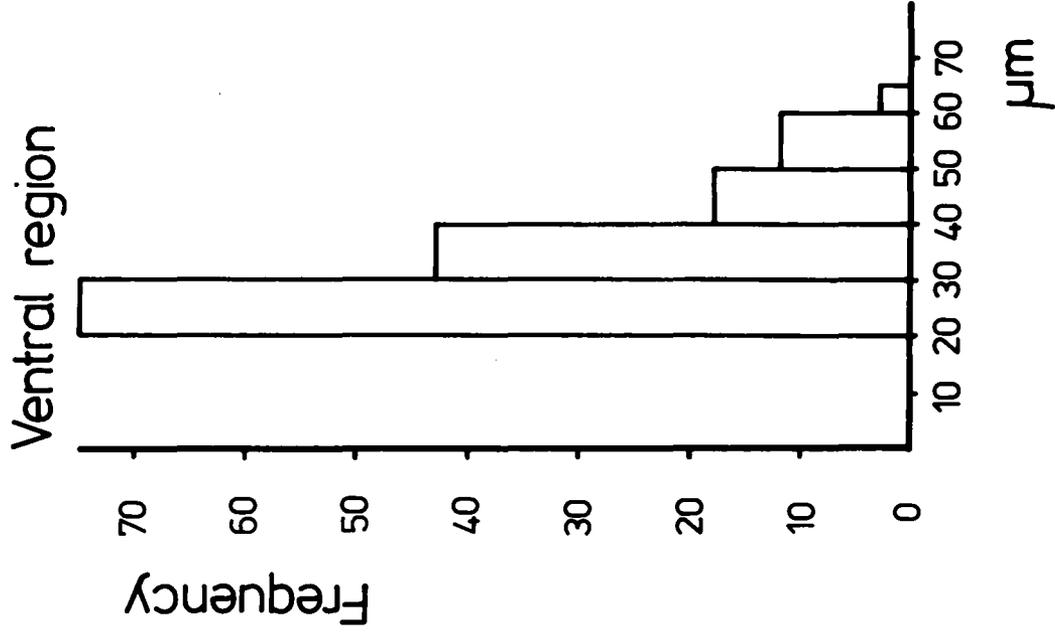
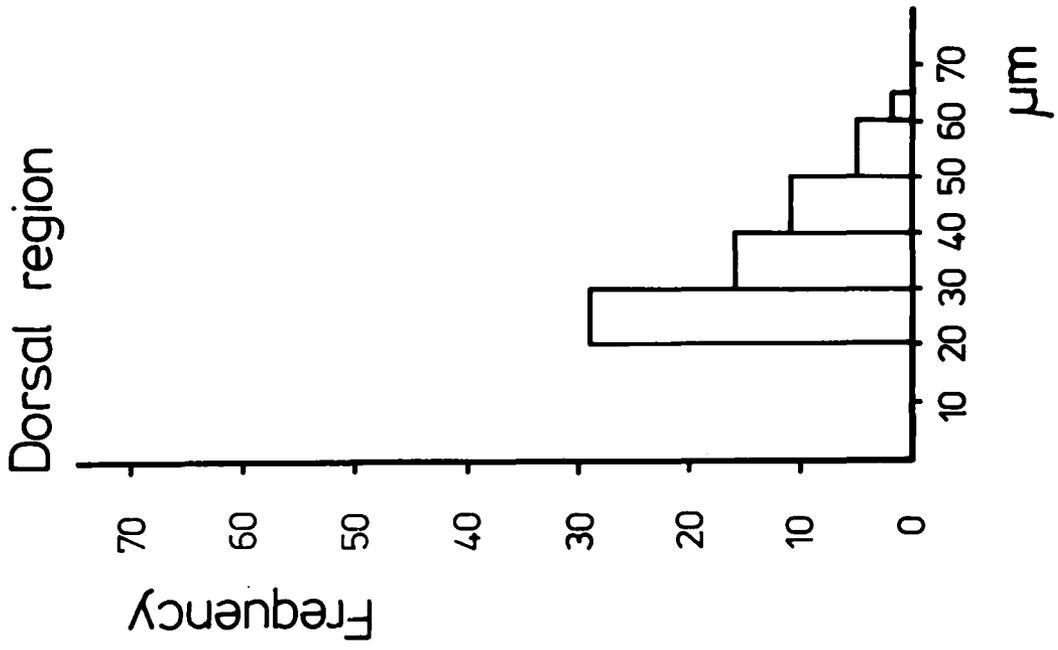
There is an obvious difference in number of cell bodies among these geographical groups, and the only groups whose cell bodies can be counted precisely are those of the middle regions on the dorsal and ventral sides of the ganglion because of their rather small number; whereas the anterior and posterior groups cannot be precisely counted because of their large numbers, especially those cell bodies whose diameters are less than  $20 \mu\text{m}$ ; (table 1.3; Figures 1.14, 1.15) of the whole ganglion. Estimation of cell bodies less than  $20 \mu\text{m}$  gives a figure around 2200. *however, a count was made of the cell bodies whose diameter was larger than  $20 \mu\text{m}$*

The groups themselves were delimited according to their regional position within the ganglion. Each group has been given a number for the sake of recognizing it. Apart from two groups which occupy the middle of the dorsal region and the ventral region of the ganglion respectively, the other groups are bisymmetrical.

1.14



1.15 The whole of the ganglion of G. b.



Groups No. 1 :

Figure 1.13 shows the location of the major cell body groups. Group No. 1 has a smaller number of cell bodies on the dorsal side than its equivalent on the ventral side (Figures 1.14, 1.15). Both the dorsal and ventral groups occupy the anterior corners of the two halves of the ganglion (Figure 1.13). On the dorsal side, the cell bodies are confined to a shallow region of the corner of the ganglion, which extends from the lateral side of the ipsilateral anterior connective to the level of  $N_2$ ; whereas the ventral group spreads over a much larger distance including the region beneath the anterior connectives back to the level of  $N_3$ . Both dorsal and ventral groups contain cell bodies of different sizes ranging from 65  $\mu\text{m}$  to less than 20  $\mu\text{m}$ . The large cell bodies are more peripheral and lateral in position and they are either ovoidal or spherical.

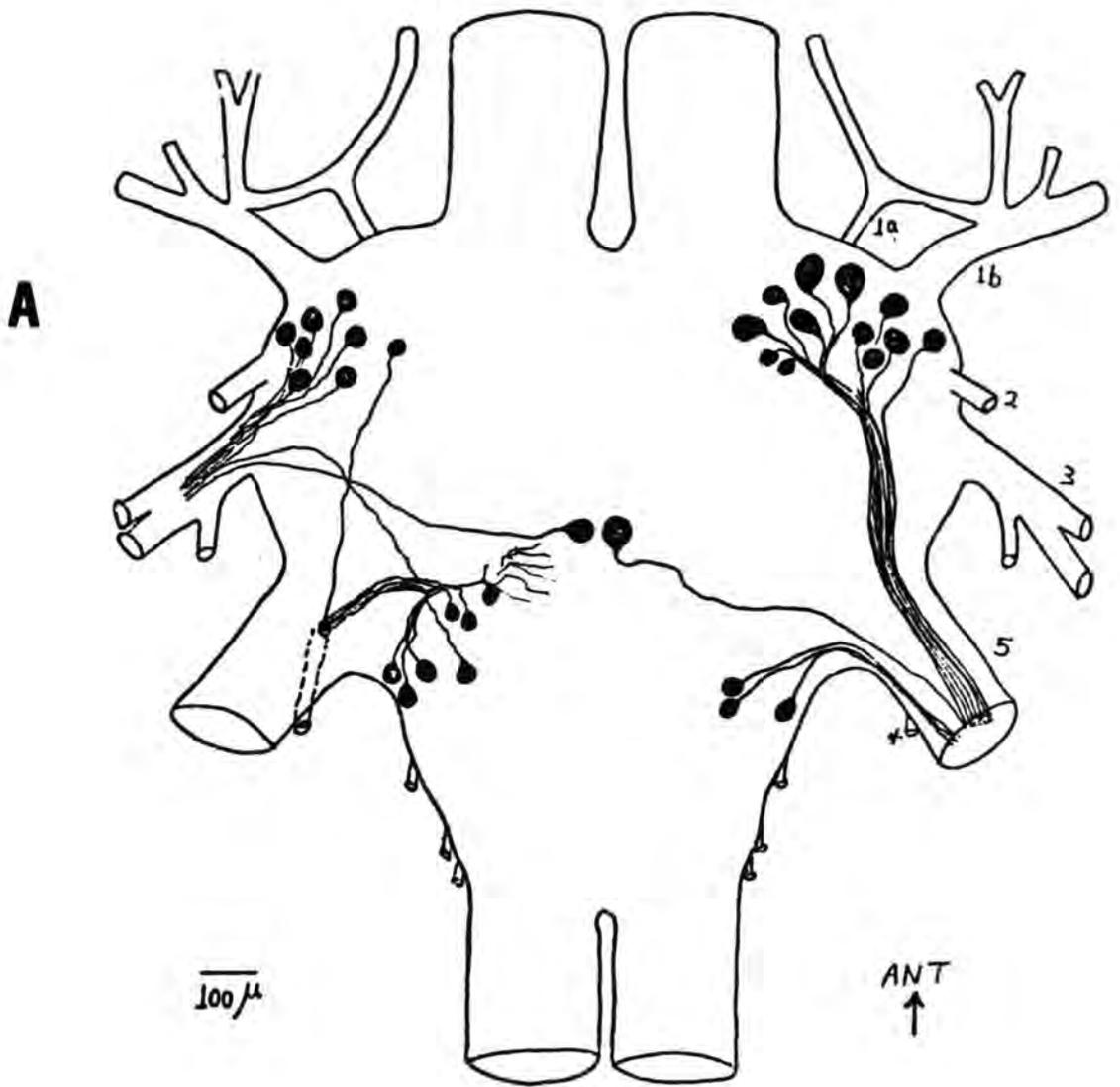
Back-filling through  $N_5$  with cobalt chloride solution showed that up to 12 cell bodies were filled, on the ventral side only of group No. 1; three of them were large, being about 60  $\mu\text{m}$  diameter, six were between 35 - 40  $\mu\text{m}$  and the other three were around 20  $\mu\text{m}$ . (Figure 1.16, table 1.4) Probably all or most of them are motor neurones that innervate the muscles of the metathoracic leg. On the other hand, back-filling through  $N_3$  revealed up to 7 cell bodies filled on the ventral side. Two of them have a diameter around 40  $\mu\text{m}$  each and the other five a diameter around 25 - 30  $\mu\text{m}$ ; and on the dorsal side two cell bodies were filled through the same nerve and their diameters were around 20  $\mu\text{m}$ .

Figure 1.16

- (A) A drawing showing the cell bodies back-filled with cobalt chloride through their cut peripheral nerves. Cell bodies of each nerve are shown only on one side to avoid intermixing.
- (B) Back-filling through N5 showing some of the cell bodies filled (arrow) in group 1 (ventral view).

Calibration : 100  $\mu$ m.

1.16



1 16

B



N5

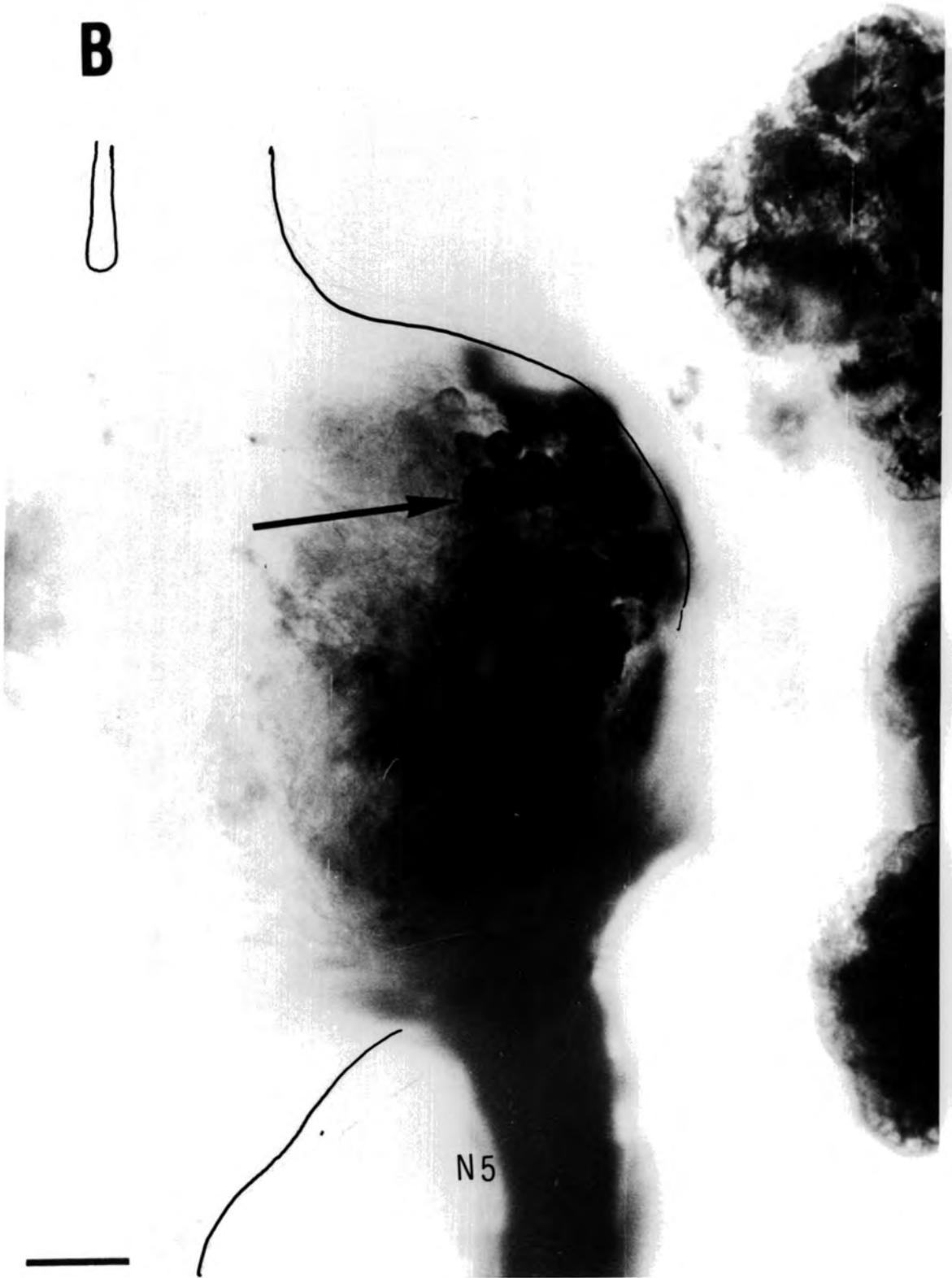


TABLE 1.4

Cell bodies filled through peripheral nerves of the ganglion of Gryllus.

Dorsal Area

Nerve trunk	Group No. 1		Group No. 2		Group No. 3	
	No. of cell bodies	diameter $\mu\text{m}'\text{s}$	No. of cell bodies	diameter $\mu\text{m}'\text{s}$	No. of cell bodies	diameter $\mu\text{m}'\text{s}$
N1	2	60,20	1	60	-	-
N2	-	-	-	-	-	-
N3	2	20	1	35	2	30
N4	-	-	-	-	-	-
N5	-	-	1	60	-	-

Ventral Area

Nerve trunk	Group No. 1		Group No. 2		Group No. 3	
	No. of cell bodies	diameter $\mu\text{m}'\text{s}$	No. of cell bodies	diameter $\mu\text{m}'\text{s}$	No. of cell bodies	diameter $\mu\text{m}'\text{s}$
N1	-	-	-	-	-	-
N2	-	-	-	-	-	-
N3	7	25.30	1	40	1	30
N4	1	<20	-	-	6	20-30
N5	12	20-60	1	50	3	30-45

Cell bodies of groups No. 1 and 3 represent only one half of the ganglion.

It had been expected that a larger number of neurones from all over the ganglion would be filled through the peripheral nerves; but despite the large number of preparations made, the number of neurones filled remained small, and it is most likely that it does not represent the real number of neurones that ought to be filled.

Figure 1.14 shows the frequency of cell bodies within different ranges of cell body sizes. It should be noted that cell bodies less than 20  $\mu\text{m}$  diameter were not included both because of their large number and because they are probably either interneurones or glial cells (e.g. <sup>see</sup> Cohen and Jacklet 1967). Table 1.4 shows the size distribution of cell bodies back-filled through the peripheral nerves of the metathoracic ganglion.

Group No. 2 :

This group is characterized by being median and unpaired; there is only one group in each of the dorsal and ventral regions. Also their cell bodies are characterized by their large size (ranging mostly from 40 to 55  $\mu\text{m}$ ) (table 1.3) and being unpaired and sufficiently small in number it is possible to count them in good preparations.

Dorsally, the group is situated almost in the middle of the ganglion over and between the two dorsal longitudinal tracts a (Figures 1.13, 1.17) and contains about 18 cell bodies, whereas the ventral group contains only about 6 cell bodies.

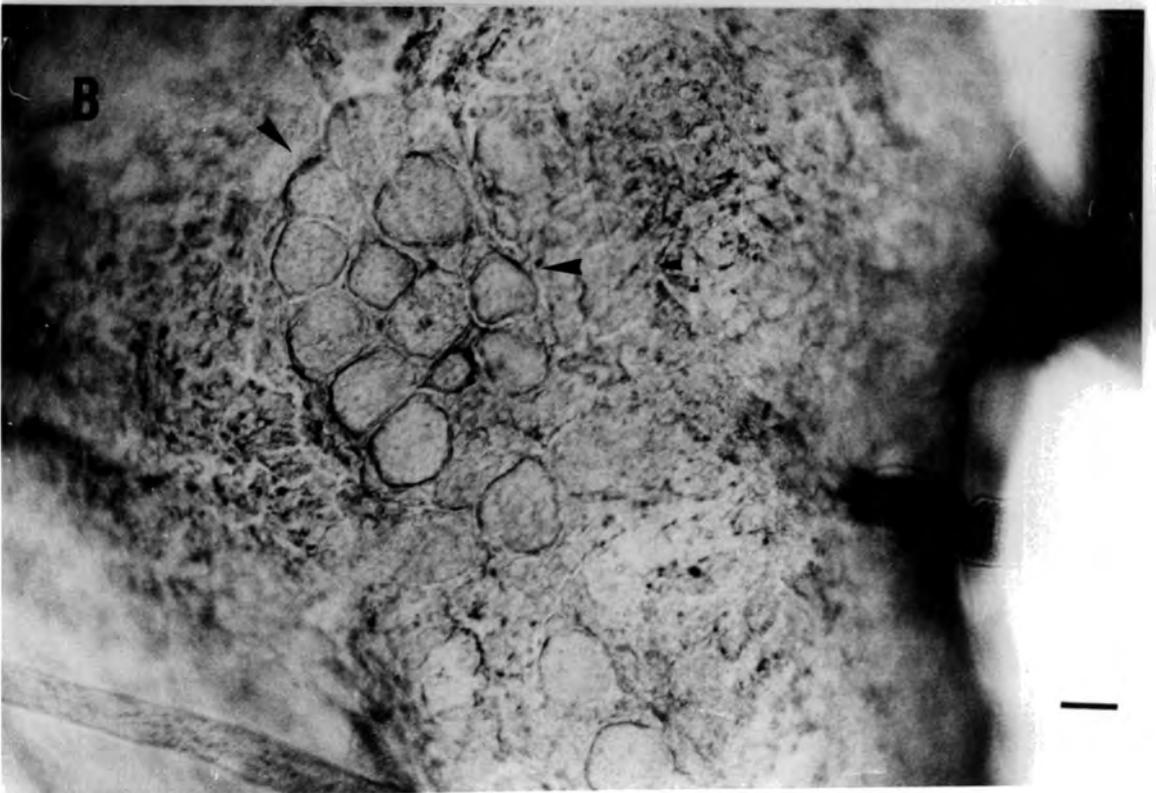
The cell bodies on the dorsal side are arranged around the dorsal longitudinal mid-line, but their arrangement is not consistently the same in all preparations. There are

Figure 1.17

(A) and (B) : Two different preparations of whole mount stained with cobalt chloride showing group No. 2 (arrows) of the dorsal area. It can be seen that most of cell bodies are of the large size and that their positions are not the same in both preparations. See also figure 1.34.

Calibration : 50  $\mu$ m.

1.17



variations from one individual of the species to another (Figures 1.17, 1.34); in some individuals, the cell bodies are grouped close to each other, in others <sup>they</sup> are arranged in lines, and in others are separated from each other. Figure 1.14 shows <sup>the</sup> distribution of cell bodies according to their size and <sup>also</sup> table 1.3. The variations cannot be attributed to methods of fixation for such variations are seen whatever fixatives and periods of fixation are used. Some variation was also observed in the number of cell bodies and it is believed that this is indeed due to actual variation in number between individuals. It seems that variation in position and in number are not linked to each other for there is no correlation between them.

Group No. 3 :

These are the cell body groups that are situated in the posterior *part* of the ganglion. As can be seen in Figure# 1.13 the middle section of the ganglion is devoid of cell bodies and appears as a clear area because the neuropile expands in the middle of the ganglion and extends up to the ganglionic sheath. Groups No. 3 lie posterior to the neuropilar "clear" area, and again as in groups No. 1, they have a smaller number of neurones on the dorsal side, and most of them augment laterally rather than in the middle, specially on the dorsal side. Also, they are mostly rather small (around 30  $\mu$ m) except for a few cell bodies, mostly on the ventral side (Figure 1.14). Cobalt filling through N<sub>4</sub> revealed a group of six cells in the ventral region usually filled through this nerve (20 - 30  $\mu$ m Figure 1.18) and dorsally two rather big (35  $\mu$ m) cell bodies were filled

Figure 1.18

(A) Back-filling through N4 revealed six neurones of group No. 3 on the ventral region. (B) drawing showing the course of these neurones. Both views are ventral of the ganglion. (From at least 5 preparations)

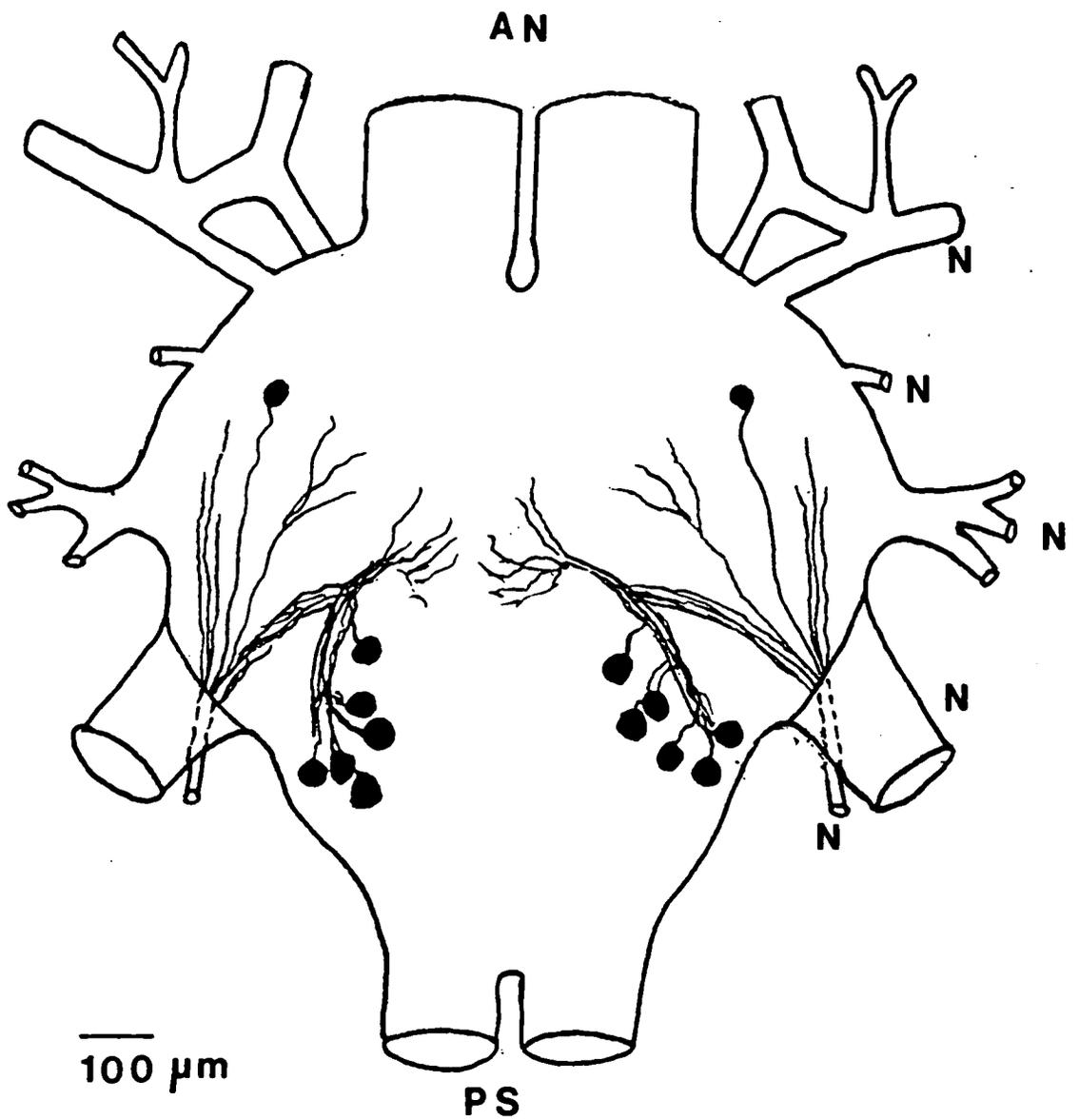
Calibration : 100  $\mu$ m.



A

1.18

# 1-18 B



through the posterior connectives, one on each side (Figure 1.19). They may be interneurons, for they were not filled through a peripheral nerve. In addition to these two big cell bodies, there are many of the smaller ones (around 20  $\mu\text{m}$ ) that were filled through the posterior connectives in both dorsal and ventral regions. However, back-filling through the connectives has the disadvantage of allowing some of the cobalt solution to leak into the extracellular medium, which resulted in preparations that were too dark (see Figure 1.19 as an example). Accordingly, attempts were made to penetrate the giant interneurons that pass through the metathoracic ganglion and inject the  $\text{CoCl}_2$  solution iontophoretically; although success was rather limited due to difficulties referred to earlier concerning  $\text{CoCl}_2$  injection, a number of successful injections <sup>(about 5% of many <sup>2</sup>hundreds of attempts)</sup> were made and Figure 1.20 shows one of them; up to eleven small cell bodies, all with diameters of less than 20  $\mu\text{m}$ , that were filled together can be seen; i.e. they were filled through the injection of one giant interneuron, suggesting the axons are syncytial. Table 1.4 shows the numbers of cell bodies filled through the peripheral nerves of the ganglion; and Figure 14 shows the distribution of cell bodies within a size range.

Group No. 4 :

This is not simply a geographical grouping of cell bodies, but is in fact a small functional group that consists of four somewhat large motoneurons (40 - 50  $\mu\text{m}$ ) and a fifth smaller one (less than 20  $\mu\text{m}$ ), that innervate the dorsal longitudinal flight muscles. They are not found in the metathoracic ganglion at all, but are situated in the

Figure 1.19

A dorsal view showing the two cell bodies (arrows) of group No. 3 (dorsal) that are filled with cobalt chloride through the posterior connectives.

Calibration : 100  $\mu\text{m}$

Figure 1.20

(A) and (B) Dorsal views of the metathoracic ganglion showing some of the cell bodies that are filled through an impaled giant axon. (C) A camera lucida drawing showing the course of the giant axon and the small cell bodies. Arrow in (C) indicates anterior.

