J. Exp. Biol. (1974), 60, 1–12
With 3 plates and 5 text-figures
Printed in Great Britain

THE ANATOMY OF A LOCUST VISUAL INTERNEURONE; THE DESCENDING CONTRALATERAL MOVEMENT DETECTOR

BY M. O'SHEA, C. H. F. ROWELL AND J. L. D. WILLIAMS*
Department of Zoology, University of California, Berkeley, California 94720, *U.S.A.*

(*Received* 23 *May* 1973)

INTRODUCTION

One of the most thoroughly studied of all interneurones is the descending contralateral movement detector (DCMD) of the locust (see Rowell, 1971*a* for review). It is possible to record this unit in the thoracic nerve cord using the most simple type of extracellular electrodes, and it is invariably the most conspicuous spike. The unit's electrophysiological availability has made it a popular subject for study, and much is known about its response characteristics (Palka, 1967*a*, *b*, 1972; Rowell & Horn, 1967, 1968; Horn & Rowell, 1968; Rowell, 1971*b*, *c*) and its output connexions (Burrows & Rowell, 1973). Despite the relative wealth of electrophysiological information, knowledge of even its gross anatomical features has been missing.

As its name implies, the unit responds to movement in the visual field of the contralateral eye. The basic response, however, appears to be to a rapid decrease of illumination over small areas of the retina, which has a short-term excitatory effect and a longer-lasting inhibitory effect. The result is a highly sensitive response to abrupt movement of small, dark objects in the visual field.

On the basis of intracellular staining with cobalt sulphide in conjunction with intra- and extracellular recording techniques, it is now possible to describe the anatomy of the DCMD in some detail. The possible identification of this cell by Satija (1957, 1958) is seen to be mistaken. The DCMD and the large protocerebral cell (Giant One or G 1) in *Schistocerca gregaria* (Williams, 1972) are, however, identical, and Williams' histological observations are confirmed and extended.

This paper has two aims. The first is to describe the morphology of the DCMD neurone, and to correlate the information with what is known of its electrophysiology and function. The second is to present the investigation as an example of the way in which modern neuro-anatomical techniques can be applied to the insect central nervous system in order to obtain intracellular preparations of neurones traditionally known only from extracellular recordings. This process is already being applied in the decapod crustacean nervous system, in which the neurones are generally larger (e.g. Remler, Selverston & Kennedy, 1968, and subsequent workers). With few exceptions (e.g. Burrows & Hoyle, 1973, and various workers on the ascending abdominal giant fibres of Orthoptera) intracellular recordings from insects have been confined to primary sensilla or to the relatively large motor cell somata.

* Present address: 26, Glebe Court, Southampton, England.

MATERIALS AND METHODS

The results are based on the observation of more than one hundred adult specimens of *Schistocerca vaga*; both sexes were used. The anatomy of the DCMD was elucidated by the application of two different intracellular staining techniques (for a review see Nicholson & Kater, 1973) in conjunction with nerve-cord splitting and intra- and extracellular recording. We give this procedure in some detail as it may be of assistance in determining the anatomy of other arthropod interneurones known only from recordings from the connectives.

(*a*) *Thoracic nerve-cord splitting and recording*

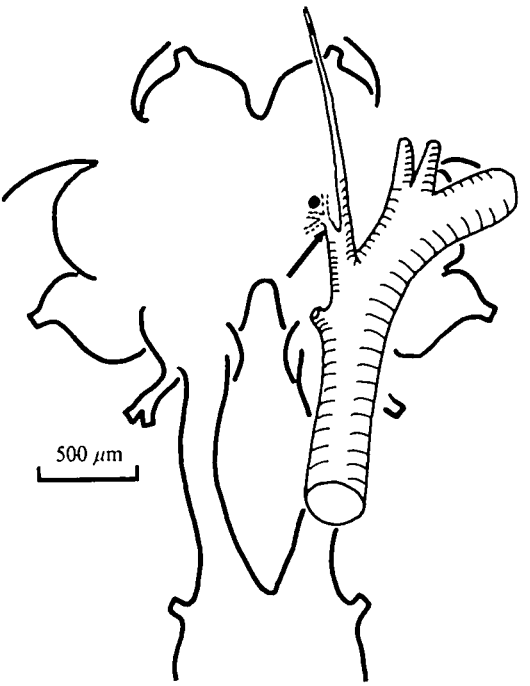
The position of the DCMD axon in the cord was determined initially by connective splitting and the use of fine suction electrodes. The unit may be recognized in an isolated bundle of axons by its well-known response characteristics. A check on the identification of the axon was provided by recording simultaneously elsewhere on the whole cord with ventrally inserted silver hook electrodes; recorded in this way the DCMD is the largest and the most easily identifiable unit. This technique was repeated along the length of the thoracic nerve cord, between each ganglion, and the results were correlated with the distribution of large axons as seen in cross-section of the cord.