Calibration : 100  $\mu\text{m}$

1.19



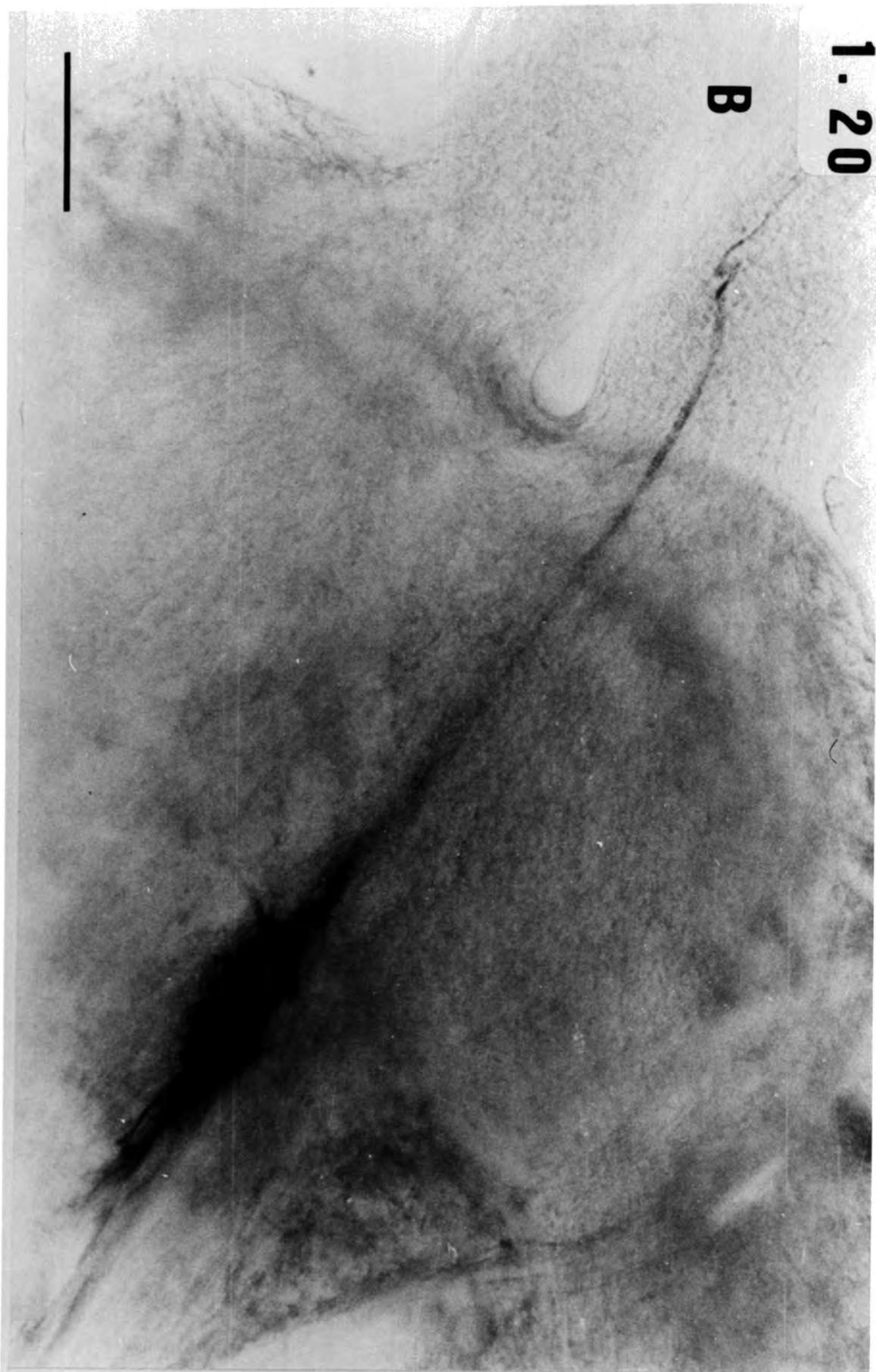
1.20

A

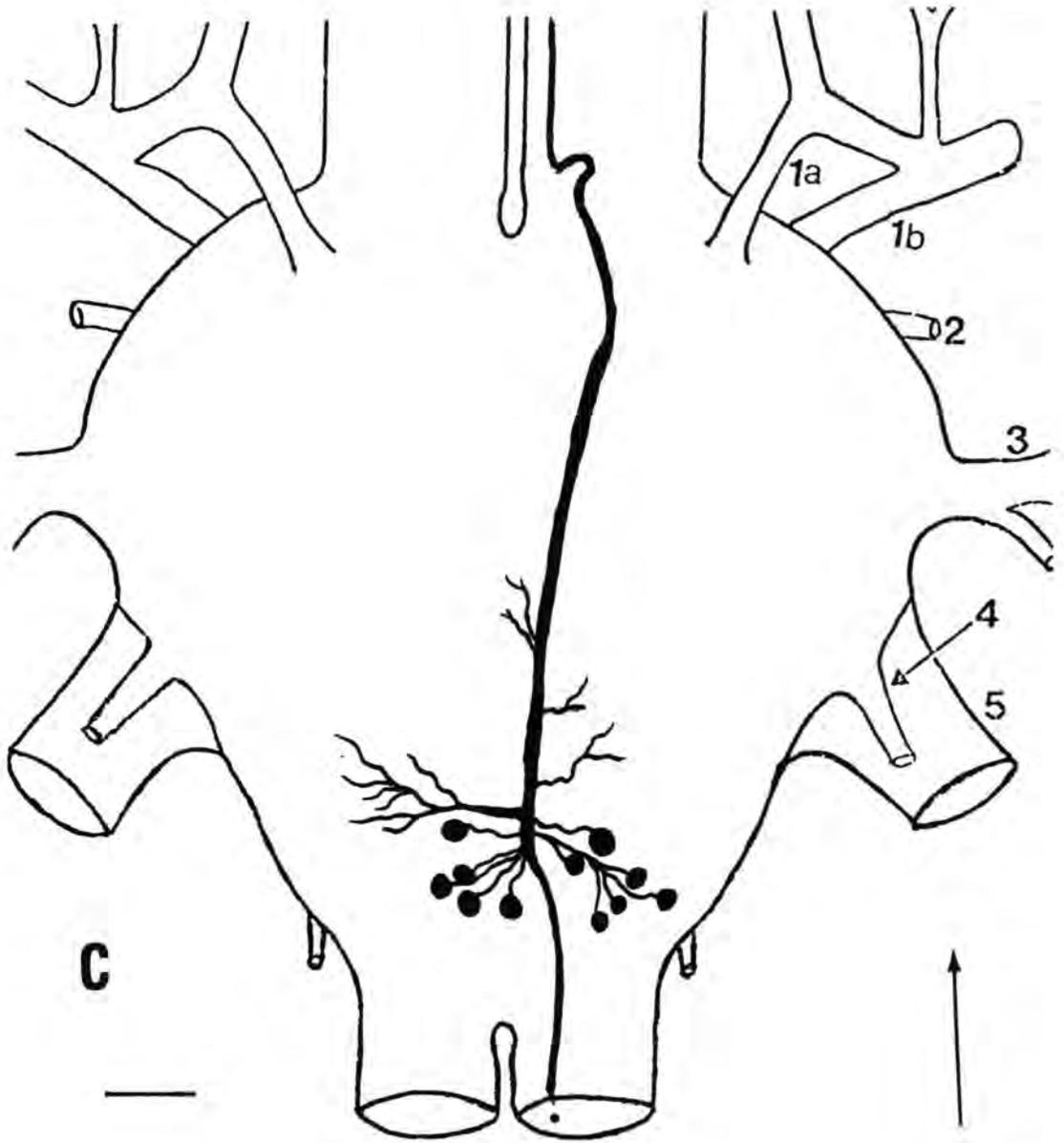


1.20

B



1.20



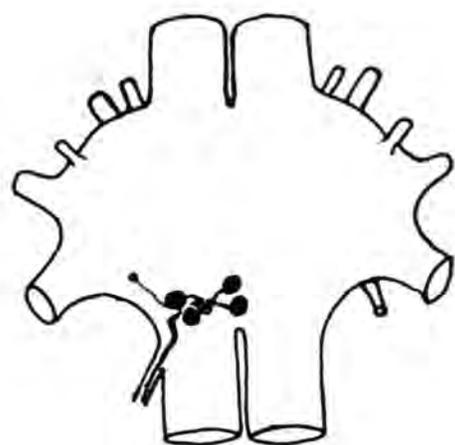
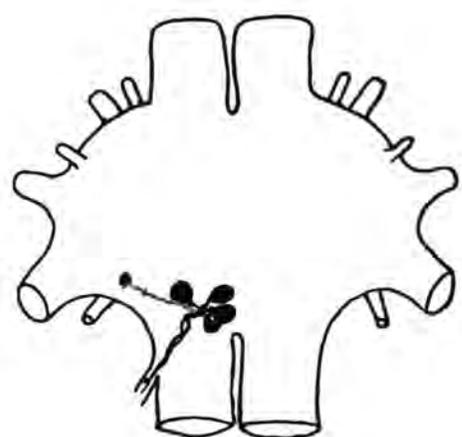
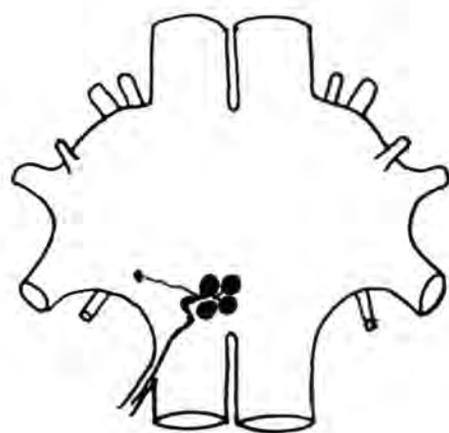
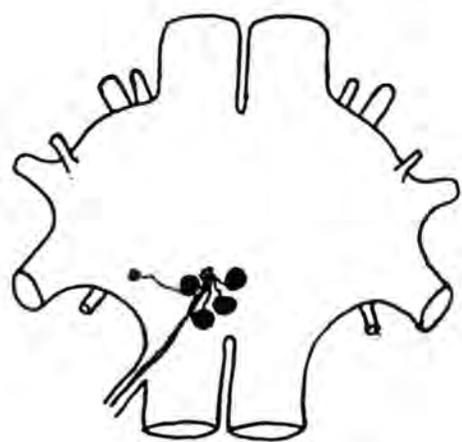
mesothoracic ganglion.

The <sup>large</sup> cell bodies are approximately of the same size and shape which is nearly spherical. The position of this group is in the posterior region on either side of the mesothoracic ganglion with their cell bodies ventrally situated. Their axons leave the ganglion almost dorsally through the ipsilateral recurrent nerve (Figure 1.32A); but the position of the cell bodies varies slightly from one individual to another: in some individuals they are found close to each other whereas in others they are found separated to a varying extent (Figures 1.21, 1.22).

This small group of cell bodies is connected with the metathoracic ganglion in that its members send their axons to their destinations through  $N_1$  of the metathoracic ganglion which joins the mesothoracic ganglion through its  $N_6$  (the recurrent nerve). Furthermore, the members of this group together with three other neurones situated in the metathoracic ganglion form a functional group that innervate the dorsal longitudinal flight muscles. The small neurone contains obvious granules suggesting that it may be neurosecretory.

This group can be identified only by staining its cell bodies with CoCl<sub>2</sub> through back-filling via their axons. There are no distinguishing properties that allow the recognition <sup>of</sup> these cell bodies reliably from each other or from other neighbouring cell bodies in serial sections or in stained whole mount, for they are situated among other similar cell bodies within the ganglion.

1.21



—

Figure 1.21

Drawings of four different preparations in which the four neurones of the group that lie in the mesothoracic ganglion were back-filled with cobalt chloride through N1 of the metathoracic ganglion. The drawings were produced using camera lucida and are showing <sup>the</sup> ventral view.

Calibration : 200  $\mu$ m

Figure 1.22

The four cell bodies group are shown stained <sup>(from another preparation)</sup> with cobalt chloride through N1 of the metathoracic ganglion and are highly magnified (arrows).

Calibration : 50  $\mu$ m

1.22



C. BACK-FILLING THROUGH  $N_1$ :

In Orthoptera,  $N_1$  is a joint nerve trunk between the mesothoracic ganglion and the metathoracic ganglion (Campbell, 1961). It is connected to the mesothoracic ganglion by the recurrent nerve ( $N_6$ ); and to the metathoracic ganglion by a single branch in locusts, but by two branches in crickets (Figure 1.9). When the cobalt chloride solution was allowed to diffuse through the cut end of  $N_1$ , two types of neural structures were revealed.

- 1 - Afferent axons (sensory) with their cell bodies in the periphery and branches in the central ganglia.
- 2 - Efferent axons (motor or neurosecretory) with their cell bodies and branches in the central ganglia and their main axons running to the periphery.

Preliminary filling through both main branches  $N_{1C}$  and  $N_{1D}$  (Figure 1.9) revealed the complexity of both afferent and efferent elements, in both the metathoracic and the mesothoracic ganglion. Back-filling through  $N_{1C}$  only revealed afferent elements running in the ventral region of the ganglion (Figure 1.23). Filling through  $N_{1D}$  only revealed nine cell bodies in the mesothoracic ganglion on either side filled through the recurrent nerve, and three cell bodies in the metathoracic ganglion. The cell bodies in the mesothoracic ganglion are situated as follows: Four large (40 - 50  $\mu$ m) cell bodies (Figure 1.22) lie ventrally at the rear of the ganglion and ipsilateral to the recurrent nerve through which they were filled. Another four cell bodies, but smaller (less than 20  $\mu$ m) lie also

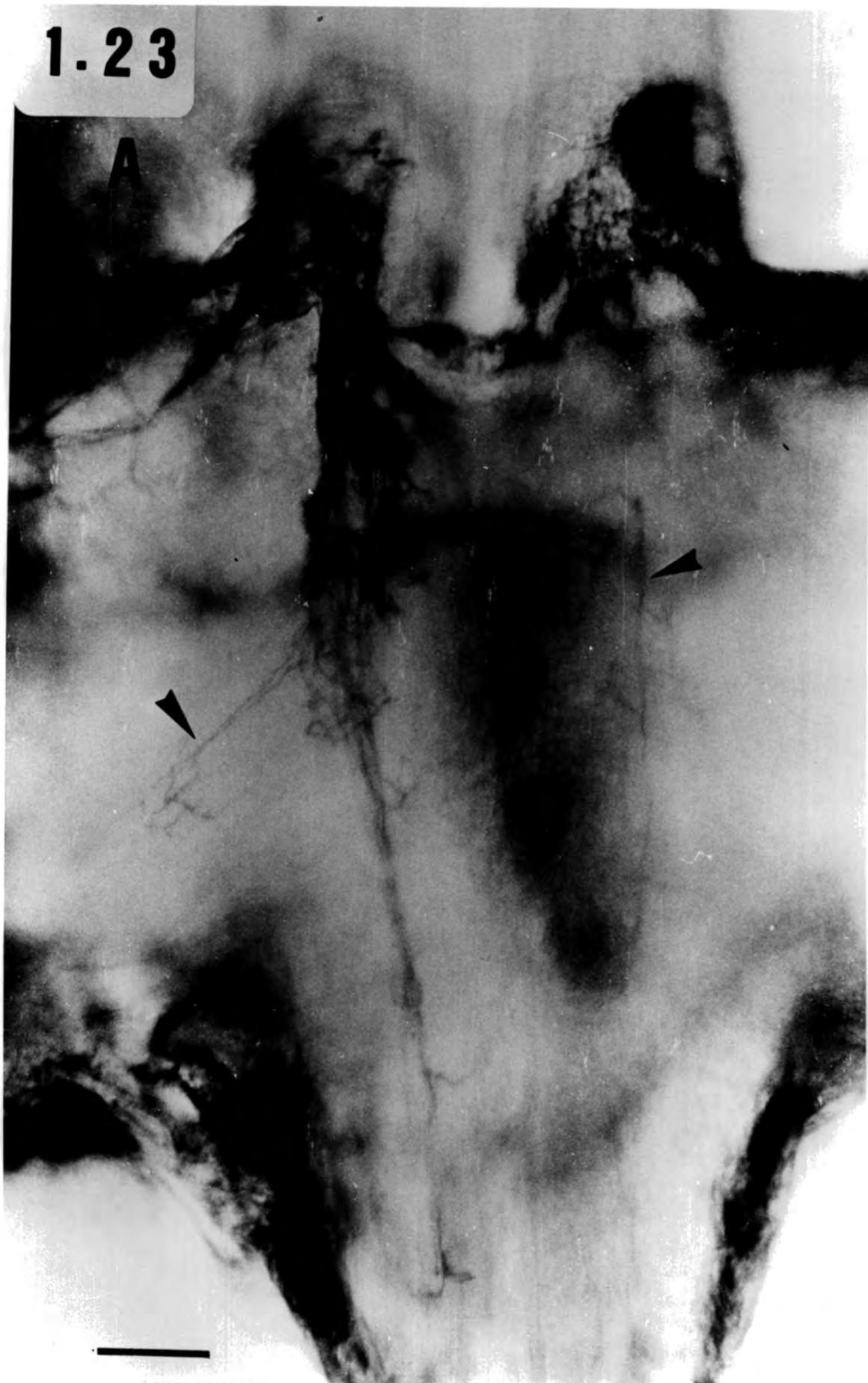
Figure 1.23

(A) Back-filling through  $N_1C$  showing sensory axons in the ventral area of the ganglion (back-filling through  $N_1D_1$  showing sensory axons of the stretch receptors on the dorsal area of the ganglion, are shown in Figure 1.33).

(B) A diagrammatic representation of (A).

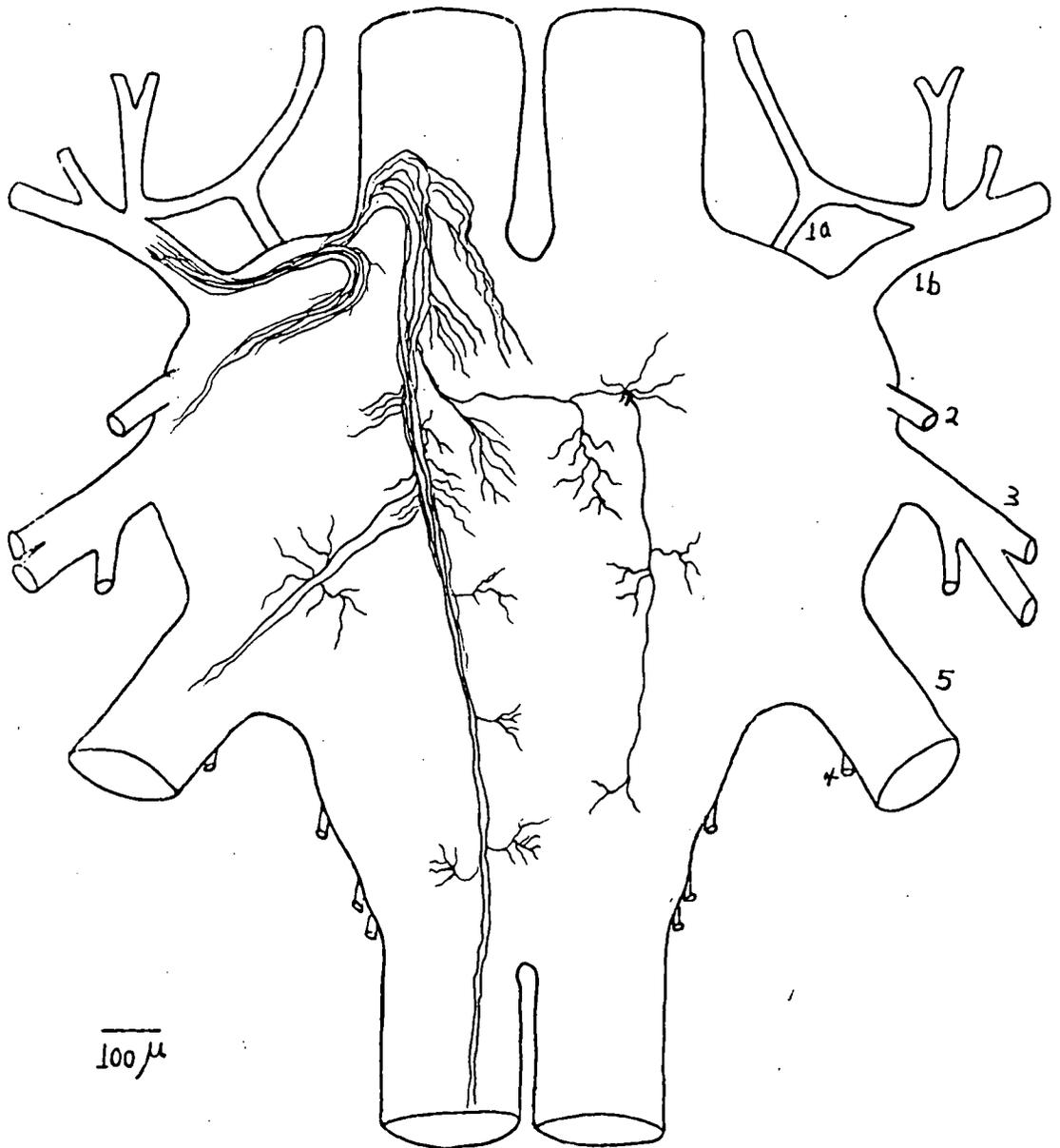
Calibration : 100  $\mu$ m

1.23



1.23

B



ipsilaterally but dorsolaterally (Figure 1.30c). The ninth one lies ipsilaterally ventral and in proximity to the large ones (Figure 1.24).

In the metathoracic ganglion there are two large cell bodies (approximately 50  $\mu$ m): one of them is unpaired and lies mid-dorsally, the other lies <sup>a</sup>contralaterally on both sides of the ganglion. A third cell body is smaller, and lies antero-ipsilaterally. Filling through  $N_1D$  also revealed afferent elements which run in the dorsal region of the ganglion. Further filling through  $N_1D_2$  showed only the dorsal afferent elements and no cell bodies filled through it. Filling through  $N_1D_1$  showed only the nine cell bodies in the mesothoracic ganglion as well as the three ones in the metathoracic ganglion. Further filling through  $N_1D_{1A}$  revealed the four large cell bodies and the single ventral one in the mesothoracic ganglion, as well as the mid-dorsal and the contralateral cell bodies in the metathoracic ganglion (Figure 1.32A); when filling through  $N_1D_{1B}$  revealed the four, dorsal, small cell bodies of the mesothoracic ganglion as well as the ipsilateral one in the metathoracic ganglion. A few attempts have been made to fill through  $N_1D_{1C}$ , but they were unsuccessful, and no more attention has been given to it.

I found that as the length of the nerve filled increases, filling was efficient only when the  $CoCl_2$  was reasonably diluted; most of the fillings through the very distal branches ( $N_1D_{1A}$  and  $N_1D_{1B}$ ) were obtained with concentrations of between 50 - 100 mM; higher concentrations produced incomplete fillings and it was difficult to make out the finer branches,

Figure 1.24

a section of the ganglion showing  
Two photographs of the small cell body that is usually  
found in <sup>the</sup> vicinity of the four motor neurones in the meso-  
thoracic ganglion. (A) Is highly magnified to show the  
contents of the cell body. (Cobalt chloride preparation,  
intensified).

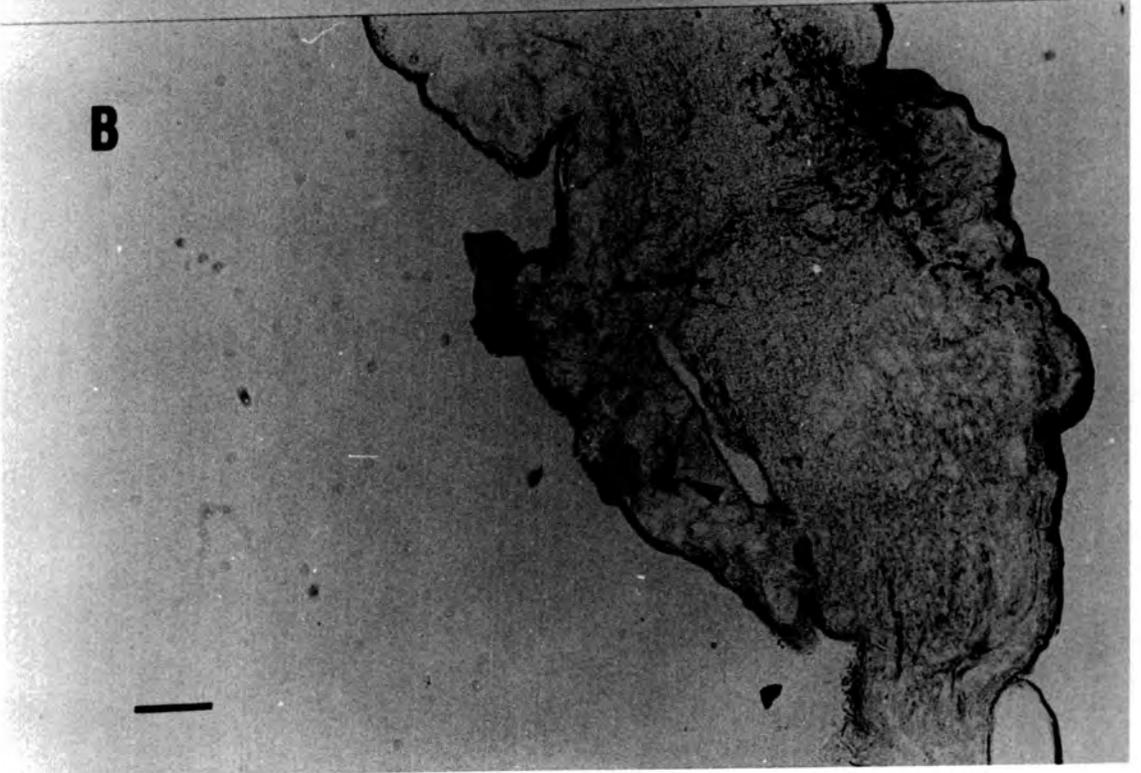
Calibration : (A) 50  $\mu\text{m}$  ; (B) 100  $\mu\text{m}$

1 24

A



B



despite the use of the intensification method of Bacon and Altman (1977) which did produce a useful enhancement of the stain.

DETAILS OF THE EFFERENT ELEMENTS :

(i) In the metathoracic ganglion :

(a) The Contralateral neurone: This neurone is unique, for it has some unusual properties : (a) its cell body is situated contralaterally to its main axon exit, (b) it has two sets of branches, one ipsilateral to the cell body being short and less extensive; the other is contralateral to the cell body but much more extensive.

In a highly magnified preparation, the cell body appears almost spherical (Figure 1.25) with a cone-like end from which the axon emerges at the rear of the cell body which has a diameter of approximately 50 - 65  $\mu\text{m}$ . In a few cobalt preparations, the cell body appeared deformed, being elongated to an unusual diamond shape 35 x 80  $\mu\text{m}$ , but still in the normal position. Its nucleus still can be seen clearly (Figure 1.26). This cell body (the contralateral dorsal longitudinal motor neuron, CDLM, Clark 1976a) was always found in the contralateral connective and laterally to it (1.27 A,B,C,D,); even the neural "through tracts" make a slight bend at that position of the connective during their Passage (Figure 1.20), and this can be seen in the silver stained preparations as well as in the cobalt ones. Guthrie (1964) showed what appears to be the homologous cell body in the locust Schistocerca gregaria, close to the midline of the ganglion; though Bentley (1970) and Tyrer and Altman (1974) found it more laterally in the locusts, Schistocerca gregaria

Figure 1.25

A highly magnified cell body of the CDLM (the contralateral dorsal longitudinal motor neurone). The rear of the cell body is cone-like and from this the axon of the neurone emerges.

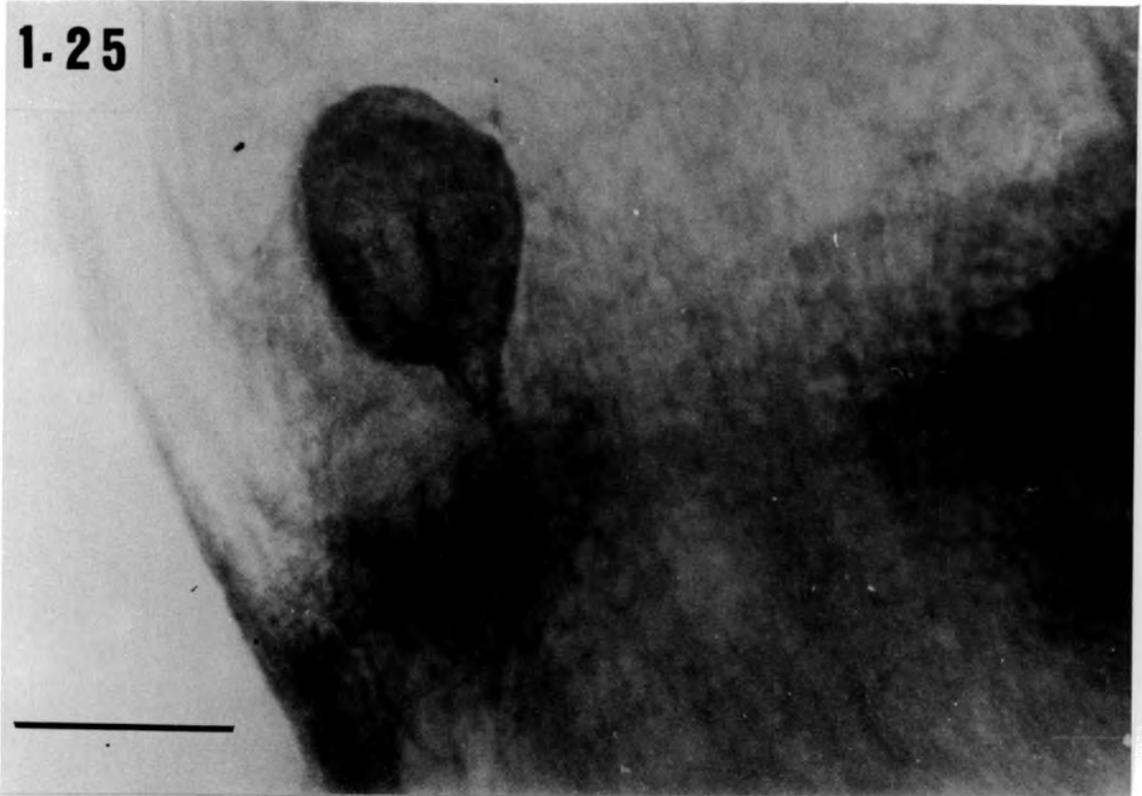
Calibration : 50  $\mu$ m

Figure 1.26

On a very few occasions the cell body was found deformed. In this figure, the dorsal unpaired median neurone (DUMDL) is shown also in the middle of the ganglion (arrow).

Calibration : 100  $\mu$ m

1.25



1.26

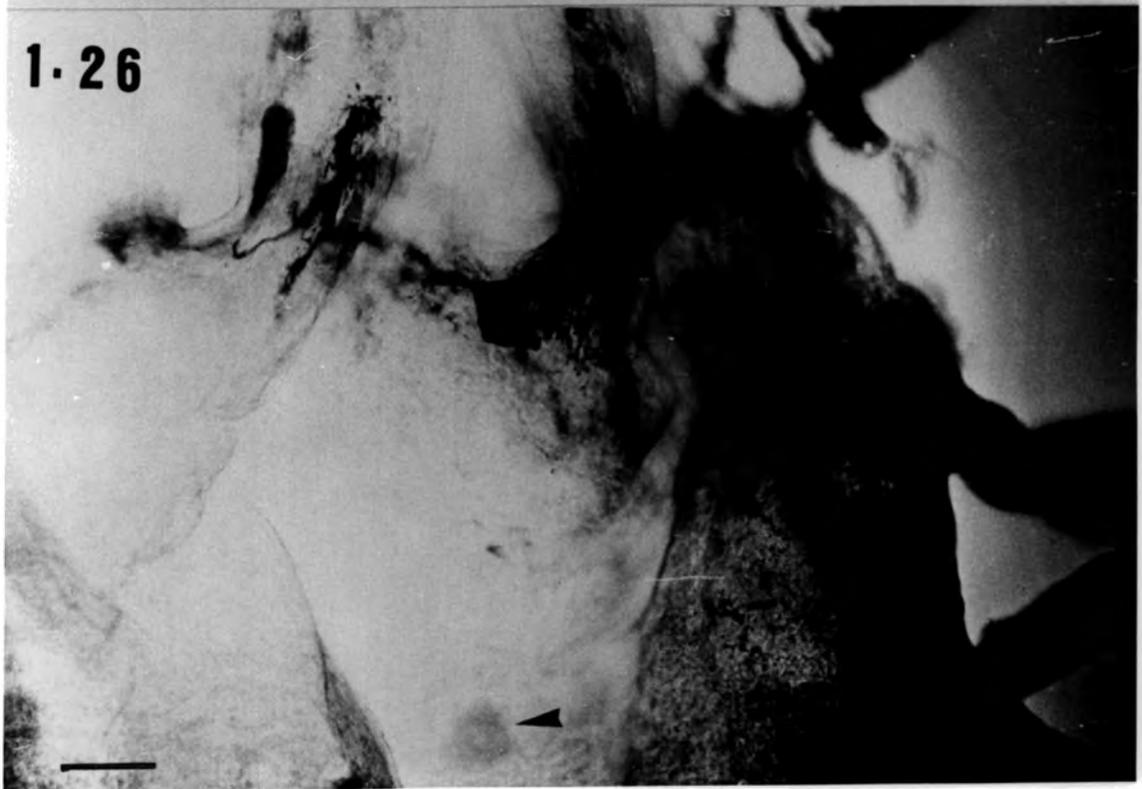


Figure 1.27

(A), (B), (C), and (D) are four different preparations showing the position of the CDLM (in the connective). In (B) the cell body is out of focus to show the branches of the neurone (arrows).

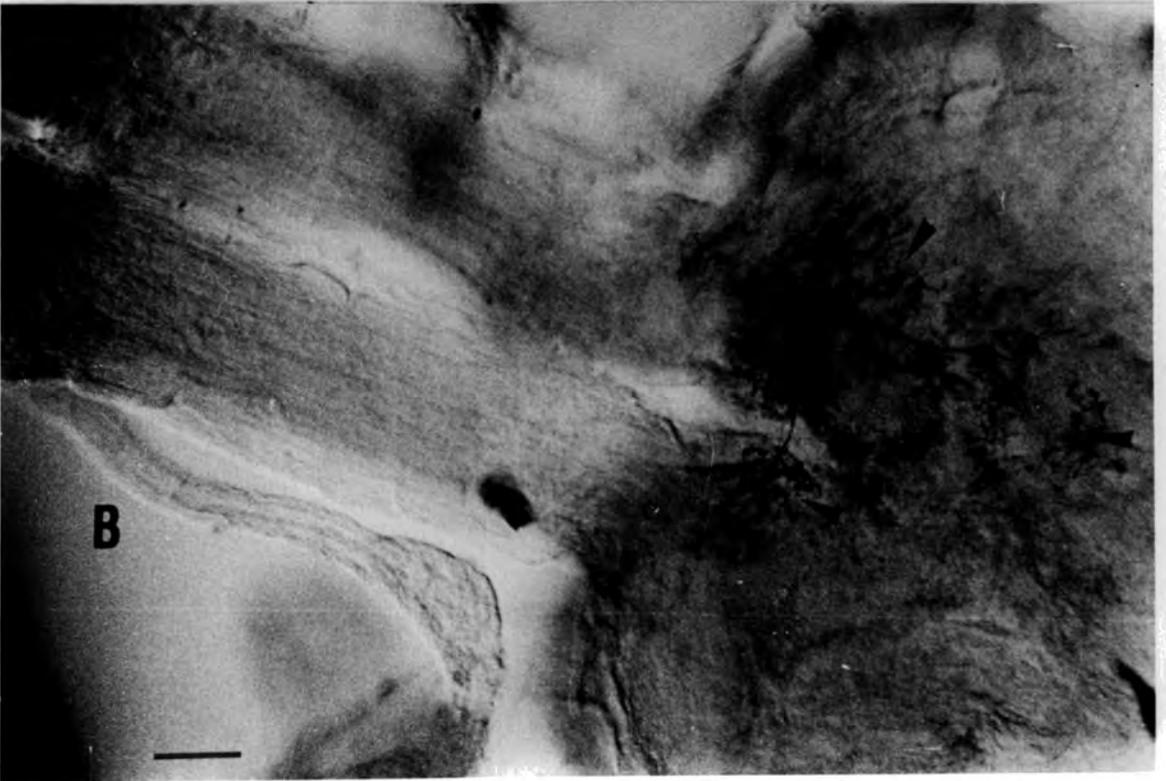
Calibration : 100  $\mu$ m

1.27

A



B

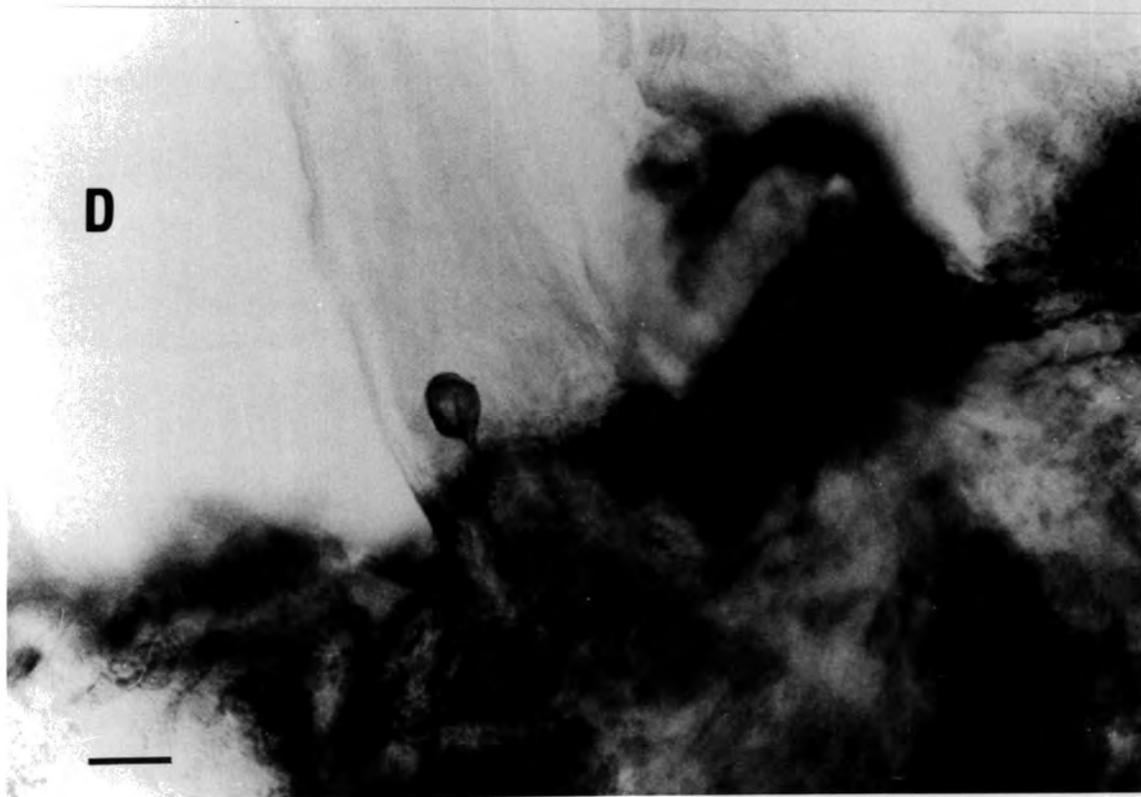


1.27

C



D



and Chortoicetes terminifera respectively; also Truman and Reis (1976) described a similar neurone in the fourth abdominal ganglion, but in the ventral region, in the tobacco hornworm moth.

The axon emerges from the cell body and follows its path backward then it bends towards the curvature of the ganglion with a slight inclination downward; then it makes a "U" turn towards the contralateral side of the ganglion towards N<sub>1a</sub>.

Two sets of branches are given off by the axon on both sides of the ganglion. The first one is ipsilateral to the cell body, shorter, and less extensive (Figure 1.27B, 1.28). The other set is contralateral to the cell body, more extensive, and consists of two main branches; one is more contralateral and longer; the other is lateral and shorter. All the main branches give rise to smaller branches, and some of them end in blebs. The density of the branches increases at the anterior part of the ganglion (Figure 1.28); the branches are running only dorsally, and no extension of them was noticed deeper than 70  $\mu$ m from the surface of the ganglion. Also in some preparations, some branches were seen emerging from the main axon, going into the connective contralaterally to the cell body; these start thick, then they terminate abruptly nearby (Figure 1.29).

(b) The dorsal unpaired median neurone (DUMDL) that innervates the dorsal longitudinal flight muscles : The cell body of this neurone is usually found almost in the middle of the dorsal region of the metathoracic ganglion (Figure 1.26). It is filled through N<sub>1D1A</sub> on both sides of the

1.28

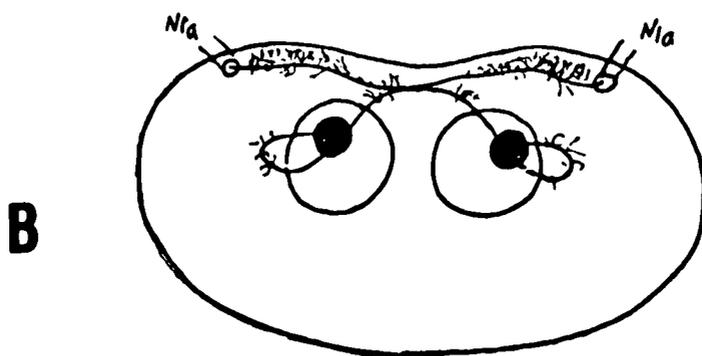
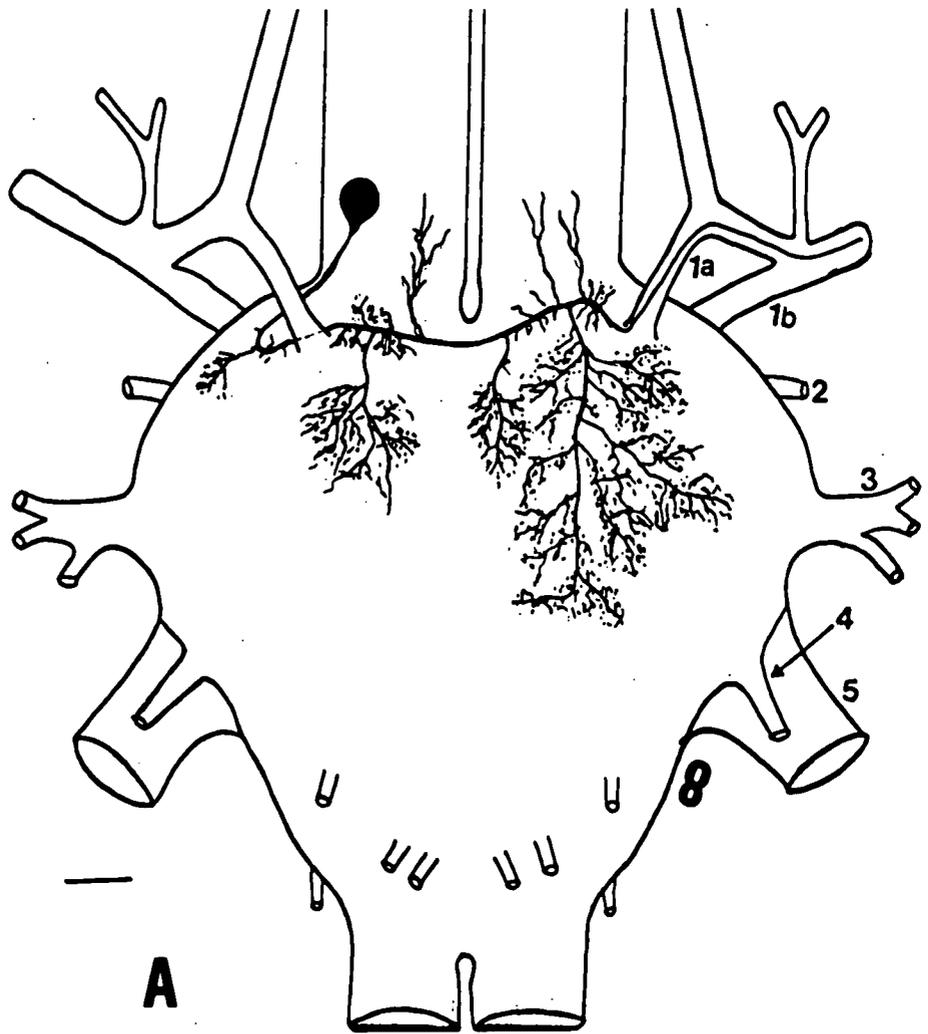


Figure 1.28

(A) A camera lucida drawing of a whole moun~~g~~ stained with cobalt chloride showing the branching pattern of the CDLM.

(B) A reconstructed cross section showing the CDLM and its axons.

Calibration : 100  $\mu$ m

Figure 1.29

A highly magnified branch of the CDLM, that goes towards the anterior<sup>end</sup> of the connective (arrow).

Calibration : 50  $\mu$ m

1.29



ganglion. The cell body is a large one (50 - 60  $\mu\text{m}$  diameter) and almost spherical in shape. Its neurite emerges from it anteriorly, posteriorly, or ventrally on some occasions and runs ventrally for between 30 to 60  $\mu\text{m}$  downwards from its emerging point (Figure 1.30); then it turns anterad beneath the cell body, and continues forward for a length of around 200 - 300  $\mu\text{m}$  after which it bifurcates, giving a branch to  $N_{1a}$  on both sides. However, during its course from the soma to the point of bifurcation, variations were found to occur in this pattern and these were generally as follows: after leaving the soma, the neurite bends and goes forward between the "through tracts" a and parallel with the dorsal side for a length of 55 - 77  $\mu\text{m}$ ; and this length usually stays straight; or slight zigzagging may occur in some preparations. Then it loops around itself several times for a length of 46.5  $\mu\text{m}$ ; during this looping, some parts of it go downward to the level of tract b, then it continues going straight forward for a length of 155  $\mu\text{m}$  before it bifurcates into two branches, each of them going to the ipsilateral  $N_{1a}$  on each side of the ganglion. Along the course of this axon between 4-7 swellings can sometimes be seen at irregular distances, varying from 6-13  $\mu\text{m}$  in thickness and 7-15  $\mu\text{m}$  in length. From these swellings very fine branches can be seen emerging in all directions and they end shortly after they emerge although some go further up to around 100  $\mu\text{m}$ ; however they are not thick or arborized; and they end usually in spiny ends. No other branches were seen emerging from other areas of the main axon except at these swellings; but after the bifurcating point, each branch gives off some rather thin and short branches (Figure 1.30).

Figure 1.30

Drawings produced using camera lucida showing variations in the course of the axon of the DUMDL.

Figure 1.31

(A) Four different preparations were drawn using camera lucida, showing a slight variation in the position of the DUMDL and the arrangement of the surrounding cell bodies. The DUMDL was first stained with the  $\text{CoCl}_2$  through  $\text{N}_1$  then the whole mount was stained with the toluidene blue method.

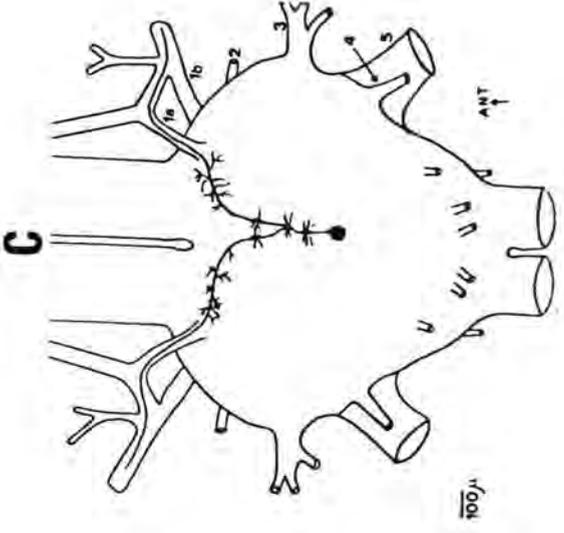
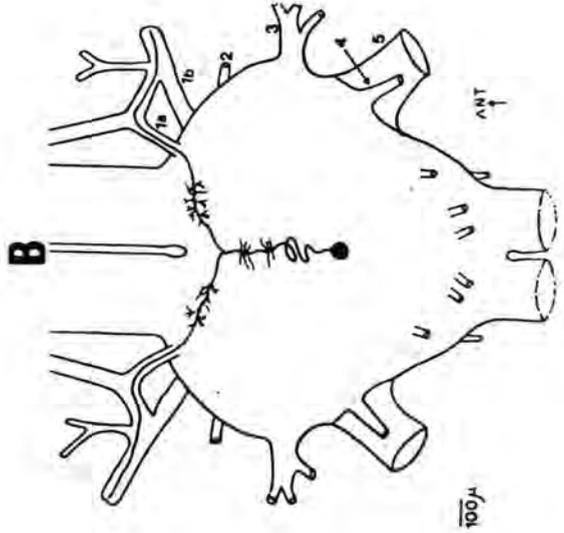
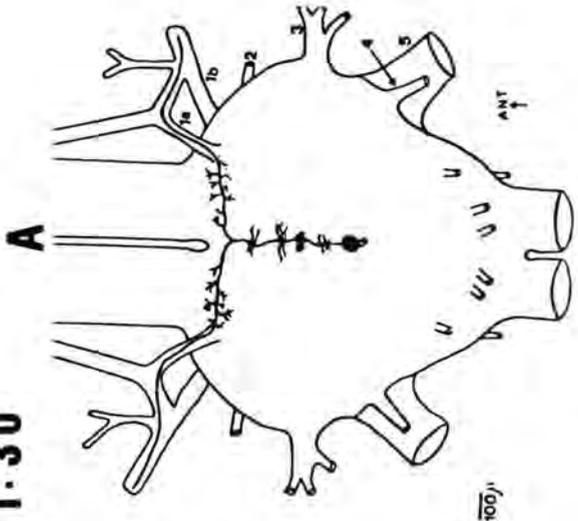
(B) The DUMDL in a cross section after being stained with procion yellow through the microelectrode.

Arrow indicates its axon.

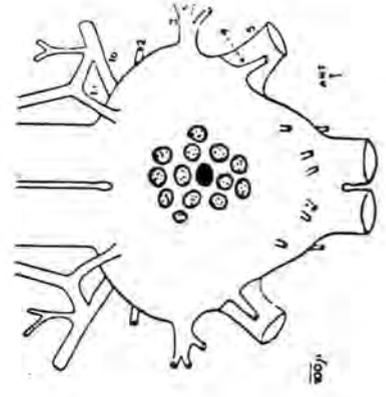
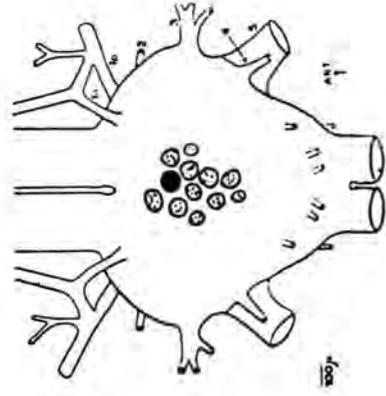
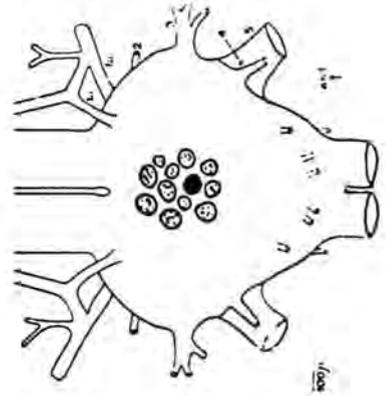
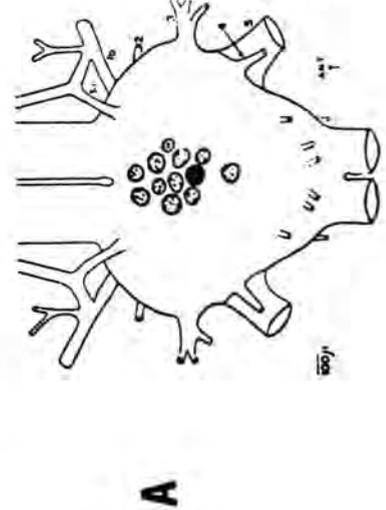
ANT = Anterior

Calibration : 100  $\mu\text{m}$

1-30

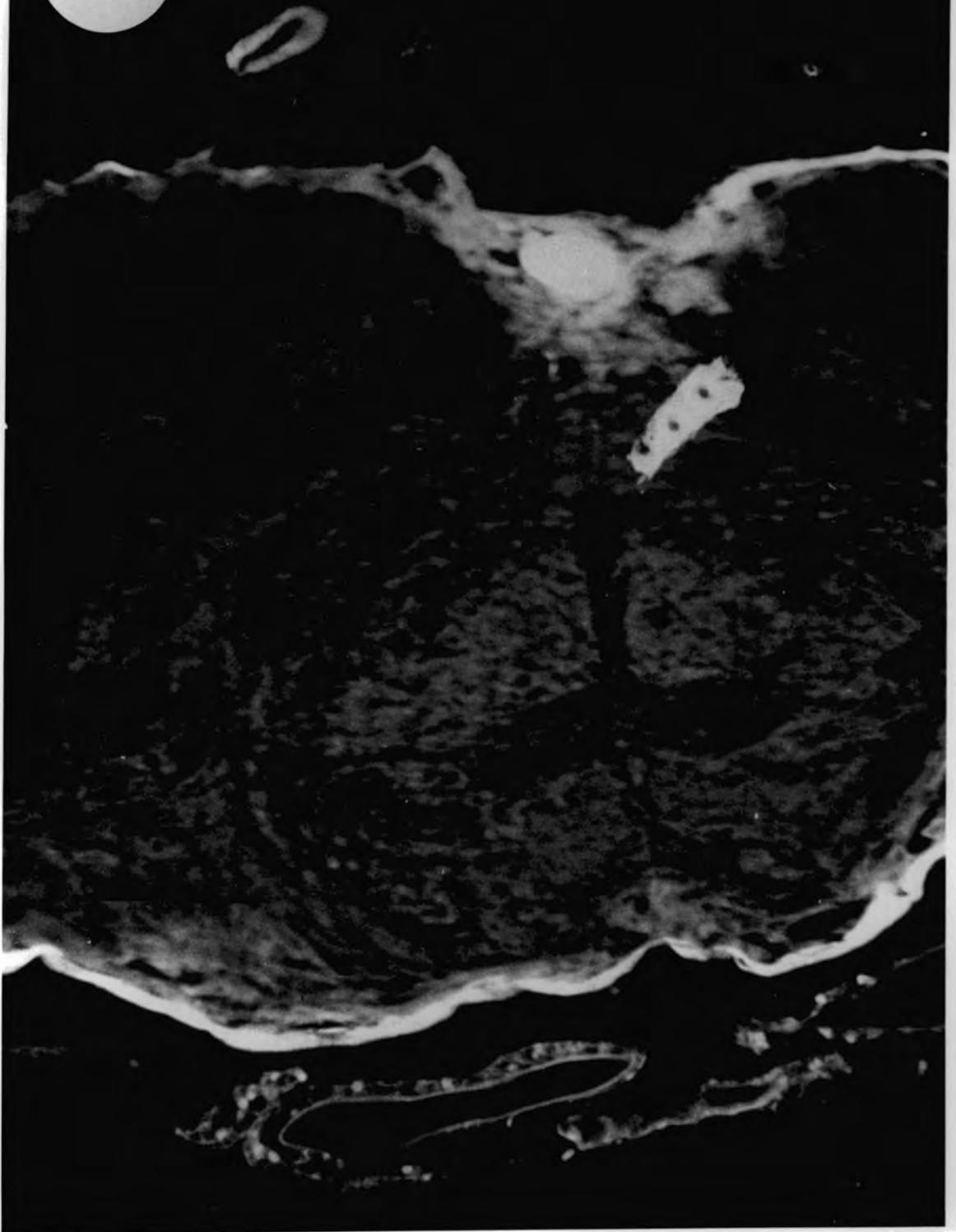


1-31



1.31

B



In some other preparations, the whole axon appears almost straight until the bifurcation point, or slightly zigzagged. The bifurcation point on some occasions was found to be more anterior (Figure 1.30A,B,C) close to the axon of the contralateral neurone, and it is most likely that these two neurones contact each other in some way for they both innervate the same muscle; although it was difficult definitely to establish such a relationship with the technique used.

Most of the fillings of this neurone were obtained only by using diluted concentrations of  $\text{CoCl}_2$  solution (50 - 100  $\mu\text{M}$ ); and this resulted in rather faint precipitation of cobalt sulphide which needed to be intensified carefully. The length of the nerve was another problem and it was not easy to fill, needing numerous attempts in order to obtain a few successful results. *(about 20% of the attempts were successful)* Other workers have reported difficulties of this sort, e.g. Bentley (1973). Hoyle (1978) suggested that in such circumstances the cell body may be degenerate, but he gave no real grounds for this and evidence is given in the next paragraph that it is indeed a functional motor neurone. Some of the difficulties could well be caused by the very complex and long pathway taken by the axon. In addition *varies* ~~and~~, the position of the cell body ~~is~~ *is* slightly ~~varying~~ from one individual to another (Figure 1.31).

Sensory axons of the wing stretch receptors of the hind wing of both sides branch in vicinity to the main axon of the DUMDL as well as to its two branches beyond the bifurcating point. This can be seen in Figures 1.32A and 1.33.

Figure 1.32

(A) Camera lucida drawings of the neural elements that innervate the DLM and which are situated in both the mesothoracic ganglion (MS) and the metathoracic ganglion (MT). In the mesothoracic ganglion : the four motor neurones that innervate the DLM can be seen (in black), their cell bodies are ventrally situated and their branches are in the dorsal region. A branch of the hind wing stretch receptor is also shown (in red).

In the metathoracic ganglion the following are shown: The DUMDL (in black), the CDLM (in green) and the branches of the hind wing stretch receptors (in red). (B), (C) and (D) showing the DUMDL filled with Procion yellow. (B) is same as (C) showing the axon and the soma, (D) is another preparation.

Calibration : 100  $\mu$ m.

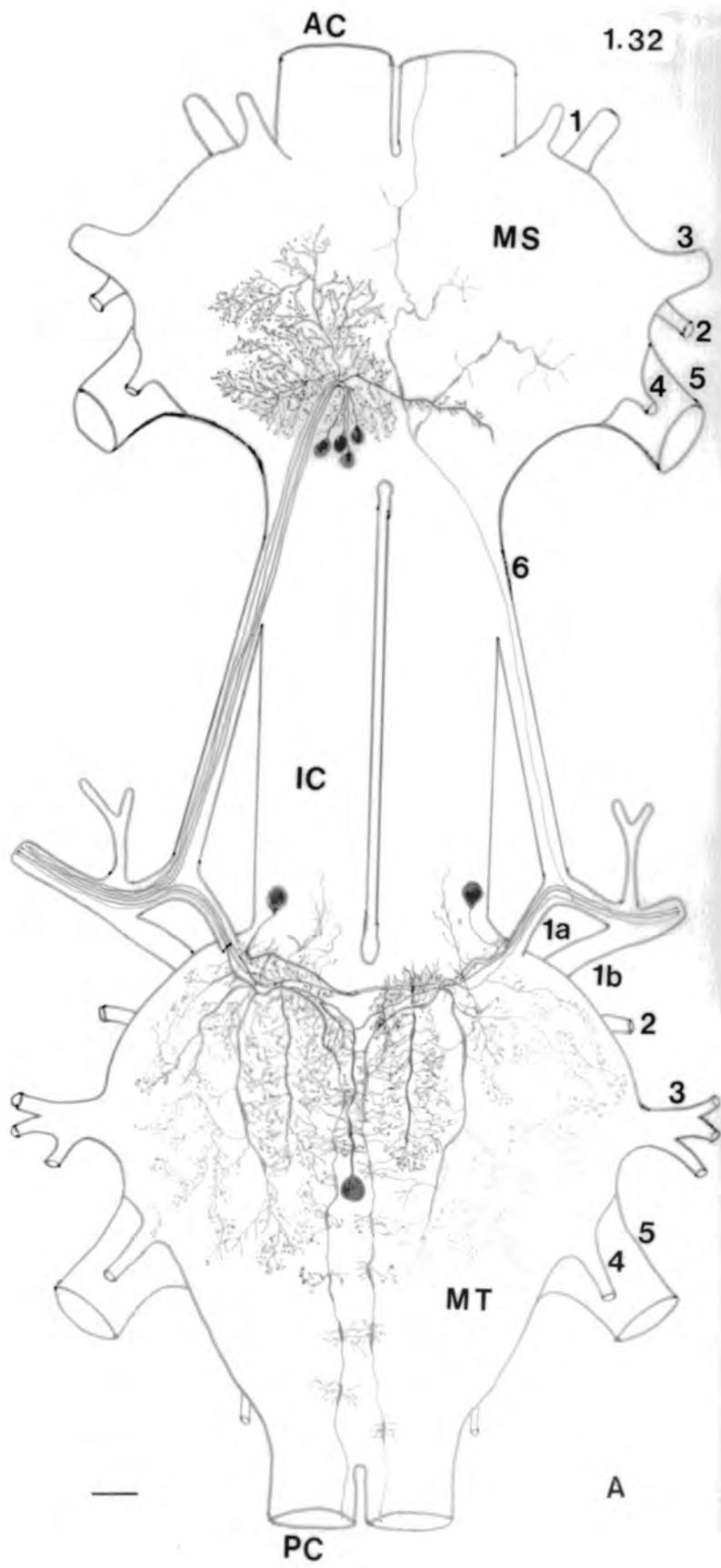


Figure 1.33

A dorsal view of the metathoracic ganglion of Gryllus showing the main branches of the hind wing stretch receptors. The axons of the CDLM are also shown (arrow). This preparation is a double-filling through N1D of both sides of the ganglion.

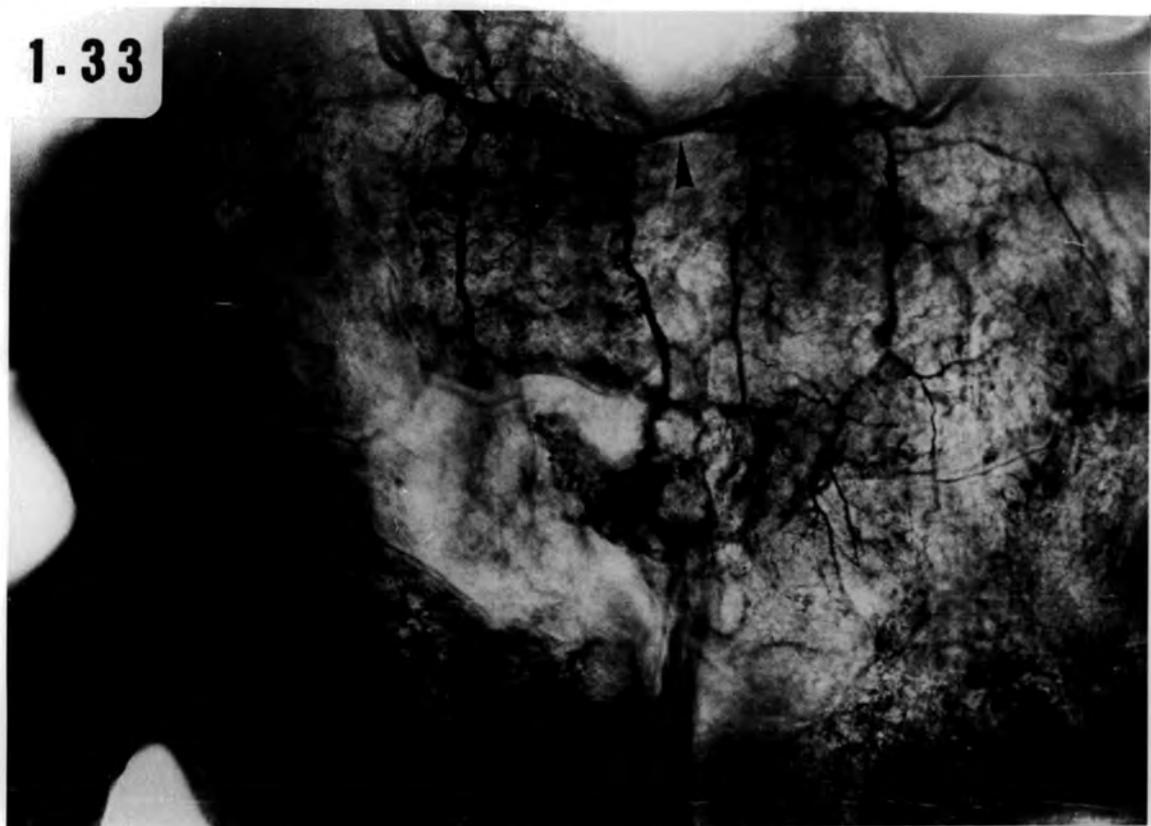
Calibration : 100  $\mu$ m

Figure 1.34

A dorsal view of the metathoracic ganglion after prolonged filling <sup>(48 hours)</sup> through N1 that resulted in filling of two cell bodies of group No. 2 dorsal, the DUMDL and another one (arrows). The ganglion was later stained as a whole mount with  $\text{CoCl}_2$  before fixation.

Calibration : 100  $\mu$ m

1.33



1.34



Figures 1.32B,C,D, show the DUMDL cell body stained intracellularly with procion yellow through microelectrodes. It has often been identified physiologically (see chapter two) when it was stimulated, and a twitch in the DLM was observed. In other words, this would appear to be a normally functioning motor neurone. Davis and Alanis (1979) have previously demonstrated extracellular action potentials along the axon of this neurone in  $N_1D_1A$  in the house cricket.

Procion yellow was not found to penetrate very far along the process from the cell body compared with other methods; and its chief use would appear to be as a marker dye for cell bodies. This neurone seems to be connected to other neurons of the dorsal group (group No. 2), for with prolonged filling some of their cell bodies were filled as well (Figure 1.34).

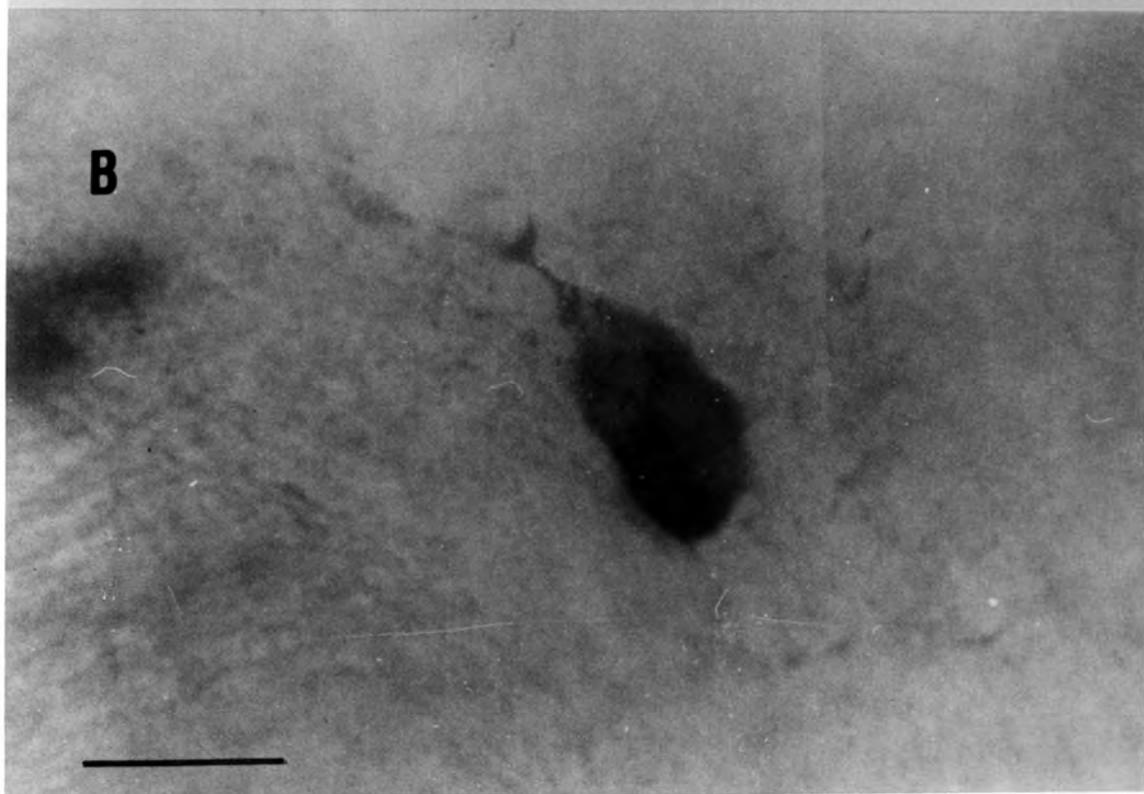
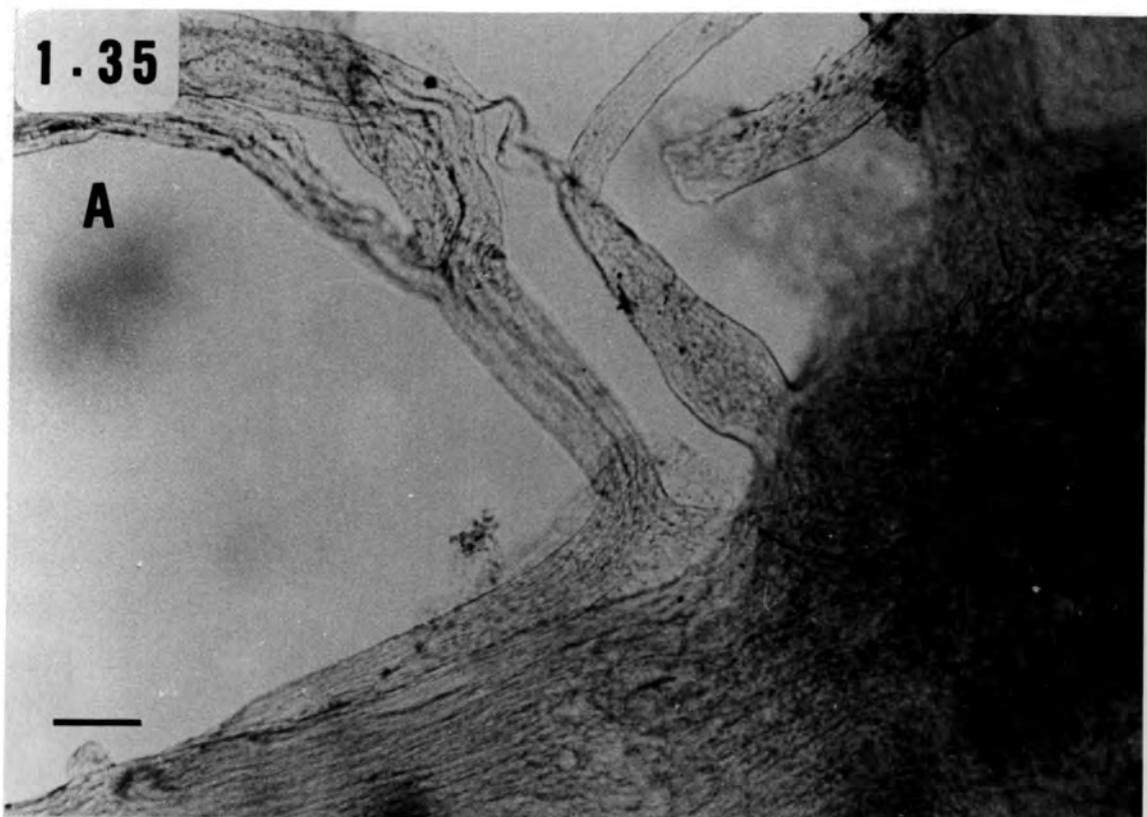
(c) The ipsilateral neurone : This neurone innervates the dorsal oblique muscle, a small muscle, described by Guthrie (1964) as "an oblique segment of the dorsal longitudinal flight muscles". Repeated filling of this neurone showed that its arborization is not extensive. As can be seen in Figure 1.35, the cell body is an elongated one, its length is 72  $\mu\text{m}$  whereas its width is 32  $\mu\text{m}$ . It gives rise to a short neurite, only 16  $\mu\text{m}$  long; this neurite splits into three fine branches after a slight thickening. The three branches are directed as follows:

- (i) The first one turns anterad where it gives three finer branches; one goes towards the ipsilateral anterior connective, where it branches and then disappears there; another one goes to the extreme lateral region of the

Figure 1.35

The ipsilateral neurone that innervates the dorsal oblique muscle, stained with the  $\text{CoCl}_2$  method through the nerve N1D1B. (2 out of 5 preparations)

Calibration : (A) 100  $\mu\text{m}$ , (B) 50  $\mu\text{m}$ .



ganglion; and the third ends near the cell body.

(ii) The second one is in the middle, and this is the main axon that leaves the cell body and goes to its exit through  $N_{1a}$  without giving branches.

(iii) The third one goes laterally, where it gives rise to two branches, but they disappear after giving off a few more branches near its branching point.

The contents of this cell body are homogeneous compared with the comparably positioned neurone in the mesothoracic ganglion (Figure 1.24A) and which contains obvious granules, suggesting <sup>a</sup> difference in functioning *between* them.

(ii) In the mesothoracic ganglion :

(a) Detailed arborization of the four large motor-neurons : These are situated in the posterior ventral corner of the mesothoracic ganglion ipsilaterally to the recurrent nerve (RN), through which they send their axons (Figures 1.32A, 1.36). The RN itself, joins the mesothoracic ganglion either directly in the ipsilateral posterior corner of the ganglion, or by joining the ipsilateral connective between the metathoracic and the mesothoracic ganglia at any point.

The four axons of these motorneurons leave the mesothoracic ganglion dorsally through the recurrent nerve; then through the connecting nerve between the recurrent nerve and  $N_{1a}$ ; then through  $N_{1D_1}$ , and from these to the branch  $N_{1D_1A}$  that leads to their destination, the dorsal longitudinal flight muscles. The filling was carried out through the branch  $N_{1D_1A}$ .

Their ventrally positioned cell bodies with axons directed towards the dorsal region of the neuropile where they

Figure 1.36

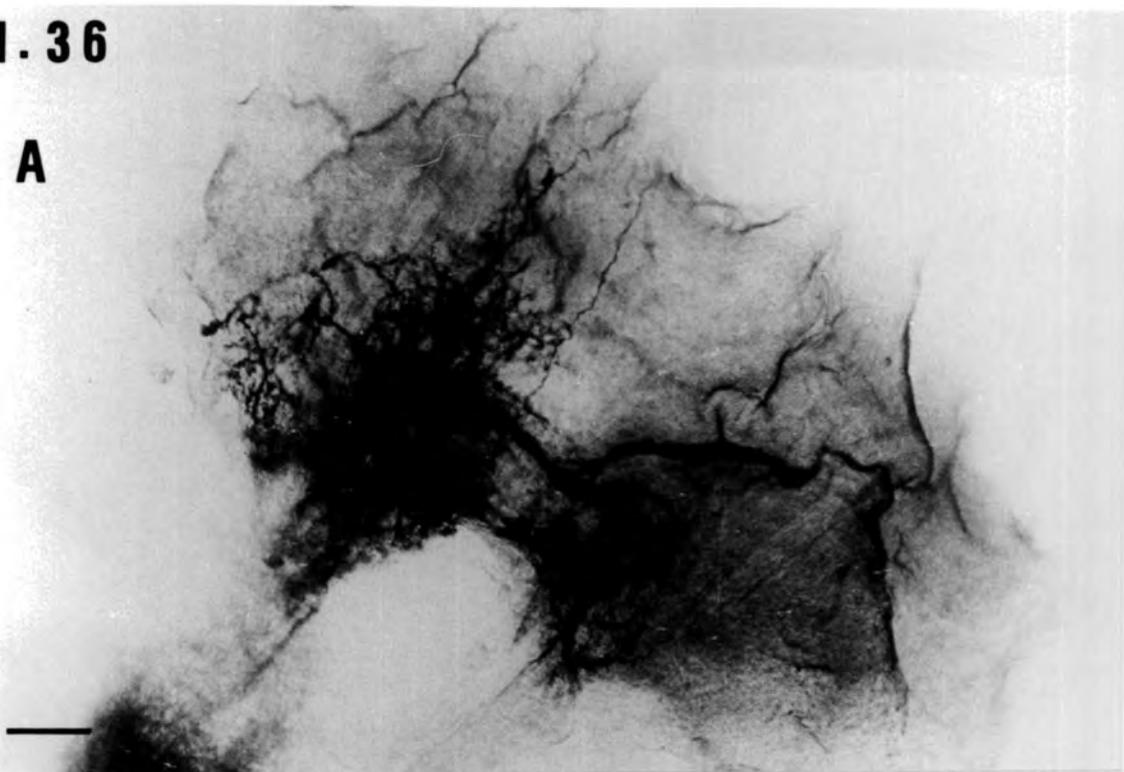
(A) Back-filling through N1D1 showing the arborizations of the four motor neurones in the dorsal region of the mesothoracic ganglion.

(B) Is a ventral view of (A) showing the position of the cell bodies (arrows).

Calibration : 100  $\mu$ m

1.36

A



B



arborize and branch, all conform to the classical description of insect motorneurones (Guthrie 1961); and also with the more modern description by Strausfeld (1976) which is "unless a nerve cell can be visualized from the neuropile to its terminal on muscle, or unless it can be shown up by retrograde dye diffusion of a motor nerve bundle to muscle, the term motorneurone is best not used".

The course of the axons of these four neurones is shown in Figures 1.36; they leave their cell bodies, which lie ventrally, and pass towards the dorsal side of the ganglion, where they branch extensively in the following pattern:

Once the axons pass into the upper half of the ganglion they start giving off branches, sparsely at first, then increasingly as they pass upward until they reach their most extensive branching at the extreme dorsal part of the neuropile (Figure 1.37). Here, the axons give off main branches as follows:

(i) One of the main branches, a neuropilar segment, crosses the dorsal longitudinal mid-line of the ganglion right to the contra-lateral half of the ganglion (Figure 1.38), where it swells more, up to 15  $\mu$ m, and gives off some branches which are much less extensive than those of the ipsilateral and almost confined to the region around the main branch itself (Figure 1.39). In some specimens, this contralateral branch gives off branches that pass posterad into the ipsilateral connective towards the metathoracic ganglion. In locusts, Kendig (1968) and in cricket Gryllus domesticus, Bentley (1969) gave physiological evidence for electrical coupling between

Figure 1.37

A cross section of the mesothoracic ganglion of Gryllus bimaculatus, showing the arborizations of the four motor neurones. It can be seen that the arborizations are confined to the dorsal region of the neuropile; they are almost completely absent near the cell bodies.

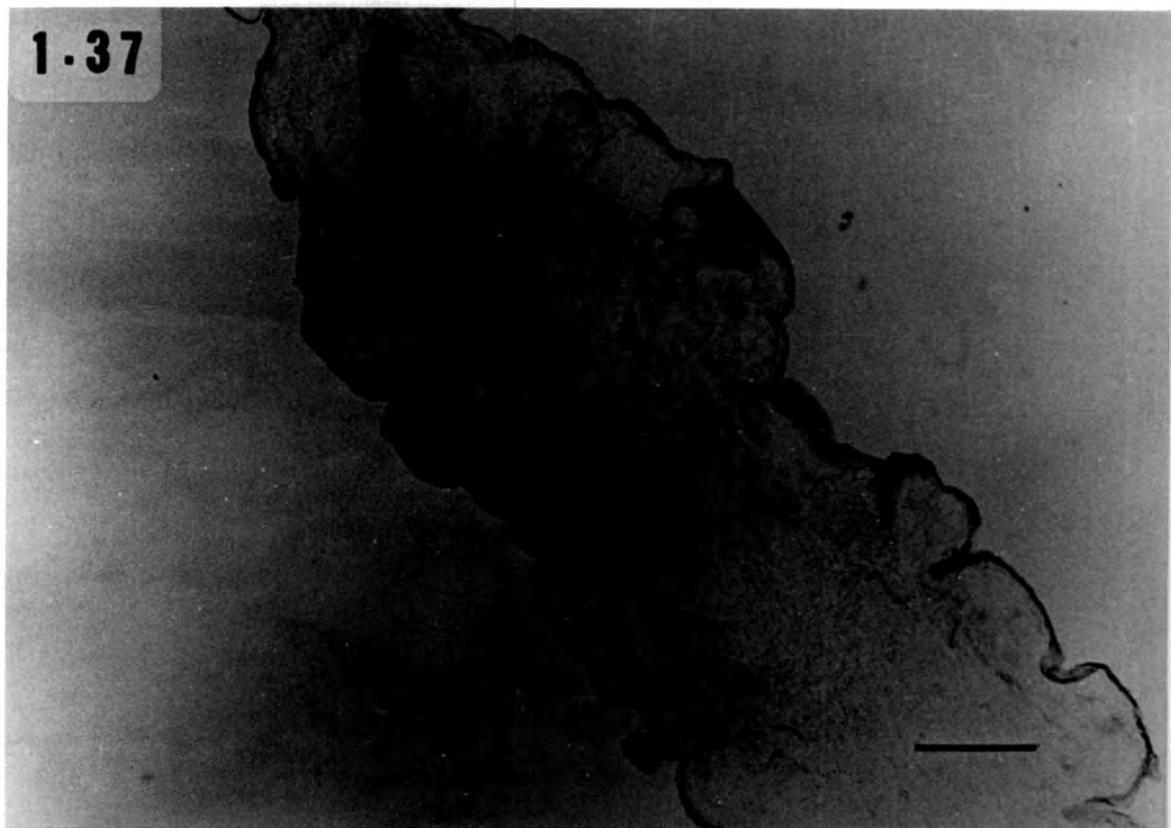
Calibration : 100  $\mu$ m

Figure 1.38

A cross section similar to Figure 1.37 and showing the axonic segment that extends to the contralateral half of the <sup>mesothoracic</sup> ganglion (arrow). It can be seen also that the axon of the cell body remains bare after emerging from it suggesting a kind of layering (see text).

Calibration : 100  $\mu$ m

1.37



1.38

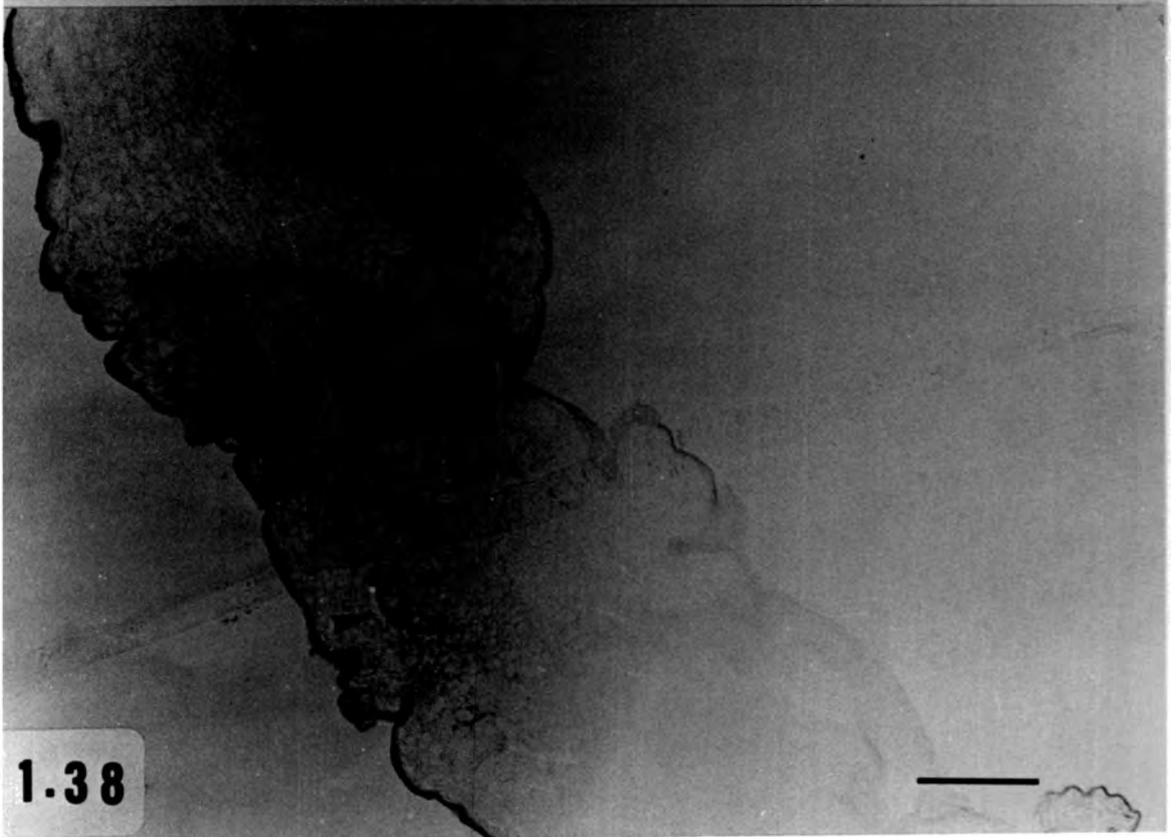


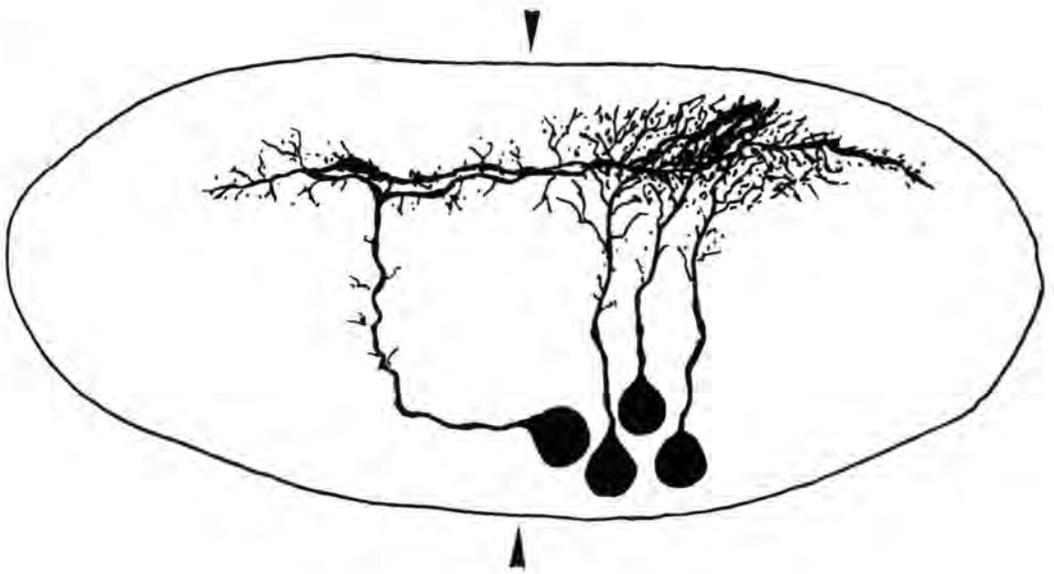
Figure 1.39

(A) A camera lucida reconstruction of the four motor neurones showing the position of the cell bodies and the course of their branching pattern <sup>in the mesothoracic ganglion</sup>. (B) and (C) are two serial sections showing some of the features shown in (A); the piece of axon in (B) indicated by the arrow is a completion of the axon in (C) (arrow). Arrows in (A) indicate, approximately, the mid-line of the ganglion. In all the three figures, the cell bodies are in the ventral region. *Two of the four small cell bodies are seen dorso-laterally in (C) (arrow) (see page 44).*

Calibration : 100  $\mu$ m

1-39

A



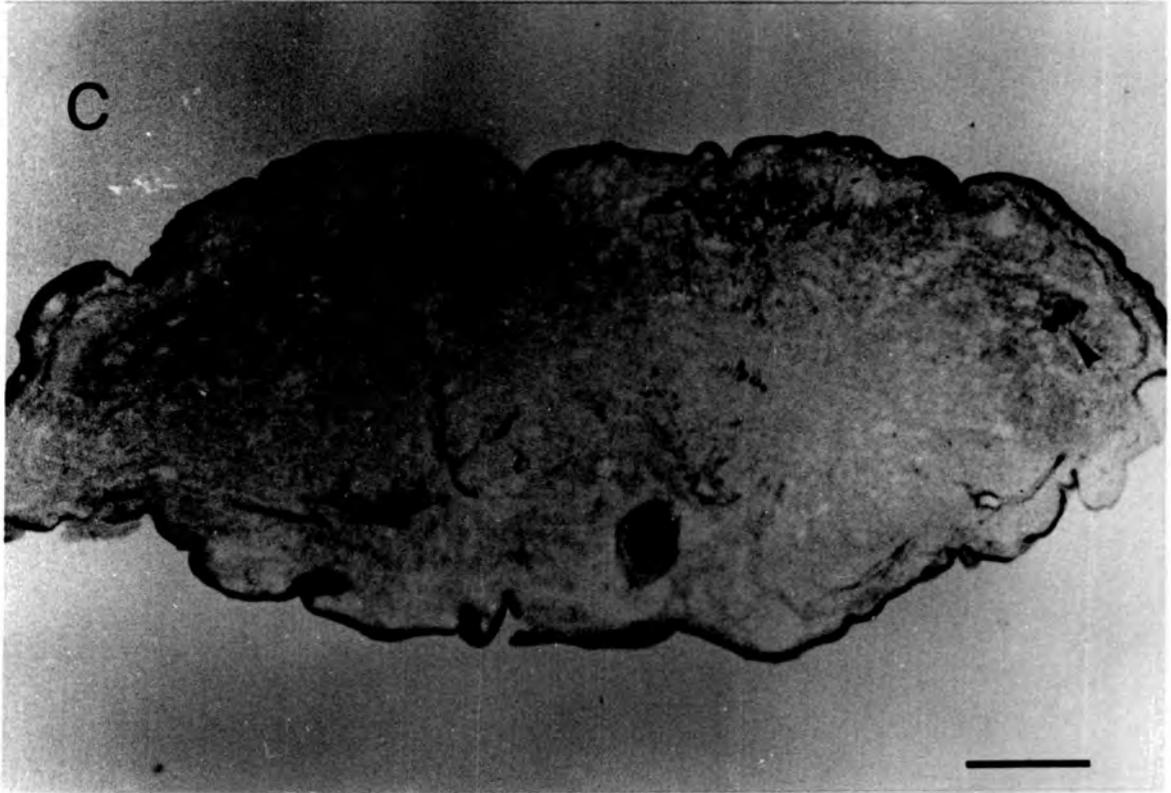
100  $\mu\text{m}$

1.39

B



C



left and right dorsal longitudinal neurones in the mesothoracic ganglion. This could explain the existence of the contralateral branches in Gryllus bimaculatus. Tyrer and Altman (1974), found similar contralateral branches in the mesothoracic <sup>ganglion</sup> of Chortoicetes terminifera when they used bias current to force the  $\text{CoCl}_2$  solution to diffuse through the cut end of the nerve trunk; but they assumed that it may be an artefact resulting from the imposed current.

(ii) Another main branch is extended laterally to the ipsilateral dorsal side of the cell bodies (Figure 1.39); and this also gives off branches mainly directed anterad and lateral.

(iii) The third main branch is in fact a band of primary branches that extend anterad and give off a branching "tree" that extends over almost two thirds of the <sup>width of the</sup> ipsilateral half of the ganglion. Its branches are quite dense (Figures 1.32A, 1.36) above the cell bodies but they get less dense as they progress towards their <sup>terminations</sup> in the anterior part of the ganglion; they become <sup>more superficial</sup> in the neuropile as they progress anteriorly. The density of these branches is so thick that they are indistinguishable from each other (Figure 1.39).

Some of the branches terminate in swellings or knobbed ends (blebs), their diameters ranging from less than a micron up to 5  $\mu\text{m}$ . These swellings presumably represent synaptic areas. Physiological evidence indicates that flight motor neurones have outputs to other neurones in locust (Burrows 1973C) and these synaptic areas could represent this kind of connection. Tyrer and Altman (1976) reconstructed vesicle aggregates from 1  $\mu\text{m}$  thick sections by light microscopy in an

attempt to determine the spatial distribution of synapses onto thoracic motorneurons in locust. They found that there are relatively few on the thicker branches *but* the finer branches have more, suggesting that synapses to the branches may be found mostly on the more distal twigs.

The blebs contain synaptic apparatus in insect brain and optic lobes (Strausfeld 1971), and similar blebs are found in the terminals of the fine branches of flight motor neurons in the locust (Tyrer & Altman 1974); but only electron microscopy can resolve their nature, and this has not been undertaken in the present study. Strausfeld (1976) stated that: "most elements certainly have trees that may be termed dendrites from light microscopy observations, but are in fact both pre- and postsynaptic."

However, there are two striking features about the branching pattern of these neurons:

- (1) The arborizations are confined mainly to a specific depth ( $\approx 50 \mu\text{m}$ ) of the dorsal side of the ganglionic neuropile.
- (2) That the axons after emerging from the cell bodies do not give off any branches and stay bare until they reach the dorsal region, this base length was called "neurite" by Tyrer and Altman (1974) and Strausfeld (1976), and was different from one neuron to another of the four but none of them starts branching in the lower half of the ganglion.

It could be considered that the general configuration of the neurons consists of three layers, although not very obvious ones, namely:

- (a) The cell body layer which occupies a ventral position. It is completely devoid of any branches.
- (b) The central areas of the axons that pass through the central neuropile and which are either bare or sparsely give off short fine branches.
- (c) The arborizations that are confined to the dorsal region of the ganglion, but spread over a large area of it.

The neurite itself is either straight and vertical or sometimes zigzagged; and there are no obvious constrictions along it.

In some preparations, one of the neurites emerges from its cell body ventro-laterally directed towards the middle of the ganglion (Figures 1.39 ) it crosses to the contralateral half of the ganglion, then it turns upwards with a right angle and slightly zigzags before joining the other axons dorsally.

Attempts have been made to inject  $\text{CoCl}_2$  solution into these neurones individually through a microelectrode, but this was not achieved because of the difficulties mentioned above (see Materials and Methods Section).

It was noticed that the axons of stretch receptors of the hind wings pass and branch (although much less extensive than their branching in the metathoracic ganglion) in the same area that the four motor neurones give off their branches (Figure 1.32A) suggesting that direct synapses between the two elements is probable.

(b) The small neurone that is found in <sup>the</sup> vicinity of the four motor neurones in the mesothoracic ganglion : As can be seen in Figure 1.24, this neurone has a small cell body

situated ventrally in the mesothoracic ganglion in the adjacent ipsilateral position of the four motorneurons described above. Its soma is an elongated ovoid shape with length of about 20  $\mu$ m and width of about 10  $\mu$ m; its axon is very thin and seems to disappear a short distance after leaving the cell body; this neurone was found to be filled in most preparations in which filling through  $N_1D_1A$  was carried out.

Examination of the cell body shows that it contains obvious small granules (vesicles) of different sizes which may mean that this cell body is neurosecretory; the granules were black stained with cobalt chloride but this substance is not, of course, a specific dye for neurosecretory contents.

### DISCUSSION

In recent years, the neuroanatomy of arthropods in general and insects in particular, has been advanced considerably because of the use of intracellular staining methods (Stretton and Kravitz, 1968; Pitman et al., 1972) as tools whereby the fine as well as the main branches of neurones can be resolved and visualized in sections and in bulk. The discovery of these new methods of staining does not, of course, ~~detract~~ <sup>from</sup> the importance of the earlier orthodox methods, of staining the neural elements, such as silver nitrate technique and methylene blue. Both methods are, in fact, of considerable value and complement one another and were used in this work.

Both kinds of staining methods were used in studying the metathoracic ganglia of both insects, the stick insect, Carausius morosus, and the cricket Gryllus bimaculatus.

Each of the two methods has its own advantages and disadvantages; for instance it is not possible to stain *only* individual neurones with the silver nitrate technique, but a general idea about the distribution of cell bodies within the ganglion could be obtained by silver staining of serial sections of that ganglion although a functional role for the neurones cannot be concluded reliably from this method. It has the disadvantage of being capricious from one species to another and even the same species on many occasions. By using the newer methods, individual neurones could be stained and ~~they~~ enable determination <sup>of</sup> whether the stained neurone is sensory (afferent) or motor (efferent). Procion yellow was found not to diffuse into fine branches less than 2µm in diameter, a difficulty found by many previous workers

e.g. Tyrer & Altman (1974). Cobalt chloride has the disadvantage of tending to precipitate with some of the components of the cytoplasm such as phosphate and <sup>bi-</sup>carbonate and this resulted in the blocking of the glass micro-electrodes when the substance was injected intracellularly, or in blocking the axons when used for back filling. This was particularly true of the stick insect preparation with which success was limited, no doubt reflecting the fact that the haemolymph of this insect contains <sup>a</sup> somewhat high level of phosphate (Ramsay, 1955; Wood, 1957). On some rare occasions, cobalt sulphide shifted from its original location in sections as previously reported (Strausfeld and Hausen, 1977).

In the present work a general map of the gross anatomical features was reconstructed from serial sections with particular attention being paid to the groupings of cell bodies within the metathoracic ganglia of both insects and eventually a group of neurones that innervate the dorsal longitudinal flight muscles of Gryllus bimaculatus was given further attention by studying their individual morphological features in an attempt to correlate it with subsequent physiological features. Because of the problems encountered with stick insect preparations, this work was finally concentrated on that of the cricket, in which these problems were less acute.

In general, the basic neuroanatomical organization of the metathoracic ganglia in both insects appeared to be consistent from one individual to another; and this was found to be in accordance with the general framework of other insect ganglia

such as the terminal abdominal ganglion of the locust Schistocerca gregaria (Forsk.) (Seabrook, 1968, 1970), the mesothoracic ganglion of the locust Schistocerca gregaria, (Bentley, 1970), the mesothoracic ganglion of Chortiocetes terminifera (Tyrer and Altman, 1974) and the metathoracic and the mesothoracic ganglia, respectively, of the cockroach, Periplaneta americana (Cohen and Jacklet, 1967, Gregory, 1974). These basic features have been summarized by Huber (1974) as follows:

"(a) A fibrous sheath, the neural lamella, built of collagen like material; (b) a cellular layer, the perineurium, situated beneath; (c) the area with the neuron cell bodies encapsulated by glial cell processes. This cortical region with grouped cell bodies of motor and interneurons is rather sharply demarcated from (d), a central complex containing axons and their branching processes, the neuropile."

Most of the ganglion appeared to be occupied by the neuropile, which is a very complex part, consisting of complexly interweaving neural fibres, and little is known about any general organization of its fine details. Most of it is so densely packed with arborizations that single processes cannot even be resolved.

Although the neuropile of the thoracic ganglia contains discrete regions (Gregory 1974) yet it does not resemble that of the brain, which is compartmentalized into special regions that are innervated by characteristic configurations of neurones (Strausfeld 1976). Such specialized innervation has not been found in the thoracic ganglia either by previous workers or in the present study. However, workers on insect nervous systems are in agreement that synaptic contacts as

well as integrating processes occur in the neuropile (e.g. see Huber 1974). This assumption is mainly based on electrostimulation (Huber, 1960; Rowell 1963) and on electrophysiological recording (Hughes, 1965; Hoyle, 1970; Burrows, 1973a,c; Burrows and Rowell 1973). Electron microscopical studies of the neuropile (Smith 1967) showed that most of the fibres and branches are rather closely packed within the neuropile, and fibre membranes are separated by a space of 10-15 nm. Also certain features of organization appear to be shared by chemically mediated central and peripheral synapses - notably close apposition of pre- and post-synaptic membranes across a narrow glial free synaptic gap and the presence of large numbers of synaptic vesicles, within the presynaptic element, characteristically aggregated into clusters alongside the membrane of the axon terminal, (Smith 1967).

Within the neuropile, bundles of fibres that pass through the ganglion can be recognised in stained sections, these were called the "through-tracts" in the locust (Tyrer and Altman 1974) and can be regarded as land-marks within the ganglion. These fibre bundles contains fibres of different diameters, apparently including the well known ones, namely the giant fibres (interneurones), that associate the head ganglia with the last abdominal ganglion. It is through these giant fibres that escape behaviour was thought to be transmitted (Roeder, 1963; Hughes, 1965); however, it was found later that other smaller fibres carry this type of information as well (Iles 1972). In Gryllus bimaculatus, the situation seems similar, for back filling through the connectives

showed that some of the fibres that pass through the meta-thoracic ganglion give off branches within the ganglion, and some of these branches even cross the mid-line to the contralateral half of the ganglion providing links between the two sides. The overall link between the metathoracic ganglion, the brain and the last abdominal ganglion was demonstrated physiologically by intracellular recording from the DUMDL cell body while stimulating sense organs on the head or the cerci (see next Chapter). The fine details of the through-tracts have not been followed in all their detail, but sufficient has been seen to suggest that in general, they appear similar to those of the locust (Tyrrer and Altman 1974) and of the cockroach, Periplaneta americana (Gregory 1974).

The ganglion, like the rest of the insect nervous system, is enveloped with a neural sheath that separates it from the surrounding haemolymph. It was found (Smith and Treherne 1963) that the neural sheath consists of two layers; a non-cellular outer layer, the neural lamella and a cellular underlying one called <sup>the</sup> perilemma. However, more complexity can be seen in the outer layer. Both electron microscopical and X-ray diffraction studies of the neural lamella have shown that this region of the sheath contains collagenous fibres mounted in a non-fibrous matrix at different directions; and the structure is similar in both the locust and the cockroach. It would thus appear that the collagenous material is deployed in a multidirectional meshwork with the long axes of the fibres generally tangential with respect to the ganglion as a whole (Smith and Treherne 1963). It is this structure

that creates so much difficulty for neurophysiologists working with glass microelectrodes. In Musca the depth of the neural lamella is between 60 and 100 nm (Strausfeld 1976). In addition to this barrier, the cells of the perilemma are joined by their adjacent surfaces by septate desmosomes forming a further unicellular sheath that invests the cell body rind (Treherne et al., 1973; Strausfeld, 1976). However, the main bar to microelectrode penetration appears to be the neural lamella, which contains collagenous fibres running in different directions. In the stick insect even the use of enzymes, including collagenase, failed to permit microelectrode penetration, and it may be that in this insect there are differences in structure and/or composition from those of other insect neural lamellae. On the other hand, it was shown by Skaer and Lane (1974) that the neural lamellae of Periplaneta americana and Schistocerca gregaria show a range in depth, being rather thicker around the ganglia of the ventral nerve cord than the interganglionic connectives. Deposits of fat body are also commonly found at intervals along the ventral nerve cord of the cricket; but always forming a continuous circumferential sheath in the stick insect that needed to be removed before penetrating the ganglion with a microelectrode; this fat body sheath was found <sup>to be</sup> much less extensive in the cockroach and the locust than it is in the stick insect (Maddrell and Treherne, 1966; Lane and Treherne 1971; Huddart, 1972; Skaer and Lane 1974). The physiological properties of the sheath were investigated by Wigglesworth (1960) and Lane & Treherne (1972) who suggested that the influx of nutrients from the haemolymph is

mediated by a complex physiological - structural link between the cellular components of the sheath and the glia, and it has been proposed that the tight junctions of the sheath cells impose a selective barrier to the exchange of metabolites and ions between the blood and the underlying ganglion. The non-cellular neural lamella is relatively permeable, even to large molecules such as inulin (Treherne, 1960) dyestuffs (Wigglesworth 1960) and some histological staining solutions, such as methylene blue.

The neurone cell body ring forms an intermittent layer of somata around the neuropile, and this differs from other insects examined, e.g. Guthrie (1961) found that the cell bodies around the ganglia of Gerris form a continuous layer of nearly equal thickness around the neuropile. In both Gryllus bimaculatus and the stick insect, they are found more aggregated in the antero-lateral and postero-lateral positions of the ganglion nearer to the exits of the nerve trunks which are mainly at the lateral sides of the ganglion. However, the general layout of the cell body layer seems similar to those of other ganglia, such as the metathoracic ganglion (Cohen and Jacklet, 1967), mesothoracic ganglion (Young 1969; Gregory, 1974) and prothoracic ganglion (Iles, 1976) of the cockroach Periplaneta americana; and to the mesothoracic ganglion of the locust Schistocerca gregaria (Bentley 1970).

Although the distribution of the cell bodies within the ganglion appears confusing at first sight, it can be fairly classified into positional or geographical groupings. However, using purely morphological criteria for the grouping of cell

bodies proved impossible except for the dorsal median group, (group No. 2). There is no particular size or shape of cell bodies that can be used to distinguish any group of cell bodies from another group; but different sizes and shapes of cells can be seen in all of the positional groups. Functionally, it has been suggested that neurones of cell bodies of diameter less than 20  $\mu\text{m}$  are interneurones (Cohen et al. 1967; Taylor and Truman, 1974; Seigler and Burrows 1979); large cell bodies are often assumed to be motor neurones or neurosecretory. On the other hand, there is a clear neuropilar area that separates the groups situated in the anterior half of the ganglion from those situated in the posterior half. However, despite the similarity in the general plan of the ganglion of both the cricket and the stick insect, there are some differences. Concerning the detailed groupings of cell bodies, both insects have the same geographical groups; but in the ventral region of the metathoracic ganglion of the stick insect appeared two extra small groups (group No. 4) which are, in fact, an extension of group No. 3 on each side, but they appear as distinct small groups (Figure 1.6B). These separate small groups were not detected in the metathoracic ganglion of the cricket. Their occurrence in the stick insect could mean that ~~their~~ members have a special functional role, but more information is needed to be sure about this, such as the organ they innervate. Despite the similarity in the general layout of the other cell bodies of both insects to each other and to that of the cockroach, there are differences in the detailed groupings.

These differences appear clearly in the middle groups that exist along the longitudinal mid-line of both dorsal and ventral sides of the prothoracic ganglion of Periplaneta americana and both Carausius morosus and Gryllus bimaculatus. It was shown by Iles (1976) that there are three distinct groups on the dorsal side found along the longitudinal mid-line, one of them lies in the anterior of the ganglion, another group in the middle and the third in the posterior; and on the ventral side there are only two groups along the ventral longitudinal mid-line. In Gryllus and Carausus the cell bodies on both the dorsal and ventral sides of the meta-thoracic ganglion group together to form only one group (group No. 2, Figure 1.13A,B), though the number of cell bodies on <sup>the</sup> dorsal side, in particular, differs in both animals, being 8 cell bodies in Carausus and up to 18 cell bodies in Gryllus.

On the other hand, as mentioned earlier, there are two small ventral groups in the methathoracic ganglion of Carausus but not in Gryllus. These two small groups could correspond to <sup>two</sup> similarly positioned groups in Periplaneta (Iles 1976, Groups PV3, Figure 26).

The number of motor neurones (neurones with cell bodies over 20  $\mu$ m diameter) in Gryllus is greater, being 215, (Table 3) than it is in Carausus, being 94, (Table 4), and this perhaps is relevant to the fact that Carausus is a wingless animal whose patterns of behaviour are less complicated than those of Gryllus. The total number of Gryllus motorneurones <sup>were</sup> that filled through the peripheral nerves was 77 (Table 3) i.e. 39% of the total number; whereas there were 31 neurones, i.e. 34% filled through the peripheral

nerves of Carausius. These figures of neurones of both animals suggest that there are 138 (215 - 77) in Gryllus and 53 (84 - 31) in Carausius interneurones, and possibly neurosecretory. Also it can be seen from Figures (1.5, 1.15) and tables 3 and 4 that whereas the numbers in the 30 - 50  $\mu$ m range is exactly <sup>that</sup>  $\frac{1}{2}$  in Carausius (42 against 84) there are also 21 neurones larger than this in Gryllus, and none in Carausius; however the largest size of cell body found in Gryllus was 65  $\mu$ m and is only in groups 2d and 1V (table 2). The largest size of cell body in Carausius was 50  $\mu$ m in groups 2d, 1V and 3V. Estimations of motoneurones (neurones with cell bodies over 20  $\mu$ m diameter) of the cockroach were 230 neurones in the metathoracic ganglion (Cohen and Jacklet, 1967), and 300 neurones in the mesothoracic ganglion (Gregory 1974). The number of neurones with cell bodies over 20  $\mu$ m diameter in the cricket (being 215) is close to those of the cockroach, but that of the stick insect (being 84) is much less; this feature may perhaps be related to the general behavioural levels of the two animals. It should be noted that only cell bodies over 20  $\mu$ m diameter were counted in all these estimations; and certainly they include some interneurones and neurosecretory neurones, for filling through the interganglionic connectives revealed some cell bodies that were not filled through the peripheral nerves, yet these were over 20  $\mu$ m in diameter (Figure 1.19). On the other hand, the smaller cell bodies less than 20  $\mu$ m in diameter are many more in number than those of 20  $\mu$ m and over. Estimations of these cell bodies in the metathoracic ganglia of both the cricket and the stick insect were 2200 and 1500 respectively. They are smaller than

those of the metathoracic <sup>ganglion</sup>/of the cockroach which were estimated at around 3400 (Cohen and Jacklet 1967). In addition to the difference in the general behavioural levels, the size of the animal may be relevant as well. However, the small cell bodies, i.e. less than 20  $\mu\text{m}$ , are considered to be mostly glial cells or interneurons. Burrows and Seigler (1979) found that all the interneurons they studied in the metathoracic ganglion of the locust, Schistocerca americana gregaria, were less than 20  $\mu\text{m}$  in diameter. Moreover, the majority of neurons in the nervous systems of vertebrates and invertebrates are interneurons (Pearson 1977).

The distribution patterns of different sizes of cell bodies appeared similar dorsally and ventrally, but with more of all sizes in the ventral regions (Figure 1.5, 1.15) in both insects. This means that most of the motor neurons are situated in the ventral part of the ganglion; and this is supported by the finding that almost all (except a few) of the filled cell bodies through the peripheral nerves are situated in the ventral region. Others have found that motor neurons such as those that control flying and walking all were found in the ventral region (e.g. Bentley, 1970; Burrows, 1973b; Burrows and Hoyle 1973; Fourtner and Pearson 1977).

If the 20 - 30  $\mu\text{m}$  group is mostly motorneurons (Figure 1.15), and there are motorneurons in the other groups, as well as neurosecretory and interneurons, then there are likely to be more motorneurons than interneurons, which

suggests that some of the neuronal systems in the ganglion may be of the "Command neurone" type, i.e. one or a few interneurons that trigger a much larger number of motor neurones to perform a sequential behavioural act (such as walking, flying, feeding- etc.) (e.g. see Kupfermann and Weiss 1978).

The pattern of the size range for the separate groups was similar to that for the whole ganglion (Figures 1.14) except for group No. 2 of both the cricket and the stick insect and group No. 4 of the latter, where there are more of the larger neurones than the smaller ones. This means that, with the possible exception of group No. 2, it is not feasible to distinguish functional groups of neurones in different regions on the basis of size, since the size distribution is comparable over the whole or most of the ganglion. This suggests that cell bodies subserving different functions may be mixed together in the different areas without being geographically grouped in any fine sense.

The medio-dorsal group, group 2d, is rather distinct from the other groups because of some unusual properties. One of the noticeable features of this group is its varying pattern of arrangement of cell bodies both as a group and in their number within the group (Figures 1.17 ). Similar variations of arrangement were also found in other areas, i.e. the four motor neurones that innervate the dorsal longitudinal flight muscles and which are situated in the mesothoracic ganglion. Such variation of the position of cell bodies may perhaps be a more common phenomenon than is

sometimes supposed in different parts of the CNS of insects, but is simply more easily detectable in the smaller groupings of cell bodies. However, it seems that these variations do not affect the functional role of the cell bodies for activity recordings from an identified neurone (DUMDL) of group 2d, were obtained from different preparations, yet they were always virtually similar. Hoyle (1978) showed similar variations in number and arrangement of cell bodies in the corresponding groups of the locust Schistocerca gregaria and the grasshopper, Romalea microptera; he explained this by suggesting that the absent cell bodies could be unusually located forwards or backwards of the main group. This could be the situation in the cricket and the stick insect, but variable degeneration of some neurones is also possible for it was found by Taylor and Truman (1977) that degeneration of neurones during developmental stages takes place and by the adult stage, reduction in the number of neurones by up to 50% can occur. It is clearly possible that either some of the degenerating cell bodies might be retained but inactive, or a varying number of active neurones could survive to maturity. The least number of cell bodies found was eight large ones. The neurones of this group are known to be unpaired (Plotnikova 1969), i.e. each of them send axons to both sides of the insect body; and because of this, they were called "Dorsal unpaired median neurones" (DUM) (Hoyle et al., 1974). The least number of cell bodies found was eight large ones, but a varying number was usual and the upper limit was eighteen cell bodies in the cricket when it was not more

than eight in the stick insect. Different numbers of the cell bodies of the corresponding group of other insects were reported as well. Plotnikova (1969) who first described these neurones as unpaired found nine cell bodies in Schistocerca gregaria; Crossman et al. (1971) reported eight cell bodies on the dorsal region of the metathoracic ganglion of Periplaneta americana, Hoyle (1978) found up to twenty one in Schistocerca gregaria and recently only four unpaired dorsal neurones were found in the lantern ganglion of male fireflies Photuris versicolor (Christensen and Carlson, 1981). It is likely that the "Dorsal unpaired median neurones" (DUM) as were called by Hoyle et al. (1974), exist at different numbers in different ganglia according to the role they perform.

The neurones of this group, namely group 2d, are known to be unpaired, i.e. each of them sends axons to both sides of the insect body (Plotnikova, 1969; Hoyle et al., 1974); but it seems more likely that only some of them, notably the large ones, are unpaired; for dye filling through the connectives of the metathoracic ganglion of Gryllus bimaculatus, revealed some of the smaller cell bodies of this group are paired. On the other hand, the cell bodies of this group appeared to be connected to each other through fine branches (intermingled), for on some occasions more than one cell body were filled over prolonged periods of back-filling through  $N_1$ . This may indicate that they are integrative neurones; but Hoyle and Dagan (1978) found no electrical coupling between similar cell bodies of the Locust and the grasshopper.

Back-filling through  $N_1$  also revealed the other five motorneurons that innervate the dorsal longitudinal flight muscles, four of which are situated in the posterior region of the mesothoracic ganglion, and the fifth in the anterior region of the metathoracic ganglion and contralaterally to  $N_1$  through which it was filled. Neville (1963) demonstrated that the dorsal flight muscles of the locust Schistocerca gregaria, Forskal phasic gregaria are innervated with five motorneurons four of which lie in the mesothoracic ganglion and the fifth lies in the metathoracic ganglion. The morphology of the group of four is conformable with the classical description of motorneurons of insects (Wigglesworth, 1959; Guthrie, 1961) by having their cell bodies situated at the ventral region of the ganglion and their arborizations in the dorsal region. In their studies of the flight motorneurons of the locust Schistocerca gregaria and Chortoicetes terminifera, Bentley (1970) and Tyrer and Altman (1974) respectively, showed the corresponding neurons in the mesothoracic ganglion of these animals, but Bentley (1970) did not show their branches; whereas Tyrer and Altman (1974) did show the arborizations of these neurons. In general, resemblance between the configuration of this group of motorneurons of Gryllus and the similar group of Chortoicetes was found. However, some differences were noticed as well, <sup>and</sup> these authors attributed the apparent branches of the group that cross the middle of the ganglion to the contralateral half of the ganglion to dye leakage and regarded them as artifacts caused by the use of bias

current. In the present study, it was found repeatedly in Gryllus that they are indeed genuine branches (Figure 1.37) and not only appear in the dorsal region, but in the ventral region as well, where on some occasions one of the four axons goes towards the other half of the ganglion after emerging from its cell body; but not more than one such axon was found in the same preparation. However, it should be borne in mind that although both Chortoicetes and Gryllus are orthoptera<sup>e</sup> and winged insects, Gryllus do not fly very much, unlike locusts.

Each of the four neurones has a part of its axon that is almost bare after emerging from the cell body and the branching ramifications appear in the upper half of the ganglion, where branches from the wing sense organs pass through and give off further branches, which again could indicate some sort of synaptic contact between the two neural elements. It was shown physiologically by Burrows (1975a) that the connection between the stretch receptors of the wings and the flight motoneurones is monosynaptic.

However, synapses in invertebrate central nervous systems do not occur on the soma as in vertebrates, but are found on the axons away from the soma (Tyrer and Altman 1976). This results in isolation of the soma from the spike initiation zones in invertebrates (Cohen 1976).

It was noticeable that the neurones studied in this work all of which innervate the dorsal longitudinal flight muscles have their branches mainly in the dorsal area of the ganglia, although some of their cell bodies are ventrally situated and others are dorsal. This could suggest that the dorsal

area of the ganglion is the main region for synaptic contact between these neurones (and probably all flight neurones) and the branches that come from the wing sense organs. This conclusion was made because all the branches of the above mentioned neural elements (efferent and afferent) are found in the same areas within the ganglia. Burrows (1975a) and Altman and Tyrer (1977a,b) showed that the branches of the wing stretch receptors are found mainly on the dorsal areas of both the mesothoracic and metathoracic ganglia of locusts.

On the other hand, the two small cell bodies that dye-filled along with the large ones are of unknown function. The cell in the mesothoracic ganglion contains obvious vesicles, suggesting it may be neurosecretory, and the other in the metathoracic ganglion is of uncomplicated branching pattern. However, it has been reported recently that there are present some sensory neurones with central small cells (Peter Brauning and Reinhold Hustert 1980).

Each of the four motor neurones has a part of its axon that is almost bare after emerging from the cell body, and the branching ramifications appear in the upper half of the ganglion where branches from the ipsilateral hind wing stretch receptor pass through and give off further branches, though they are not as extensive as those given off in the metathoracic ganglion by the same stretch receptor (Fig. 1.32A), which again could indicate some sort of synaptic contact between the two neural elements. Branches from the ipsilateral fore wing stretch receptor were shown in the corresponding area of the mesothoracic ganglia of the locusts Chortoicetes

terminifera (Altman and Tyrer, 1974, 1977a,b) and Schistocerca gregaria (Barrows 1975a). In the present study, no filling was made through  $N_1$  of the mesothoracic ganglion of the cricket Gryllus bimaculatus but similar branches of the fore wing stretch receptors of locusts are most likely to be found in the cricket. This is because great similarity between the branches of the hind wing stretch receptors in both the mesothoracic and metathoracic ganglia of the cricket and locusts was found, though Clark (1976a) reported that the branches of the hind wing stretch receptors in the metathoracic ganglion of Teleogryllus oceanicus were short and did not show similarity to those of the locust shown by Burrows (1975a). However, these stretch receptors were found to control wingbeat frequency (Wilson and Gettrup, 1963; Gettrup, 1963; Burrows, 1975b,c; 1977).

As has been previously mentioned, the cell bodies of the four motor neurones are situated in the ventral region of the mesothoracic ganglion away from their arborizations. Synapses in invertebrate central nervous systems do not occur on the soma as in vertebrates, but are found on the axons away from the soma (Tyrer and Altman, 1976). This results in <sup>particularly in insects</sup> isolation of the soma from the spike initiation zones in <sup>and Crustacea,</sup> invertebrates (Cohen, 1976).

The sixth neurone of the group that innervates the DLM is the contralateral dorsal longitudinal motor neurone "CDLM" (Clark, 1976a) that is situated in the metathoracic ganglion. Its striking properties are: (a) its cell body lies in the anterior connective contralateral to  $N_1$  through

which it sends its axon to the DLM; (b) most of its ramifications are found contralateral to the cell body, in the dorsal area of the ganglion (Fig. 1.32A). So, the cell body appeared isolated inside the anterior connective and away from most of its ramifications. It is not known yet, whether or not, this position of the cell body has any physiological significance. However, Bentley (1970) was unable to excite the DLM of Schistocerca gregaria by stimulating the corresponding cell body of the mesothoracic ganglion. He attributed his failure to the distance of the cell body from the spike initiation zone. However, the position of the cell body, the CDLM, is different <sup>in Gryllus</sup> from that of locusts. Guthrie (1964) found it almost in the middle of the anterior part of the mesothoracic ganglion of Schistocerca gregaria; Bentley (1973) and Clark (1976a) found it more lateral, anteriorly in the ganglion of Teleogryllus oceanicus similar to that found by Tyrer and Altman (1974) in Chortoicetes terminifera, but none of these authors reported it in the connective as was found in Gryllus bimaculatus. The axon of the CDLM of each side of the ganglion is very close to each of the two branches of the DUMDL axon (beyond the bifurcating point) and from which some branches are given off (Figure 1.32A). However, direct contact between them has not been found. But Davis and Alanis (1979) reported that the DUMDL remains inactive during a burst of the CDLM. On the other hand, the arborizations of the CDLM are only dorsal and found in the same area that has extensive sensory branches from the wing stretch receptor suggesting that connection between them may exist.

It was noticeable that the neurones studied in this work, and which innervate the DLM, have their branches mainly in the dorsal area of the ganglion, although some of the cell bodies are ventrally situated and others are dorsal. This could suggest that the dorsal area of the ganglion is the main region for synaptic contact between neurones. This conclusion is supported by the finding that most of the cell bodies of the ganglion as a whole are found in the ventral region, indicating a kind of functional layering. No indication has been found in the course of the present work of any obvious layering in the neuropile.

It was suggested by Bentley (1970) that neurones that innervate the same muscle are grouped together in the ganglion. The conclusion to be drawn from the histological and anatomical work presented here is that very little such grouping of neurones occurs, and this seems to be especially true of the motor neurones, e.g. those that innervate the dorsal longitudinal flight muscle. No doubt this is to some extent a reflection of the fact that all the synaptic contacts occur away from the soma. Such grouping as there may be would be more likely to occur within the neuropile; but the complex and tortuous routes followed by many neurites may suggest that even in the neuropile the anatomical organization is not such that it will be readily worked out by histological methods.

CHAPTER 2 : NEUROPHYSIOLOGY

1. Introduction

The dorsal longitudinal flight muscles of the orthopteroid insects are known to be innervated by five motor-neurones for each side. Four of these neurones are situated ipsilaterally in the mesothoracic ganglion and the fifth is situated contralaterally in the metathoracic ganglion (Nevile, 1963; Guthrie, 1964, Bentley 1970). In addition to these five motorneurones, it was shown later (Bentley, 1973; Altman and Tyrer 1974) that there is a sixth neurone situated in the metathoracic ganglion that innervates both sides of the dorsal longitudinal flight muscles. This neurone is a member of a group (group 2d) of neurones whose cell bodies are situated in the dorsal area of the metathoracic ganglion. The neurones of this group have unusual properties compared with the rest of the neurones of the central nervous system of the insect and are found in all of the thoracic and abdominal ganglia of the nerve cord (Rowell, 1976). These neurones are unpaired; they do not occur as bilaterally symmetrical homologues. Each of these cell bodies gives rise to a single somewhat short neurite that bifurcates into two symmetrical processes each of which goes to the ipsilateral side of the ganglion to exit through peripheral nerve trunks on each side. This group of neurones was described first by Plotnikova (1969) in Locusta migratoria and she called them polyaxonal neurones. Crossman et al. (1971b) showed that the soma of these neurones of Periplaneta americana and Schistocerca gregaria give rise to bilateral axons that exit through most of the nerve trunks of the meta-

thoracic ganglion; but found no sign of axon innervation from these cells to the nerve that goes to the dorsal longitudinal flight muscles and suggested that these neurones may be involved in the co-ordination of leg movements or the maintenance of posture.

Investigations have been carried out by some workers of the electrical and physiological properties of these neurones. One of their striking properties is that they are electrically excitable, the soma of these neurones being capable of carrying large, overshooting action potentials that are of longer duration than is usual in insect neurones (Crossman et al. 1971a, 1972). This excitability was not found in other neurones of insects (Hoyle, 1970; Hoyle and Burrows 1973). Hoyle et al. (1974) described seven such cells from the metathoracic ganglion of Schistocerca and showed that one of them sends axons bilaterally to the fast extensor tibiae muscle of the metathoracic leg, and that the terminal showed an EM profile of large (60 - 170 nm) electrondense vesicles typical of some neurosecretory cells. It was found that this neurone inhibits the slow spontaneous rhythm of contraction and relaxation that is characteristic of this muscle and is itself of unknown function (Hoyle and O'Shea 1974; Hoyle 1974). Because these neurones are unpaired they were called the dorsal unpaired median (DUM) neurones (Hoyle et al. 1974) and that which innervates the fast extensor tibiae muscle was called DUMETI.

From the whole cluster of the (DUM) neurones, the destinations of only two of them are known so far; the

DUMETI and that which goes to the dorsal longitudinal flight muscles, the DUMDL.

Only DUMETI of the locust has been studied in detail, *in papers that have appeared* mostly during the course of the present work (Hoyle et al. 1974; Hoyle 1978; Hoyle and Dagan 1978; Evans and O'Shea 1978; Heitler and Goodman 1978). The DUMDL that innervates the dorsal longitudinal flight muscles (DLM) was less studied in the adult stage. Davis and Alanis (1979) showed some of its functional activities using extracellular recording techniques in Gryllus domesticus; and general properties of the DUM neurones of Schistocerca were presented, again during the course of the present work, by Hoyle and Dagan (1978). It was found that the effect of the DUMETI on the rhythmic contraction and relaxation of the jumping muscle can be mimicked by the application of low concentrations of octopamine to the isolated muscle (Hoyle 1974; Evans and O'Shea 1977, 1978) and the soma of the DUMETI has been shown to contain octopamine (O'Shea and Evans, 1977). Also, there was no mechanical response obtained when the DUMETI was stimulated. This in addition to the finding of neurosecretory vesicles in its axon terminals led to the suggestion that it is a neurosecretory cell body, a suggestion which was expanded to include the whole cluster of the DUM neurones.

On the other hand, the electrical activities of the DUMETI were related to different spike initiation zones (Hoyle and Dagan 1978; Heitler and Goodman 1978). Some electrical and pharmacological properties were suggested for the DUMDL by recording its activity extracellularly (Davis and Alanis 1979).

This work has been carried out as a compliment to the histological information obtained in the first chapter to extend our knowledge about the functional role of the DUMDL of Gryllus bimaculatus. Attempts have been made to find out if there is a link between the DUMDL and some sensory organs such as the Caudal Cerci and those of the head. Also, the effect of direct (electrical) and indirect stimulation on the DUMDL and the dorsal longitudinal flight muscles has been studied. Intracellular recordings were <sup>also</sup> obtained from other neurones that innervate the dorsal muscles.

Pharmacological experiments were carried out using cholinergic drugs to see their effect on the activity of the DUMDL since cholinergic system components are widespread in the CNS of many insects (e.g. Pichon, 1974; Sattle, 1980).

Activities of the other motoneurones that innervate the DLM were also recorded and the possibility of <sup>their inter-</sup>relationships with the DUMDL is discussed.

## II. Materials and Methods

The animal was put in a waxed dissecting plate in the natural position and was immobilized with strips of plasticene around the legs. The wings were removed, and a cut was made along the dorsal longitudinal mid-line from the neck right to the posterior end of the animal. The viscera, fat, connective tissues, and muscles lying over the abdominal nerve cord were removed to expose it. Tracheae were left intact to supply oxygen, and the two flaps of the body were pinned on each side. The metathoracic ganglion was gently lifted and a rigid plastic platform was slipped beneath it either passing between the metathoracic-mesothoracic connectives or from the lateral side. The ganglion was secured on the platform by means of fine pins passing close to it or through the tracheae or connective tissues. The platform was attached to a micromanipulator, so it could be gently and precisely adjusted to the level wanted. The platform provided a solid support from beneath the ganglion during impaling with a microelectrode. Although the cell bodies are invisible, and their position is somewhat variable, experience made it possible to predict the position of the DUMDL which is usually almost in the middle of the dorsal region of the metathoracic ganglion, in line with  $N_5$  of both sides of the ganglion. During the preparation of the ganglion for penetration, it was kept moistened with insect saline. Other insect salines were tried and usually Fielden's (1960) saline was used having the following composition:

7.5g NaCl, 0.1g.KCl, 0.2g.CaCl, 0.2g.NaHCO, distilled H<sub>2</sub>O 1000ml. Its PH was kept at 6.8 using 1.0mM Tris - HCl buffer.

However, when CoCl<sub>2</sub> was used to stain the neurone, saline containing chlorides only was eventually used to avoid blocking of the electrode. Eventually the whole ganglion was flooded with the saline.

A glass microelectrode was attached to a holder and this in turn attached to a micromanipulator that was pushed towards the predicted position of the cell body, and the penetration was carried out blindly. Sometimes many attempts had to be made in order to find the desired cell body. The glass microelectrode was filled with an electrolyte, 2M potassium acetate following Hoyle and Burrows (1973). Potassium chloride was used at the beginning, but its precipitation at the top of the glass tube led to some problems. On some occasions, the glass microelectrode was filled with 4.5% Procion yellow or 5% cobaltous chloride (CoCl<sub>2</sub>). Microelectrodes had a resistance of 20-40 MΩ when filled with potassium chloride or potassium acetate, and 40-60 MΩ when filled with Procion Yellow or Cobaltous Chloride. At the end of the experiment, dye was injected into the cell body electrophoretically through the recording microelectrode by means of a bridge circuit fitted into the amplifier. Procion Yellow was injected by negatively charged direct or pulsed constant current (12nA) and the CoCl<sub>2</sub> was injected by pulses of positively charged current (12nA, 500 m sec. repeated every second). After dye injection was accomplished histological processes were carried out as previously described in the first chapter.

Microelectrodes were connected to a High-Gain differential pre-amplifier through a cathode follower and the neural activity was displayed on a Tektronix dual beam oscilloscope (Type 502A). Recordings of the activity were made by means of a Tektronix oscilloscope camera using a Kodak 249 RAR film. Intracellular injected current was delivered from a Grass 104A stimulator or from a pulse generator. A silver/silver chloride electrode served as an indifferent electrode.

Pharmacological agents were prepared by dissolving them in the insect saline solution.

Air puff stimulation was delivered from mouth or from an aquarium pump and was directed to the cerci or head through a narrow glass tube.

### III. Results

#### A. The dorsal unpaired median neurone innervating the dorsal longitudinal flight muscles (DUMDL):

As has been shown in the first chapter of this work (Figure 1.32A) the DUMDL cell body is situated almost in the middle of the dorsal area of the metathoracic ganglion of the cricket Gryllus bimaculatus, as a member of group No. 2d (the dorsal unpaired median neurones).

Anatomically it has a bifurcating axon that innervates both right and left sides of the dorsal longitudinal flight muscles (DLM).

##### (I) Identification of the DUMDL neurone

###### (i) The Position:

The position of the cell body is slightly variable, but the extent of this variation has not prevented penetration of its soma by microelectrodes on the majority of occasions on which this has been attempted.

###### (ii) Response of DUMDL to electrical orthodromic and Antidromic stimulation:

A direct check on correct penetration was the twitching of the dorsal longitudinal flight muscles of both sides following stimulation of the cell body with depolarizing constant current (3-15 nA for a duration of 0.1 - 500 m sec., although sometimes direct constant current was used for varying periods). A single stimulating pulse of depolarizing current causes a twitch in the dorsal longitudinal flight

muscle; but these twitches disappear with continuous repetition of stimulating pulses when applied for 3-5 minutes. The twitches can be elicited again if the animal is allowed to rest for a period of 15-30 minutes; but stronger current is required and the twitches are less in number than those originally obtained. The times involved make it unlikely that we are dealing with a peripheral fatigue phenomenon. It seems more likely that there is a change in membrane properties at the site of impulse generation of the neurone caused by the artificial stimulation of the soma but since impulse generation occurs away from the soma it is not possible to be specific about this.

The position is further complicated by the fact that this twitch response was not obtained on all the occasions when it was otherwise evident (by allowing its electrical properties to be observed and distinguished from another active cell body) that the soma had been penetrated. This variability of response may reflect differences in physiological condition between animals and/or the neurones involved. It is not known, for example, what effect the synaptic relationships between DUMDL and other neurones might have on impulse generation; however, the resting potential level of this neurone was comparable between different animals, and fluctuations in its level, that might have represented such distant synaptic influences, were never observed. It will presumably not simply reflect damage caused by the microelectrode to the penetrated cell body.

In addition to its response to electrical orthodromic stimulation DUMDL responded to antidromic electrical

stimulation through  $N_1$ . However, DUM neurones in general respond to antidromic stimulation through most of the ganglionic peripheral nerves (Crossman et al., 1972; Hoyle and Dagan, 1978).

(iii) Distinguishing the DUMDL's activities from those of the other DUM neurones:

Successful searching for the DUMDL cell body among the DUM neurones required a large number of penetrations of cell bodies of the DUM group (Group No. 2d, Figure 1.13A).

Penetration of any cell body in this group was indicated by a steady resting potential and/or membrane activity.

Usually two cell bodies of the DUM neurones are found either spontaneously active, or can be relatively easily activated by either direct stimulation of the cell body, by injecting a small amount of depolarizing current (3-15nA); or by external, indirect, stimulation such as air puff on the cerci, gentle tactile stimulation of the abdomen, loud noise or sudden shadow. These two kinds of stimulation, direct and indirect, elicit somewhat different types of response as will be discussed below.

Each of the two cell bodies has its own pattern of activity which makes it distinguishable from the other one as follows:

(a) The first cell body always exhibits the same pattern of spiking (Figure 2.1A), being distinguished by its single spikes with rather pronounced undershoots. The spikes of this cell body usually occur at a regular frequency which is rather low (1-4.5HZ) when spontaneously active, but increasing up to 23HZ (Figure 2.1B) if the animal was excited by direct or indirect stimulation.

Figure 2.1

(A) and (B) Intracellular recordings showing patterns of firing of the DUMETI neurone. (A) spontaneous and (B) induced by air puff stimulation on the caudal cerci.

(C) - (H) Intracellular recordings from the DUMDL showing pattern of firing of this neurone. Irregularity of spiking is shown. The undershoot is absent compared with that of the DUMETI. All recordings are from different preparations.

Voltage calibration, (A) and (C) - (H) 20 mV.

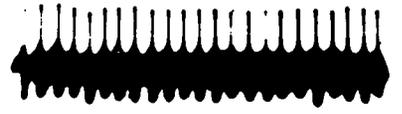
(B) 40 mV. Time calibration (A) and (C) - (H) 400 m sec.

(B) 200 m sec.

2-1



A



B



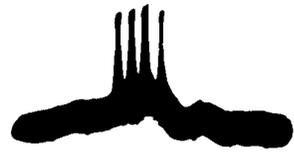
C



D



E



F



G



H



The nature of the activity of this cell body suggests that it may be the corresponding cell body to the DUMETI neurone that innervates the extensor tibiae (the jumping muscle) of the hind legs of the locust Schistocerca gregoria and which has received a good deal of investigation from many workers (see Introduction). This conclusion arises from the fact that there are common properties between these two neurones in the cricket and the locust, as follows:

- (i) Both are members of the DUM group of neurones of the metathoracic ganglion.
- (ii) The low rate of frequency, in Cricket 1-4HZ; and in locust from 0.5HZ (Heitler & Goodman 1978) to 1-3HZ (Hoyle and Duggan 1978).
- (iii) The spikes are usually single; doublets were seen during the firing of this cell body only on a very few occasions.
- (iv) The spikes of this cell body have a pronounced undershoot.
- (v) It can be stimulated through N<sub>5</sub> (the nerve to the leg).

(b) The second active cell body has its own different pattern of spiking. This pattern is distinguishable by spikes that usually appear in bursts of spikes, each consisting of 2-5 spikes; however, single spikes were seen occasionally but could be preceded or followed by bursts of doublets, triplets or more (Figure 2.1 C-H).

The firing frequency is thus irregular, at 1-30 per minute. Regular firing was found in some preparations but

even here the frequency varies from one individual to another. It has no after-potential or a very small one compared with that of the first cell body, the DUMETI. On the other hand, twitching in the DLM was produced when this cell body was stimulated by depolarizing current and the cell body itself could be stimulated through  $N_1D_1$ . So it was concluded that this is the cell body which innervates the DLM (Bentley, 1973) and which was termed the "DUMDL" (Hoyle and Dagan, 1978) in locusts.

(ii) The electrical properties of the DUMDL

The cell body has a resting potential ranging from 45-55 mV in different individuals, being negative with respect to the outside of the ganglion. The differences may reflect the physiological condition of the animals. Overshooting action potentials were obtained in about 50% of the <sup>preparations studied,</sup> sometimes induced and at other times spontaneous, reaching up to 90 mv in size (Figure 2.2 A-D) with a duration ranging from 20-50 ms (it is noticeable that the spontaneous ones have a longer duration). The action potential has either no undershoot or rather small undershoot (compared with that of the DUMETI); though it reached nearly 7 mV on some occasions (Figure 2.2A).

Bursts of action potentials as groups of spikes is a common feature of the DUMDL neurone and there was no preparation without this kind of activity. The number of spikes found in each burst varied between one to five spikes per burst when the cell body was spontaneously firing (Figure 2.1 C-H) but it could be increased by stimulation such as gentle tactile stimulus or air puff; or by chemical stimulation by applying Acetylcholine (ACh) which increased it to more than twenty spikes per burst (see section on effect of ACh). During the firing of a burst of action

Figure 2.2

(A) - (D) Intracellular recording from the DUMDL showing overshooting action potentials from different preparations. (A) A sudden brief tactile stimulus to one of the caudal cerci initiated an overshooting action potential. (B) and (C) are spontaneous action potentials. It can be seen in (A) and (B) that there is an initial slow rise in membrane potential before the spike fires. (D) Is an action potential initiated in response to an intracellular stimulus (10 nA, 20 m sec. duration). (E) Intracellular recording from the DUMDL showing spontaneous spikes with after potentials that were abolished by applying direct depolarizing current (arrow) and which also increased the spike frequency.

Voltage calibration : (A) - (D), 40 mV. (E) 20 mV.

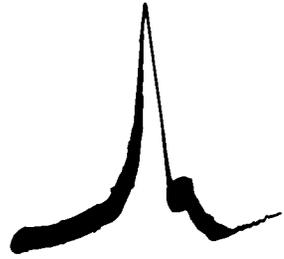
Time calibration : (A) 40 m sec; (B) - (D) 100 m sec.

(E) 200 m sec.

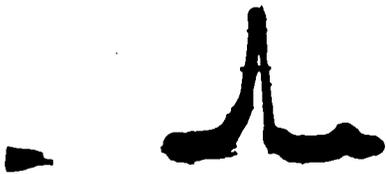
2.2



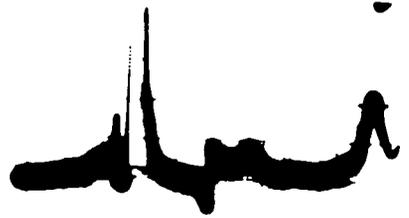
A



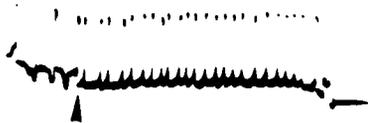
B



C



D



E



potentials, the underlying membrane potential shifts towards depolarization (e.g. Figure 2.1 E-H). This kind of firing was never seen in the other cell body, the supposed DUMETI neurone.

(III) Synaptic input and spontaneous activity of DUMDL

As stated above, the DUMDL was found spontaneously firing on some occasions. When such activity occurred it lasted the whole life of the preparation (up to two hours). This spontaneous activity had an irregular frequency of 1-30 per minute. If the animal was excited by directly injected depolarizing current (3-15 nA) into the soma, or indirectly by gentle tactile stimulation or air puff on to the caudal cerci, the frequency increased up to 16 HZ (Figure 2.2 E is an example).

The neurone receives a continuous bombardment of both inhibitory post synaptic potentials (ipsp's) and excitatory post synaptic potentials (epsp's) which are clearly visible <sup>in the cell body</sup> when spontaneous activity is not present (Figure 2.3); the ipsp's usually being common in the unexcited animal, and sometimes ipsp's and epsp's were produced by the same DUMDL alternatively (Figure 2.3 F). The epsp's sometimes give rise to an action potential or a burst of action potentials.

Both ipsp's and epsp's were at varying amplitudes, indicating the input of many synaptic sites to the neurone at varying distances from the soma. Reference to the morphology of the neurone (first chapter Figure 1.30) shows that some projections, spiny and swollen ended, emerge from the main axon at relatively short distances from the soma and these

Figure 2.3

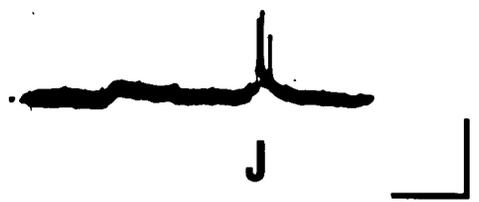
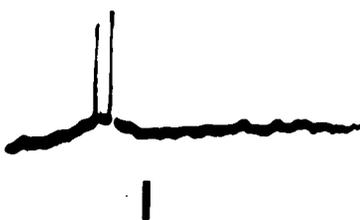
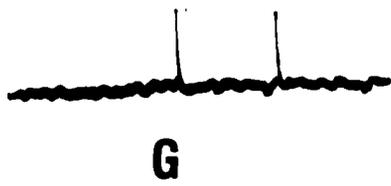
Intracellular recordings showing different kinds of both ipsp's and epsp's : (A), (B) ipsp's, (C) epsp's, (D) and (F) show *both* ipsp's and epsp's. (E) *two examples of* epsp's which occur at regular intervals. (G) - (J) show epsp's give rise to different numbers of spikes in each burst.

Voltage calibration : (A) - (E) and (G) - (J), 20 mV (F) 10 mV

Time calibration : (A) - (E) and (G) - (J) 400 m sec.

(F) 200 m sec.

2-3



may represent synaptic contacts made with other neurones. In view of their promixity to the soma, it is likely that synaptic activity in this region would be recorded in the soma. Also from the first section of this work, it is noticeable that there are many afferent elements that terminate or branch and pass close to the axon of the DUMDL and its branches (Figure 1.32A).

#### IV. Functional properties

Direct and microscopical observations were made of the DLM of Gryllus bimaculatus in most of the preparations to see whether there were any contractions similar to the "intrinsic rhythmic contractions" found in the locust jumping muscles. This rhythmic contraction of the locust jumping muscles was found by other workers to be abolished by artificial saline and reduced in frequency and amplitude or completely inhibited when the DUMETI was stimulated (Hoyle, 1974; Evans and O'Shea, 1978). Nothing similar was observed in the cricket's DLM although the observations were made both before and after the addition of saline. However, on some occasions some contraction of the DLM were observed during electrical stimulation of the DUMDL and the four motor neurones of the mesothoracic ganglion, although these were not always obtained.

It was suggested by Davis and Alanis (1979) that the DLM of Gryllus domesticus are bifunctional, serving both in flight and in more generalized intersegmental movements like ventilation. Coincidence of firing of the DUMDL, of Gryllus bimaculatus, with the respiratory movements of the body was noticed on some occasions; but it did not last a whole life of a preparation neither was it found in every one.

V. Effect of decapitation and deafferentiation:

When the animal was left intact and an air current was applied on its head, the activity in the DUMDL cell body was either completely suppressed or slowed down very much with little shifting of the membrane potential towards hyperpolarization (Figure 2.4A).

On the other hand, the DUMDL of headless and legless animals tended to be hyperactive (Figure 2.4 B-E). This indicates that the DUMDL is under inhibitory control by neurones in one or both of the head ganglia. It was shown histologically (First Chapter; Fig. 1.11) that axons pass through the metathoracic ganglion to the anterior and posterior parts of the animal; some of these axons give branches in the metathoracic ganglion and some others terminate into it, and Huber (1965) found that inhibition of kicking in Gryllus campestris was caused by stimulating the loci in the head and that neurones which seem to be involved in suppressing the cercal response could be localized within the glomerular region and the lobe system of the mushroom bodies which also control the sound production.

VI. The responsiveness of the DUMDL to the indirect stimulation

Crickets, like many other insects, exhibit evasive responses. That the head has an inhibitory influence on DUMDL has already been established and it seems likely that this is related to such evasive responses. It was therefore decided to stimulate the animal under experiment in another way which might mimic natural stimuli that could produce such evasive responses, while recording from the DUMDL cell body.

Figure 2.4

Intracellular recordings from the DUMDL. (A) Regular bursts of spikes; the one that should occur between the two arrows was suppressed by applying air current on the head. (B) - (D) examples of a hyperactive DUMDL of a decapitated animal; the activity continues for up to 2 hours and on some occasions for the whole life of the preparation and towards the end of the preparation the amplitude of the spikes becomes low (E). (D+E from same preparation).

Voltage calibration : 40 mV.

Time calibration : 400 m sec.

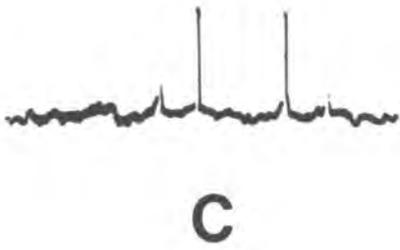
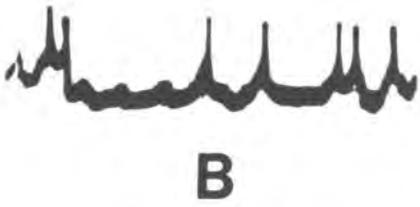


TABLE 2.1

Frequency of stimulus (seconds)	Period in which habitation occur (mean)	S.D.
5	25 sec.	3.6
15	1.5 min.	0.5
30	6.5 min.	1.5
60	15 min.	2.8

The effect of air jet (approximately 1 second duration) on the activity of the DUMDL. The air jet was applied on the caudal cerci. The mean of the time from 5 preparations. Repetition of stimulus resulted in lowering the amplitude of spikes as well as their frequency on some occasions (e.g. Figure 2.5E).

min = minutes

sec = seconds

S.D. = standard deviation

By analogy with the well-known response to air movements in the anal cerci of the cockroach. The animal was excited through the caudal cerci, by directing air puffs onto them through a narrow glass tube.

In crickets and cockroaches, the evasive response is mediated by cercal sensory neurones, giant fibres in the abdominal connectives, and thoracic motor neurones (Roeder 1963; Huber 1965). Palka (1979) stated that "the abdominal cerci of orthopteroid insects are purely sensory appendages arising from the 10th abdominal segment, and they are regarded as major sense organs that provide input to the largest interneurones in the central nervous system". The stimulus applied to the cerci was either continuous or interrupted. The response to this kind of stimulation was found to be optimal when it was applied to an animal that had remained undisturbed for at least 15 min. Examples of the activity recorded are shown in Figure 2.5 A-F. It will be seen that the effect of the air puff is to excite the neurone.

If the air puff was applied continuously, the response recorded in the DUMDL cell body stopped completely within half a minute, that is the animal became habituated to the stimulus. The stoppage usually was preceded with few changes in the activity before the final disappearance (Figure 2.5 E,F). The longer the intervals and shorter the duration of the stimulus, the more slowly did habituation occur (Table 2.1). Repetition of this stimulus resulted in lowering the amplitude of spikes as well as the number of spikes on some occasions (Figure 2.5E). Habituation lasted from 15 minutes up to 40 minutes.

Figure 2.5

(A) - (D) Intracellular recordings from the DUMDL in response to air puff on the caudal cerci of different animals.

(E) same preparation as (D) but after 20 minutes. Some of the preparations respond with long duration spikes after repetition of stimulus (F); and repetition of stimulus for longer time resulted in habituation to stimulus, (G) represents a refractory period of a DUMDL. (H) and (I) showing activity recorded from the DUMDL but unaffected by indirect stimulation (see text).

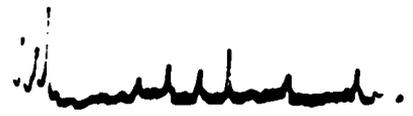
Voltage calibration : (A) - (F) 40 mV; (G) - (I) 20 mV.

Time calibration : 400 m sec.

2.5



A



B



C



D



E



F



G



H



I



Removal of one of the cerci and stimulation of the remaining cercus produced similar results to stimulation of both, but with less amplitude, suggesting that each of the cerci has its own pathway to the cell body, and that the amplitude of the activities which is recorded in the DUMDL cell body as responses to stimulation of both cerci is probably the summation of the response due to each pathway (Figure 2.6 A,B).

If the stimulus was applied while the DUMDL was spontaneously firing, the frequency increased (Figure 2.6 C,D), but if the stimulus was repeated continuously, the firing sometimes stopped for a while and then resumed spontaneously, and at varying amplitudes (Figure 2.5 H,I) and further stimulation to the cerci does not affect the firing, indicating that the spontaneous activity is due to inputs coming from other sources and that the site of the habituation previously observed is likely to be the synapses in the cercal pathway. This conclusion is supported both by the behaviour of habituation observed in the cricket and other insects and the physiological work on similar systems in other animals. For example, in the crayfish it was found that the escape reaction (tail flip) soon habituated upon repetitive stimulation. Zucker (1972b) reported that the synaptic sites between tactile afferents and non-giant interneurone are very vulnerable to habituation upon repetitive stimulation. This habituation was demonstrated to be caused by a powerful inhibitory effect of these synapses as a result of activity in the lateral giant neurone by Krasne and Bryan (1973), who suggested that the

Figure 2.6

Intracellular recordings from DUMDL :

(A) A response to air puff on both cerci while they are intact.

(B) response to air puff on one cerca while the other was removed.

(C) Another preparation in which DUMDL shows single spikes whose frequency increased (D) in response to cercal stimulation (continuous puff)

Voltage calibration : 40 mV.

Time calibration 400 m sec.

2.6



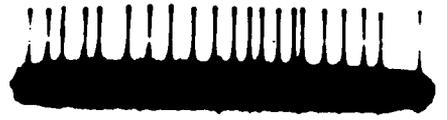
A



B



C



D



inhibition was exerted against the sensory terminals themselves, reducing the production of transmitter liberated by the activity evoked by the tail flip. Kennedy, Calabrese, and Wine (1974) demonstrated that the site of inhibition is indeed presynaptic, by recording with microelectrodes from the afferent terminals themselves.

In the cricket Murphy and Palka (1974) have shown that there is a central inhibition of the synapses made by the sensory receptor of the cerci in the last abdominal ganglion when the animal engages in spontaneous walking activity; this inhibition is reported to come from the metathoracic ganglion, for it was abolished by transection of the nerve cord at any point between the metathoracic ganglion and the last abdominal ganglion, but it was not abolished by transection of the connectives anterior to the metathoracic ganglion.

#### VII. Effect of the direct stimulation on DUMDL

As mentioned above, the DUMDL cell body is excitable and responds directly to a stimulating depolarizing current in most cases.

The application of depolarizing current during the firing of the cell body increases the frequency of the firing (Figure 2.2 E). If the cell body was depolarized while receiving inhibitory post-synaptic potentials, the amplitude of the i.p.s.p.'s increased. *This*

— conforms with the Hodgkin-Huxley model.

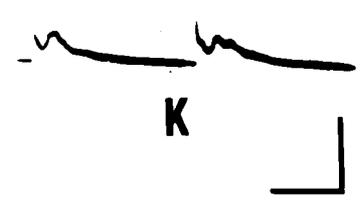
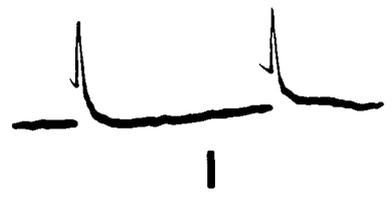
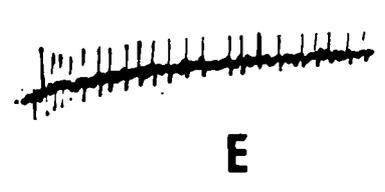
On the other hand, hyperpolarizing current suppresses or slows down the spontaneous activity of the cell body. If the hyperpolarizing current was stopped after being injected

Figure 2.7

(D) the firing was inhibited by hyperpolarizing current; and after the ceasing applying current, the cell body resumed firing with higher frequency which returns to its original one gradually; also it developed after potential which disappears gradually as well. (F) - (K) show the effect of direct stimulation. (see text). In (H) - (K) the response is preceded by a stimulus artefact.

voltage calibration : (D) - (K) 40 mV.

Time calibration : ~~(A) 200 m sec.~~ (B) - (K) 400 m sec.



into a firing cell body, immediately after cessation of the applied current, the cell was seen to fire at a higher frequency than originally with undershoots that progressively disappear and the firing soon slowed down to its former level. This is an example of the well-known phenomenon of "post-inhibitory rebound" (Figure 2.7E).

Stimulation of a non-spontaneously active DUMDL soma, directly with a depolarizing current produced two kinds of response:

(i) In most preparations usually there was not a one-to-one spike response to each stimulus; but when a depolarizing current with amplitude of 3-15 nA (varying from one preparation to another) and for a short duration ranging between 1-20 ms, was injected into the soma, it triggered it to spike, this spiking on some occasions continued for a long time until the preparation as a whole finally failed to function, i.e. up to two hours. On other occasions it produced only a few spikes.

(ii) The second kind of response is the one-to-one response to each stimulus: this kind is rare, but was obtained on some occasions. Figure 2.7 shows different stages of this kind of response. In Figure 2.7F the cell body is showing post-synaptic activity. 3nA depolarizing current of 5ms duration did not evoke <sup>a</sup> response (Figure 2.7G) but by increasing the amplitude of the injected current up to 5nA, a sign of <sup>a</sup> response appeared (Figure 2.7H); and further increase in the injected depolarizing current up to 9nA produced a clear response of nearly 40mV (Figure 2.7I).

On other occasion increase in the amplitude of the injected depolarizing current did not result in a bigger response, but it produced a double component response (Figure 2.7J,K). Double component spikes were frequently seen during activity of DUMDL.

#### VIII. Effect of Acetylcholine and eserine on DUMDL

Acetylcholine is one of the commonest neurotransmitter substances in animals including insects.

Attempts were therefore made to study the effect of Acetylcholine (ACh) on the DUMDL cell body while recording intracellularly from it. Different concentrations ranging from  $10^{-6}$  M ACh were tried to determine the threshold concentration by flooding the metathoracic ganglion overall with the solution under test while the ganglionic sheath was left intact. The threshold concentration was found to be between  $10^{-3}$  M- $10^{-2}$  M; within this range, ACh produced an increase in both the frequency and the amplitude of spikes (see Table 2.2). Table 2.2 shows the effect of ACh at concentrations of  $10^{-3}$  M and  $10^{-2}$  M on the activity of DUMDL cell body. It had a resting potential of nearly 55mV, and was not spontaneously active but shows post-synaptic activity. It was triggered to spike by a gentle tactile stimulus applied to the Caudal cerci. The spiking was typical of the manner in which the DUMDL usually fires and which was found, in this preparation, as regular bursts of firing (Figure 2.8 A,B) with little depolarization of the background membrane potential during bursts, followed by a period of inhibition.

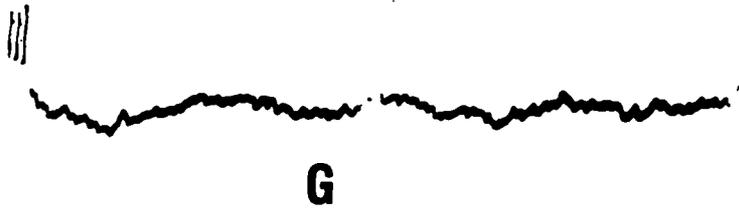
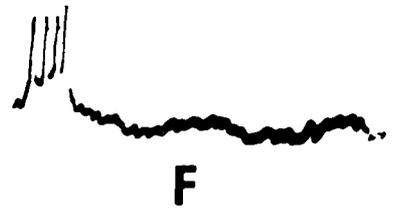
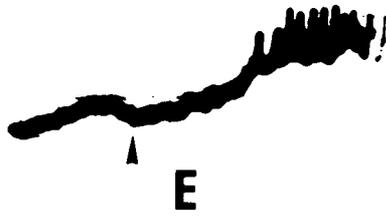
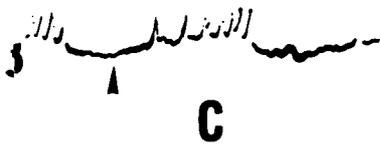
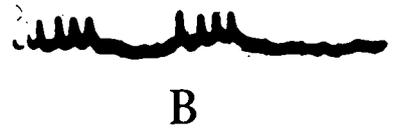
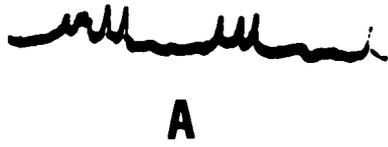
Figure 2.8

Intracellular recording from DUMDL to show the effect of ACh on its activity. (A) and (B) Regular firing of bursts triggered by indirect stimulation. (C) Increase in number of spikes after applying ACh at  $10^{-3}_M$  concentration. (D) - (H) Successive traces to show the effect of  $10^{-2}_M$  ACh; (arrows in (C) and (E) mark the start of the depolarization). The burst sometimes failed to appear as in (G) but was followed by a longer one. (All from same preparation).

Voltage calibration : 40 mV.

Time calibration : 200 m sec.

2.8



Characteristics of the firing in normal saline are shown in Table 2.2. The action of ACh at a concentration of  $10^{-3}M$  is shown in Figure 2.8C and Table 2.2. As can be seen, the effect of  $10^{-3}M$  ACh is small compared with that of  $10^{-2}M$  (Figure 2.8 D-H and Table 2.2). The increase in the depolarization of the membrane potential during the firing is small at  $10^{-3}M$  whereas it becomes more than double at  $10^{-2}M$ . It can also be seen that this depolarization takes place gradually (e.g. Figure 2.8 C,E) until it reaches a threshold at which spikes appear. The depolarization then continues during the burst duration (e.g. Figure 2.8 D,F) until it is suddenly suppressed. This strong suppression suggests that the DUMDL cell body is under the influence of strong inhibition. It was noted also that the number of spikes in each burst increased up to two and a half times at  $10^{-3}M$ , but increased up to nearly eight times at  $10^{-2}M$ ; this increase in number of spikes was accompanied by doubling of amplitude at  $10^{-2}M$  but not at  $10^{-3}M$ , and a decrease in duration to about a third at  $10^{-2}M$ . The intervals between spikes increased at  $10^{-3}M$ ; but the effect at  $10^{-2}M$  was variable, the interval decreased to half between some and increased to double between others at  $10^{-2}M$ . Changes in the burst duration took place as well, it was increased by about 100ms at  $10^{-3}M$  and by about 1300ms at  $10^{-2}M$  from time to time. The intervals between bursts increased only at  $10^{-2}$  by nearly 1300 ms. Thus it was found that the effect of ACh at  $10^{-2}M$  was generally greater compared with its effect at  $10^{-3}M$ . These are relatively high concentrations to use, but it must be remembered that the ganglion sheath is left intact.



Figure 2.9

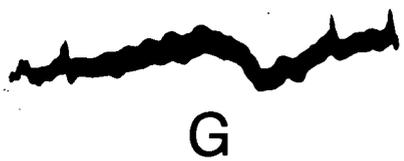
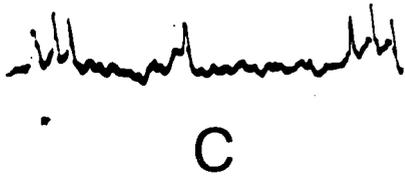
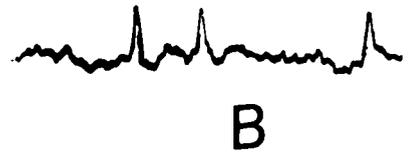
Intracellular recordings from the DUMDL showing the effect of eserine. (A) The cell body was showing epsp's before applying the drug. (B) and (C) After applying eserine at  $10^{-5}_M$  concentration.

(F) and (G) showing the effect of  $Ca^{++}$  free saline containing 20 mM  $Mg^{++}$  (arrow).

Voltage calibration : (A) - (C) 20 mV; (D) - (G) 40 mV.

Time calibration : 400 m sec.

2.9



of eserine at  $10^{-5}$ M resembles that of ACh at  $10^{-3}$ M. On the other hand, concentrations higher than  $10^{-5}$ M of eserine were found to block the activity altogether. The block was reversed on washing with saline. This is because eserine is one of the nicotinoids (Leake and Walker 1980). They bear a close relationship to ACh, and bind to cholinoreceptors causing depolarization and excitation and at high concentrations this leads to depolarization block (Eldefrawi et al., 1970).

Pretreatment of the ganglion with eserine  $10^{-6}$ M -  $10^{-5}$ M lowered the threshold concentration of ACh to  $10^{-4}$ M. This was observed on many preparations before and after the treatment. The treatment usually preceded the application of ACh by 5-10 minutes.

On the other hand, the effect of a calcium-free saline with the relatively high concentration of 20 mM  $Mg^{++}$ , resulted in a progressive decrease in the activity of the DUMDL, which finally disappeared (Figure 2.9 F,G). This was tried to show that transmission is chemically mediated for it is known that saline lacking  $Ca^{++}$  and high in  $Mg^{++}$ , blocks <sup>Central</sup> chemical synaptic transmission in insects.

B. The four motorneurones that innervate the DLM and which are situated in the mesothoracic ganglion.

As has been shown in the first chapter of this work, the cell bodies of these neurones are situated in the posterior ventrolateral part in either side of the mesothoracic ganglion. Because of the variable arrangement of their position, and since intracellular stimulation does not evoke contraction always in the DLM, it was impossible to recognize each of

them <sup>as having</sup> unique characteristics. However, intracellularly recorded activity from different preparations showed some common properties such as response to stimulation of  $N_1D$ , rather similar resting potentials, lack of large action potentials, occasional twitches in the DLM when the impaled cell body was intracellularly stimulated, and some similarity in the pattern of firing (e.g. Figure 2.10) as well as their position (see Figure 1.32 A, first chapter).

The cell bodies have a resting potential ranging from 50 - 60 mV in different individuals, being negative with respect to the outside of the ganglion; and unlike the DUMDL overshooting action potentials were never obtained from these neurones.

Post synaptic potentials were much less common than those of the DUMDL and mostly are excitatory (epsp's), but ipsp's were also seen but less frequently. This suggests that these neurones may not be involved in integrating events.

Stimulating the impaled soma with depolarizing current usually produces single spikes (Figure 2.10 A,B) of which the biggest amplitude observed was around 40 mV (Figure 2.10 C). Most of the responses were below threshold, though twitches in the DLM were seen on some occasions when depolarizing current was applied.

On the other hand, <sup>the</sup> response of these cell bodies to indirect stimulation appeared different from the response of the DUMDL to this sort of stimulation. Figure 2.10 D-J show their response to air puff on the head and on the cerci. The response to air puff was found more frequently in animals whose

Figure 2.10

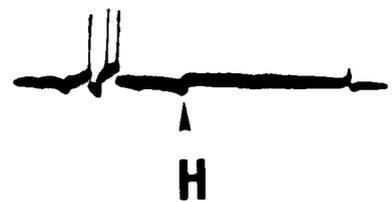
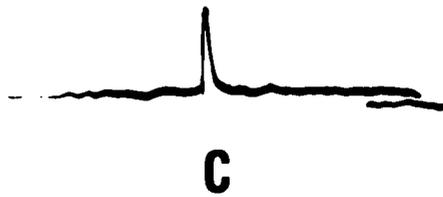
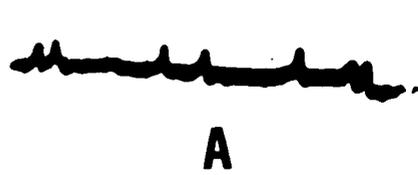
Intracellular recordings from the four motoneurons of the mesothoracic ganglion.

(A) The impaled cell body was stimulated with direct depolarizing current (3nA). The activity frequency was increased (B) when the strength of the injected current was increased to (9nA). (C) From another preparation at which 3nA direct current was injected. (D) - (F) show flight patterns, a burst of 3 spikes (D), (F) or 5 spikes (E) followed by strong inhibition (arrows). [(D), (F) from the same preparation, (E) from another preparation]. (G) show response to air current on the head from another preparation, some of the spikes start with a regular sharp increase in the depolarization of the membrane potential (e.g. the first spike) until the spike fires. Inhibition is still seen (arrow) *delaying* some spikes (e.g. the inhibitions follow the arrow). (H) is another preparation at which an inhibition (arrow) similar to that of (G) followed with no spikes. (I) another preparation at which the inhibition is weak in preventing spikes, but air puff on the cerci stopped them (J).

Voltage calibration : (A) - (C) 40 mV; (D) - (J) 20 mV.

Time calibration : (A) - (D) and (G) - (J) 400 m sec.

(E), (F) 200 m sec.



legs were removed. This perhaps is because of the removing of inhibitory influences coming from the leg sense organs, the tarsi in particular. Applying air current on the head produced flight pattern activity similar to that found by Bentley (1969) and which showed progressive increase in depolarization followed by strong inhibition (Figure 2.10 D-F) and each burst consists of 3-5 spikes. On other occasions, air current on the head produced single spikes but strong inhibition still can be seen after each three or two spikes (Figure 2.10 G) and sometimes the spikes stopped completely following this inhibition (Figure 2.10 H). On the other hand, air puff on the cerci inhibited the activity (Fig. 2.10 I,J); though, it produced few spikes <sup>and only</sup> on rare occasions.

C. The contralateral dorsal motor neurone (CDLM)

As has been shown in the first chapter (e.g. Figure 1.32 A) the CDLM, that also innervates the DLM, is unusually situated in the connective of either side close to the metathoracic ganglion. Several attempts were made to penetrate its soma but the mechanical resistance of this area was much greater than other parts of the ganglion. Figure 2.11 shows some of the activity recorded from it.

It is impossible to relate this activity to any specific physiological function without a great deal of further work.

The CDLM has a resting potential of about 60 mV. Spontaneous firing was found on some occasions but progressively disappeared, perhaps indicating damage of the cell body caused by the microelectrode.

Figure 2.11

(A), (B) and (C), three intracellular recordings from the CDLM of three different preparations. In (A) and (C) the frequency is higher than it is in (B), however, progressive decline occurred in the activity of the cell body soon after penetrating it with the microelectrode.

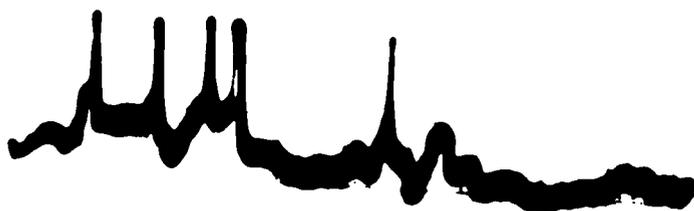
Voltage calibration (A), and (C) 40 mV, (B) 20 mV,

Time calibration : 200 m sec.

2.11



A



B



C



Clark (1976b) and Davis and Alanis (1979) showed the activity of the CDLM of Teleogryllus oceanicus and Gryllus domesticus respectively. These authors used extracellular recording technique, however, so their results are not directly comparable with those presented here.

## DISCUSSION

It has been established in the first chapter of this work that the DUMDL neurone (the dorsal unpaired median neurone innervating the dorsal longitudinal flight muscle, DLM) of the metathoracic ganglion of the cricket Gryllus bimaculatus, is morphologically very comparable to analogous neurones in Teleogryllus oceanicus (Bentley 1973) and in the locust Schistocerca gregaria (Hoyle 1978), but with the difference that the DUMDL of Gryllus bimaculatus was repeatedly back-filled with dye in the adult stage, though difficulties similar to those reported by these authors were encountered on some occasions (discussed in the first chapter). However, apart from a study during the course of the present work by Davis and Alanis (1979) in which they used the isolated metathoracic ganglion of Gryllus domesticus and extracellular recording technique, DUMDL neurones as such have not been investigated physiologically, but the properties of DUM neurones (dorsal unpaired median neurones) in general have been studied in Schistocerca gregaria and the cockroach Periplaneta americana (Crossman et al., 1971a, b; 1972) and in the locust Schistocerca gregaria and the grasshopper Romalia microptera (Hoyle and Dagan 1978) and the locust Schistocerca nites (Heitler and Goodman 1978). However, in these studies work was mainly concentrated on the DUMETI which innervates the jumping muscle.

It can be seen from these previous studies that the physiological characteristics of the DUMDL neurones of Gryllus bimaculatus presented in this work showed similarities with some of these. From the whole cluster of the DUM neurones, however, there are only two whose destinations

are known, the DUMDL and the DUMETI. Both the DUMDL and the DUMETI were identified in Gryllus bimaculatus, and although both of them were spontaneously active or could easily be activated by direct or indirect stimulation, their patterns of spiking were sufficiently different from each other to enable the particular cell body that is impaled to be recognised through its pattern of spiking. One of the two neurones the DUMETI always exhibits only single spikes which undershoot. The DUMETI of the locust sends its axons to the hind legs through N<sub>5</sub> (nomenclature of Campbell 1961) as it does in the cricket Gryllus bimaculatus. In addition there is a strong resemblance between the spiking patterns of this neurone <sup>and that of</sup> the locust, where it was reported that doublets, triplets and multiple groups are completely absent (Hoyle and Dagan 1978) and in Gryllus. All these observations suggest that this active neurone in the cricket is the corresponding one to the DUMETI of the locust.

On the other hand, the second active neurone DUMDL, was found to innervate the DLM through the stimulation of the cell body of the DUMDL itself where it produced twitches in the DLM on some occasions when it was stimulated with depolarizing current, this as well as the back-filling of the DUMDL with dye through N<sub>1</sub>D<sub>1A</sub> was regarded as direct evidence that DUMDL innervates the DLM. On the other hand, the pattern of spiking of the DUMDL differs from that of the DUMETI as it appears as bursts of spikes that make it distinguishable from the DUMETI which fires single spikes only. Also, another noticeable difference between the two neurones is the pronounced undershoots that appear after

each spike of the DUMETI, however, although <sup>an undershoot</sup> was either completely absent or rather small in the spontaneous spikes of DUMDL, it was well developed when the DUMDL was injected with hyperpolarizing current, when it became evident after cessation of the hyperpolarizing current, but soon returned to its original level. In the DUMETI of Schistocerca gregaria, the undershoot was attributed to increased potassium conductance associated with the recovery of the soma spike for it was reduced, abolished, and reversed by progressive hyperpolarization and enhanced by depolarization (Hoyle and Dagan 1978). This would seem to be the case in the DUMDL of the Cricket also, for hyperpolarization abolished the activity, and ipsp's were increased in amplitude when the DUMDL was depolarized (Figure 2.7A).

The twitches observed in the DLM were not obtainable in all preparations although the neurone responded electrically to stimulation. This situation perhaps indicates that there is a specific physiological condition in which these muscles are responsive to stimulation of the DUMDL. It is well known that the flight muscles of crickets are not highly developed for flying. Although these insects fly occasionally, they are not strongly flying insects, as is the locust; but an additional use for wings in crickets is the production of sound by scraping one of the forewings over a rasp-like series of ridges on the other forewing (Huber 1962) perhaps for courtship or fighting. Huber (1965) has shown that the behaviour and sound production of the cricket Gryllus campestris differ at different sexual stages. He also showed that the mushroom bodies and the central body (in

the brain) are necessary for the control of sound production. It is clear that the DUMDL is indeed under inhibitory control from the head ganglia. This effect was clearly seen when the animal was decapitated, when hyperactivity of the DUMDL occurred indicating that the neurone was disconnected from an inhibitory source. A similar effect of decapitation was found in the cockroach periplaneta americana by Hughes (1965) and in the metathoracic ganglion of Schistocerca gregaria and Melanoplus differentialis by Hoyle (1965) and these also were presumed to be due to interruption of some descending inhibitory pathways. So possibly the inhibitory influence of the head could be abolished during sound production or flight but not at other times. This suggestion is supported by the finding that when a current of air was directed on the head, the firing of the DUMDL was stopped and the membrane potential shifted towards hyperpolarization, at the same time as the same stimulus caused flight spiking patterns in the four motor neurones that innervate the dorsal longitudinal flight muscles and which are situated in the mesothoracic ganglion. In addition, Davis and Alanis (1979) noticed that the CDLM neurones (the contralateral longitudinal motor neurones) which also innervate the dorsal longitudinal flight muscles (DLM) fire during the suppression of the DUMDL. However, no activity was recorded of the CDLM of Gryllus bimaculatus that would indicate the functional role of this neurone due to difficulty in penetrating its soma. It has been shown in the first chapter that the neurites of both the DUMDL and the CDLM lie in close proximity to one another in the neuropile (Figure 1.32A). This could suggest that the DUMDL may be linked to the neurones that innervate the DLM

in both the metathoracic and the mesothoracic ganglia.

The question that raises itself here is: how does the DUMDL that lies in the metathoracic ganglion affect the four motorneurons that lie bilaterally in the mesothoracic ganglion? It seems that there are two explanations:

(1) via the internuncials that pass through the thoracic ganglia. It has been shown in the first chapter that dye filling through the interganglionic connectives as well as intra-axonic dye injection through microelectrode revealed many neural fibres that pass between the ganglia and branch or terminate in the thoracic ganglia (Figures 1.11) apparently including the well-known giant fibres that link the head ganglia with the last abdominal ganglion.

When the puff of air is directed onto the head, the DLM motor neurones are excited, but the DUMDL is inhibited. At first sight, the DUMDL could be seen as directly inhibiting the mesothoracic motor neurone (or CDLM neurones). When the inhibition of the motor neurones is lifted through inhibition of the DUMDL by the head ganglia, the motor neurones are enabled to fire spontaneously; alternatively, interneurones that innervate these motor neurones may fire spontaneously, to excite the motor neurones.

However, a puff of air directed onto the caudal cerci, causes excitation of the DUMDL and excitation of the DLM's. Thus, excitation of the flight muscles by puffs of air directed onto opposite ends of the body, produce opposite effects on the DUMDL but the same effects (or similar ones) on the DLMS. This shows that the DUMDL cannot be linked with the motor neurones in any direct way. If it produces its effect

on the DLMS through the motor neurones, the pathway or pathways must be indirect, and capable of modulation or suppression by the more direct effect of head or tail stimuli on the motor neurones. Behaviourally, this is to be expected: the flight or escape reaction must be direct and override more subtle effects. (2) Another alternative site for the effect of the DUMDL on the other neurones that innervate the DLM is directly upon the muscles themselves by modulating their action through varying amounts of its neurosecretion. In the locust, Evans and O'Shea (1977, 1978) found that the DUMETI directly modulates the myogenic rhythm that was found in the extensor tibiae muscle. This kind of command function of the DUMDL, if it exists, seems different from that of the conventional command neurones, which are usually interneurones and more obvious in other invertebrates such as crustaceans and molluscs rather than insects (e.g. Davis 1977; Kupfermann and Weiss 1978).

In their work on Gryllus domesticus, Davis and Alanis (1979) suggested that the dorsal longitudinal flight muscles may play a role in ventilation; if so, this could be another explanation for the hyperactivity of the DUMDL that resulted from decapitation, for Huber (1960) has shown that in crickets the ventilation cycle is initiated from a centre in the sub-oesophageal ganglion. Certainly the firing of the DUMDL did coincide with the body movements due to ventilation for prolonged periods of time on many occasions. However, spike bursts synchronised with the ventilatory rhythm in a nerve innervating a non-ventilatory muscle have

also been observed in Schistocerca (Miller, 1960; Hoyle, 1964), and Burrows (1975b) reported that some flight motorneurons are continually depolarized during ventilatory rhythm although they produce no spikes and contribute nothing towards ventilation in the experimental situation.

In his work on Schistocercagregaria, Burrows (1975b,c) has shown that there are at least two interneurons whose somata are probably within the metathoracic ganglion, and that make widespread connections with 50 flight and ventilatory motorneurons. These impose two different rhythms upon the flight motorneurons; a slow rhythm in time with ventilatory movements and a faster one whose period is similar to that of the wingbeat in flight. The activity in the motorneurons which are affected by these two interneurons is grouped in bursts similar to those normally recorded from the DUMDL in the cricket, and this may indicate that DUMDL is connected with similar interneurons. These interneurons were also found to be in connection with the motor neurons that innervate the dorsal longitudinal flight muscles from the mesothoracic ganglion. It has already been established in the first chapter that the dorsal longitudinal flight muscles of the cricket Gryllus bimaculatus are similar to those of the locust in their innervation pattern.

Each of the dorsal longitudinal flight muscles on either side consists of five units innervated by five motorneurons. Four of these motorneurons are situated ipsilaterally in the mesothoracic ganglion and the fifth is situated contralaterally in the metathoracic ganglion (Figure 1.32 A); this is in addition to their innervation

by a sixth neurone, which jointly innervates both sides of the body, the DUMDL. As for the precise innervation of the DLM, it is not known which unit or units is innervated by this latter neurone. However, it was reported by Ikeda (1977) that two units of the dorsal longitudinal flight muscles on each side of dipteran insects such as Drosophila are innervated by the same neurone. In the case of the cricket Gryllus bimaculatus there may be a similar innervation pattern or more likely the pattern is one of innervation of the whole unit, since all the units of the DLM were demonstrated to be innervated each with one of the five motor-neurones mentioned above (Neville 1963). So, it is unlikely that DUMDL innervates only some of the units unless they have a special role and since the DUMDL supplies both sides of the body its action must be synchronized on both sides.

The resting potential of the DUMDL (45 - 55 mv) falls among the normal range of resting potentials for insect neurones and in particular of the DUM neurones (Hoyle and Dagan 1978). The differences in the resting potential from one animal to another, may indicate differences in the physiological conditions of the neurone, although damage caused by the recording electrode to some neurones cannot be discounted. The duration of action potentials of the DUMDL is relatively long (5 - 50 ms) a feature which is characteristic of neurosecretory cells (Gosbæ et al., 1968; Wilkens and Mote, 1970. Maddrell and Nordman 1979), and it has been shown that DUM neurones, in general, are neurosecretory (Evans and O'Shea 1977; 1978). This long duration (compared with other motorneurones and which is around 4 msec.

(e.g. Gwilliam and Burrows 1980) may reflect a slow conduction through the axon . It was found that slow conduction axons in the cockroach Periplaneta americana have longer duration <sup>action potentials</sup> (Pearson et al., 1970) and this is in agreement with the finding of Paintal (1966, 1967) for small mammalian fibres and suggests that there is a systematic relation between <sup>size of</sup> action potentials, <sup>their</sup> duration and conduction velocity.

The DUMDL gave rise to overshooting action potentials in the cell body, a phenomenon that is believed to occur in insects only in the DUM neurones. It was suggested that the insect cell bodies are inexcitable (Hoyle 1970) and that the absence of overshooting action potential in other motor-neurones was related to the fact that spike-initiation zones are distant from the soma (Hoyle and Burrows 1973). However, it should be borne in mind that DUM neurones are unpaired and their action potential must be effective on both sides of the body and this probably requires larger action potentials than those of ordinary neurones, since these are not propagated action potentials, <sup>and</sup> their ability to pass over a distance will depend on their size. However, <sup>large</sup> overshooting action potentials were not always obtained and this failure could not be attributed to the condition of the preparation since it occurred in some preparations of long life only a few times and did not occur in other good preparations at all.

The occurrence of overshooting action potentials will presumably be related to the diameter of the main neurite. Calculations on Aplysia neurones (Graubard 1975) which are

of larger size than those of insects but generally similar in morphology, indicate that severe voltage attenuation, particularly of transitory potentials, will occur during passage from a small to a large process. This also has been demonstrated in the locust, (Burrows 1977). In the cricket it was found that the diameter of the neurite is larger than that of the axon branches, so when a spike comes from one of the branches, it will suffer a significant attenuation due to the diameter of the neurite, unless the branches fire in synchrony to raise a strong action potential in the soma. However, although Heitler and Goodman (1978) related the failure of the soma spike in some events to this cause, they also pointed out that the ionophore composition of the membrane may be such that the membrane is limited in its ability to support a full action potential under conditions in which spikes fail.

The activity of the DUMDL usually appeared as bursts of spikes grouped together and accompanied by slight depolarization in the underlying membrane potential. These bursts consisted of varying number of spikes which were of different amplitudes on some occasions, suggesting that these spikes were coming from synaptic areas at varying distances from the soma. In their studies on the DUMETI of the locust and the grasshopper, Hoyle and Dagan (1978) and on the locust Heitler and Goodman (1978), related spikes at different amplitudes to different spike-initiation zones: the soma, the neurite, and the two main axon branches. All of these workers related the overshooting spikes to the soma, whereas spikes of up to 40 mv are neurite spikes and smaller

ones are axon spikes. As for the spikes of the DUMDL, it is not evident that they show such clear-cut limits in their amplitudes as to enable them to be related definitely to specific spike-initiation zones; although the amplitudes of the spikes which occur in the same preparation and even within the same burst of spikes do indicate that these spikes are coming from varying distances. However, they may perhaps be coming from a bigger number of spike-initiation zones than four.

It seems that the DUMDL is in connection with a great number of other neurones through synaptic areas. This conclusion was made because the neurone can be excited through different modalities including visual, auditory, and tactile ones; and from the varying amplitudes of post-synaptic potential of both kinds, excitatory and inhibitory. However, it has been shown that in the ventral nerve cord of the locust the largest axons which run between the brain and the thoracic ganglia are those of a pair of neurones called the descending contralateral movement detectors (DCMDs), each of which responds with a vigorous burst of spikes when a small object moves suddenly in the visual field of the compound eye contralateral to its axon (Palka, 1967; Rowell, 1971; Rowell and O'Shea, 1976). It was also found that spikes are elicited in a DCMD by loud noises (O'Shea, 1975). It was found also that a DCMD mediates epsp's and ipsp's in the jumping motor neurones through identified interneurones (Pearson et al., 1980; Pearson and Goodman, 1981) and these authors suggested that the DCMDs are involved in initiating the jump that locusts often make when startled and which is similar to

that of crickets. Pearson and Goodman (1979) and Simmons (1980) have found that a DCMD mediates epsp's in some unidentified flight motoneurons. Anatomically, there are branches from the axon of a DCMD in all three thoracic ganglia of the locust (O'Shea et al., 1974; O'Shea and Rowell, 1977) which is similar to the cricket (Palka, 1969). All these evidences indicate that there is an input from the visual system to the thoracic ganglia and to neurones that innervate the flight muscles, and probably others, and this explains the responsiveness of the DUMDL to visual stimulation. However, the responsiveness of this neurone to diversity of stimulation shows the complexity of the DUMDL connections with the different sense organs. This can be accounted for by looking at the morphological picture of the DUMDL where extensive branches of afferent elements terminate in its vicinity, or give off branches in this region during their passage through the metathoracic ganglion (Figures 1.32A, 1.33). However, although it is difficult to pin-point the precise areas of synapse, it was apparent from the  $\text{CoCl}_2$  preparations referred to earlier that, in addition to the fine branches given off by the two lateral main branches of the axon, there are between 3-7 swollen areas in the main neurite from which fine spiny and bouton-ended branches emerge. On the other hand, the arborizations around the DUMDL axon branches and close to it are so profuse that many problems arise which are far from being resolved. For example, Burrows (1977) asks,

"In how many anatomical synapses is one physiologically defined synapse represented? What proportion of branches are inputs compared with outputs, or can a distinction be made between the two? Can different parts of a neurone act independently?, and what effect does a postsynaptic potential in a distant branch have on the impulse initiation zone". We are still far from precise answers to these questions.

DUMDL showed rapid responsiveness to air puff and gentle touch on the caudal cerci. It is well known that many arthropods, including the crickets exhibit escape behaviour; this escape behaviour was found to be conducted to the upper central nervous system through the ascending giant fibres found within the nerve cord (Raeder 1963; Hughes 1965; Huber 1965; Parnas and Dagan 1971). The response to cercal stimulation recorded in the DUMDL is consistent with the finding (Chapter one) that the fibres that come from the posterior part of the nerve cord, give off branches in the metathoracic ganglion, which may provide synapses with the neurone. It was reported however, that the dorsal giant fibres terminate in the thoracic ganglia of the cockroach (Farley and Milburn 1969).

Although DUMDL remained firing, on some occasions, for up to an hour, it showed rapid habituation to stimulation of the cerci; in other words, the response to tactile and air puff <sup>stimulation</sup> on the cerci recorded in DUMDL declined on repetition. This could be accounted for by the work of Zucker (1972a,b) on the crayfish which exhibits many similarities with the systems in the cricket (Murphy and Palka 1974).

Zucker suggested that habituation of the lateral giant system is due to intrinsic depression of the individual synapses between tactile afferent and interneurons. Moreover, there is preliminary evidence that the synaptic depression is, at least in part, specifically due to changes of the pre-synaptic element at these functions (Zucker, 1972b; Krasne 1976). Confirmation of the presynaptic inhibition during habituation come also from the work of Kennedy, Calabrese, and Wine (1974), recording intracellularly from the afferent terminals themselves. In the cricket, Murphy and Palka (1977) have shown that there is a central inhibition of the synapses made by these receptors when the animal engages in spontaneous walking activity. So the habituation process of the DUMDL seems to take place far away from the soma itself. Murphy and Palka have found that most of this inhibitory influence on the LGI and MGI was abolished by sectioning of the connective ipsilateral to the axon of interest, while cutting the contralateral connective had only a small effect. In contrast, the smaller ascending giant interneurons which fire tonically receive their inhibitory control equally from the two connectives. They demonstrated that this inhibitory influence was coming from the metathoracic ganglion. In Gryllus bimaculatus, I have shown, that the response recorded in the DUMDL was reduced to about half its amplitude when one of the cerci was cut off while the other was kept intact and stimulated. This indicated that DUMDL is in direct connection through both connectives, that the response of the two cerci is conducted ipsilaterally and that the response recorded in the DUMDL is the summation of

that conducted through both connectives. However, it was found that the giant fibres send branches to the contralateral side of the last abdominal ganglion where the afferent fibres synapse that come from the cerci (Roeder 1963; Callec and Boistel 1965); but it is not known whether the DUMDL is connected to the last abdominal ganglion through the large giant fibres or through the smaller ones. However, escape behaviour is a rapid action and first priority; and it was suggested that the large giant fibres existed to provide a fast velocity route for that purpose (Murphy and Palka 1974), in addition to serving as integrating interneurons (Parnas and Dagan 1971).

In addition to the induced excitation of the DUMDL by various sensory modalities the DUMDL was shown to exhibit spontaneous activity for prolonged periods of time as well as receiving epsp's and ipsp's. This spontaneous activity is presumably coming from intrinsic mechanisms of the DUMDL itself. This assumption is supported by the finding that on some occasions, after the DUMDL have shown no more response to stimulation of the caudal cerci, i.e. become habituated, spontaneous firing was resumed and even at varying amplitudes on some occasions; moreover, this resumed firing was no longer affected by further stimulation of the cerci within a refractory time. However, the activity of the DUMDL vanished in a saline lacking  $Ca^{++}$  and high in  $Mg^{++}$ . This suggests that the activity of the DUMDL is chemically mediated from other interneurons, but it is possible that the synaptic blocking agents also have a direct effect on the DUMDL.

The DUMDL showed response to ACh and eserine applied on the ganglion overall. DUM neurones, in general, were shown to be octopaminergic by Evans and O'Shea (1977; 1978). The afferent synapses to the DUMDL may be cholinergic for the above-mentioned reason, i.e. response to ACh. Although Dale's hypothesis that each neurone contains and releases only one transmitter was challenged by reports that there are some neurones containing more than one transmitter (e.g. in Helix pomatia, Gershenfeld, 1973; Hanley and Cottrell 1974; Hanley et al. 1974; Cottrell, 1977, and in Aplysia, Brownstein 1974 etal.) this has not been established in insects. On the other hand, although ACh is a postulated transmitter substance within the CNS of insects, it is not so at the neuromuscular junctions (e.g. Pichon, 1974; Leak and Walker, 1980) unlike vertebrates. The release of acetylcholine from insect ganglia by presynaptic nerve stimulation has not so far been demonstrated, but there is a high ACh content in insect central nervous systems, and pharmacological experiments have revealed a high sensitivity of insect central neurones to locally-applied or bathed-applied Ach following the inhibition of endogenous acetylcholinesterase (Kerkut et al., 1969a,b; Shankland et al., 1971; Callelec, 1974; Sattle et al., 1976;) and this explains the effect of ACh and eserine on the DUMDL.

Evans and O'Shea (1977) have shown that the DUMETI of locusts can modulate the intrinsic rhythmic contraction of the tibial extensor muscle, and Hoyle et al. (1974) have shown large numbers of dense core vesicles of neurosecretory type at the muscle fibres that receive terminals from the DUMETI. However, Evans and O'Shea emphasized that the function of

DUMETI and other DUM neurones is unlikely to be concerned solely with the modulation of the muscle rhythm. Observation on the DLM resulted in finding no such rhythm in this indirect flight muscle. In the mouse neuromuscular junctions, Winlow and Usherwood (1975, 1976) showed apparent lack of correlation between vesicle aggregates and the occurrence of abnormal miniature EPP discharges, inferring that these discharges were probably induced by changes unrelated to the spatial arrangement of the synaptic vesicles observed in electron microscope sections, although the simultaneous occurrence of aggregated vesicles and silent functions could be causally related events.

Function for DUMDL :

Twitch production in the dorsal longitudinal flight muscles in response to intracellular stimulation of DUMDL with depolarizing current has been already established; also spontaneous activity of the DUMDL has been demonstrated. However, it is difficult to relate these activities with certainty to a specific functional role. Several suggestions have been made by some workers concerning the function of DUM neurones in general and DUMETI in particular. In their work on the cockroach Periplaneta americana and the locust Schistocerca gregaria Crossman et al. (1972) suggested that the distribution of the mediodorsal cell bodies probably favour a motor function rather than a sensory one. Also some function involving the co-ordination of both sides of the animal was suggested by them. They added that "the possibility exists, therefore, that the mediodorsal neurones are leg motorneurones. Since most of the other leg motorneurones

appear to be situated ventrally and to have different electrical properties from the mediodorsal cells, a somewhat specialized function seems likely". Also, according to their proposed configuration of axon distribution through nerve trunks of the ganglion, which was concluded from extracellular recordings and which was found to coincide with the axon distribution of the "Common inhibitor neurone" suggested by Pearson and Bergman (1969), they postulated that the mediodorsal cell bodies may be functionally related to the common inhibitory neurones. However, the axon distribution proposed by Crossman et al., (1972) was not found in the locust (Hoyle 1978) nor have I found it in the cricket Gryllus bimaculatus. However, in his work on Schistocerca gregaria, Hoyle (1974a) found that the axon of the DUMETI accompanies the fast axon, but not the slow one which is unusually accompanied by the common inhibitor, and he suggested two possible trophic roles for DUM neurones. One is the maintenance of good functional condition in the absence of excitatory nerve impulses. The other is that they are responsible for the differentiation between muscle fibres that are innervated only by fast axons compared with those that also receive a slow and common inhibitor neurone; i.e. fast versus slow muscle fibres, respectively. Hoyle (1974b; 1975) also found that DUMETI of Schistocerca gregaria and the grasshopper Romalea microptera has an inhibitory effect on the rhythm. It causes long-term inhibition of the slow intrinsic rhythm of contraction of the muscle by releasing a neurohumoral agent. Large (600 - 1900A), dense-

core vesicles are present in the nerve terminals. The inhibitory action was mimicked by infusion into the leg of a minute (0.01 ml) drop of locust saline containing a very low concentration ( $10^{-6}$ M) of dopamine or noradrenaline, or an extraordinarily low concentration of octopamine ( $2.5 \times 10^{-9}$ M). Accordingly it is suggested that this neurone and the other DUM neurones are octopaminergic. Hoyle and Baker (1975) found that DUMETI produces octopamine but neither noradrenaline nor dopamine was detected. Evans and O'Shea (1977; 1978) confirmed that DUM neurones are octopaminergic for they selectively stained with the dye neutral red, a dye known to stain monoamine containing neurones, and through chemical assay. However, they emphasised that the function of DUMETI and other DUM cells is unlikely to be concerned solely with the modulation of the muscle rhythm, for the axon of DUMETI projects to parts of the metathoracic extensor tibiae muscle that do not exhibit the rhythm, and the rhythm in the extensor tibiae muscle is confined to the metathoracic segment and in the cockroach is absent altogether (Hoyle and O'Shea 1974).

Observations were made to see if there is any link between the activity of the DUMDL neurone and the ventilation movements of the body. Although some synchrony was noticed on some occasions between the spikes and the body movement of ventilation, this was not always found. However, the pattern of the DUMDL sometimes does appear as single tonic spikes, and similar tonic spikes were recorded extracellularly going to the dorsal longitudinal flight muscles of the house cricket Acheta domesticus (Davis and Alanis 1979)

and in Teleogryllus oceanicus (Clark 1976b). These spikes were believed to come from the DUMDL and the authors suggested that they are tonic motor impulses probably related to ventilation movements and posture. Davis and Alanis suggested that DUMDL may represent slow innervation to the dorsal flight muscles; but these muscles are believed in most insects, to have only fast motor innervation and are phasic (Wilson 1968).

Since the dorsal longitudinal flight muscles play a role in the sound production as well as flight, and probably ventilation; so the function of the DUMDL could be a command one to control all the neurones that innervate the dorsal muscles to organize their roles and coordinate the required action by the muscle according to the situation of the animal. For instance, if the animal was producing sound, when an escape behaviour suddenly was imposed, the neurone should coordinate the function of the dorsal muscles with the other effective organs in this process and give the escape behaviour first priority to sound production. Thus, the command function, as already demonstrated, could not be a simple one, and its effect may well be indirect and subject to modulation by a variety of sensory inputs.

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APPENDIX

Fixing solutions used:

(i) A.A.F. fixative (Formol-acetic acid-ethanol):

40% Formaline	10 ml.
Glacial acetic acid	5 ml.
100% Alcohol	85 ml.

(ii) Bodian's No. 2 fixative:

Formaline	5 ml.
Glacial acetic acid	5 ml.
80% Ethanol	90 ml.

(iii) Bouin's fixative:

Picric acid saturated aqueous	75 ml.
Formaline	25 ml.
Glacial acetic acid	5 ml.

(iv) Alcoholic Bouin's fixative:

80% Ethyl alcohol	150 ml.
Formaline	60 ml.
Glacial acetic acid	15 ml.
Picric acid crystals	1.0 gm.

(v) Carnoy's fixative:

Absolute ethanol	60 ml.
Chloroform	30 ml.
Acetic acid	10 ml.

(vi) Glutraldehyde Formaldehyde pH<sub>4</sub>:

25% Glutraldehyde	0.1 ml.
Formaldehyde	0.4 gm.

(solid paraformaldehyde was weighed out, heated to 90°C in distilled water, and 1 drop NaOH was added).

Buffer 5 ml. (0.1 M acetate pH<sub>4</sub>)

Salts 2.5 ml. [salts contain : 2.0%  $\text{CaCl}_2$ ,  
12%  $\text{NaCl}_2$ , 12% surose, all (w/v)].

Wood's (1957) insect saline solution:

Na	15
K	18
Mg	50
Ca	7.5
$\text{H}_2\text{PO}_4$	6
$\text{HPO}_4$	4.5
Cl	133

(all mM).

Figure A1

A diagram of the amplifiers circuit used in this work.  
Circuit modified from Colburn and Schwartz (1972).

Amplifiers A1, A2, A4, A5 = J.FET op. amp. (R.S. Components).

Amplifiers A3, A6, A7 = 741 op. amp. (R.S. Components).

BAL. = Balance

CU.IN. = Current input

D.C.Of. = D.C. Offset.

A1