Having determined the approximate position of the DCMD in the nerve cord, we probed that area with microelectrodes. The nerve cord was trans-illuminated and was not de-sheathed, but was kept under mild tension. When we penetrated the unit, its identity was confirmed as before by its response characteristics and by a comparison of the intracellular record with a simultaneous extracellular recording from the whole cord. The axon was marked by the intracellular injection of Procion Yellow (Stretton & Kravitz, 1968) or of cobalt ions (Pitman, Tweedle & Cohen, 1972) through the recording electrode; the latter gave more satisfactory results in our hands.

(*b*) *Axonal iontophoresis*

A modification of the technique first described by Iles & Mulloney (1971) was used to locate the DCMD cell body and to elucidate the neurone's pattern of branching in each ganglion.

We exposed the thoracic nerve cord by ventral dissection of unanaesthetized animals; the extraneural fat-body sheath and the tracheal supply of one connective were removed. The cleaned connective was cut transversely close to a ganglion, and petroleum jelly (Vaseline) was used to construct a cup-like container around the longer portion of the cut connective, so that its cut end projected through the floor and into the cavity of the cup. A few drops of a 250 mM solution of cobaltous chloride were placed in the cup to cover the cut end of the connective. If a current of about 5×10^{-7} A is now applied between the animal's blood and the cobaltous chloride pool, making the pool positive, the cobalt ion will flow into the axons where it can later be precipitated. Using this technique it is possible to fill axons for a distance of 5–8 mm in 4 h at room temperature. We found, however, that rather better fills, albeit slower, were obtained without the use of applied current. Some preparations were left to fill for as long as 72 h and it was necessary in such cases to take precautions against desiccation. The cobalt ion was precipitated in the filled neurones by the application



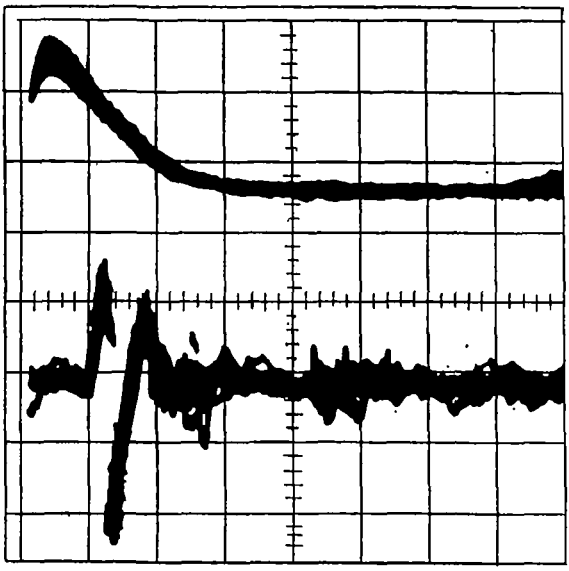
Text-fig. 1. Posterior view of the brain of *S. vaga*. On the right hand side is shown the major trunk serving the cephalic region. The arrow indicates the point of entry of the main protocerebral trachea, and the dotted lines show branches within the brain. The position of the DCMD soma is indicated by a black spot.

of a 0·05% solution of 44% ammonium sulphide in insect saline. Those parts of the central nervous system which contained filled neurones were dissected out, washed in saline, fixed in Carnoy's for about 10 min, dehydrated and cleared in methyl benzoate. Axons and their branches can be seen easily in whole, cleared ganglia.

The technique of cord splitting has also been used to make axonal iontophoresis more selective. The axons contained in a small segment of the nerve cord can be filled selectively simply by stripping out that bundle of axons and treating them in the same way as described above for the whole cord.

(*c*) *Identification of the DCMD soma; microelectrode injection of cobalt into the soma*

Axonal iontophoresis of cobalt anteriorly into the thoracic nerve cord displayed the cerebral anatomy of many neurones; these included a large cell body in the upper posterior part of the brain, contralateral to the filled connective. The brain was exposed posteriorly and microelectrode penetrations of this area were made. The brain was supported on a steel platform under light tension; the electrode was inserted through the neural lamella without de-sheathing. As far as possible the tracheal supply to the brain was left intact. This not only contributed to the health of the preparation but also provided a useful guide to the location of the cell body (Text-fig. 1). Electrical activity was recorded in the soma, and simultaneous extracellular recording from the contralateral connective allowed us to identify the intracellular recording as coming from the DCMD (Text-fig. 2). The cell body was marked by electrophoretic injection



Text-fig. 2. Simultaneous recording of a DCMD action potential from the cell soma (upper trace, intracellular) and the cervical connective (lower trace, extracellular). Superimposed multiple sweeps. Calibration: 2 msec. and (for upper trace only) 5 mv./division.

of cobalt through the recording electrode, and in favourable preparations it was possible to fill the entire cerebral projection of the neurone. The structure visualized in this way confirmed the results obtained by iontophoresis up the descending axon.

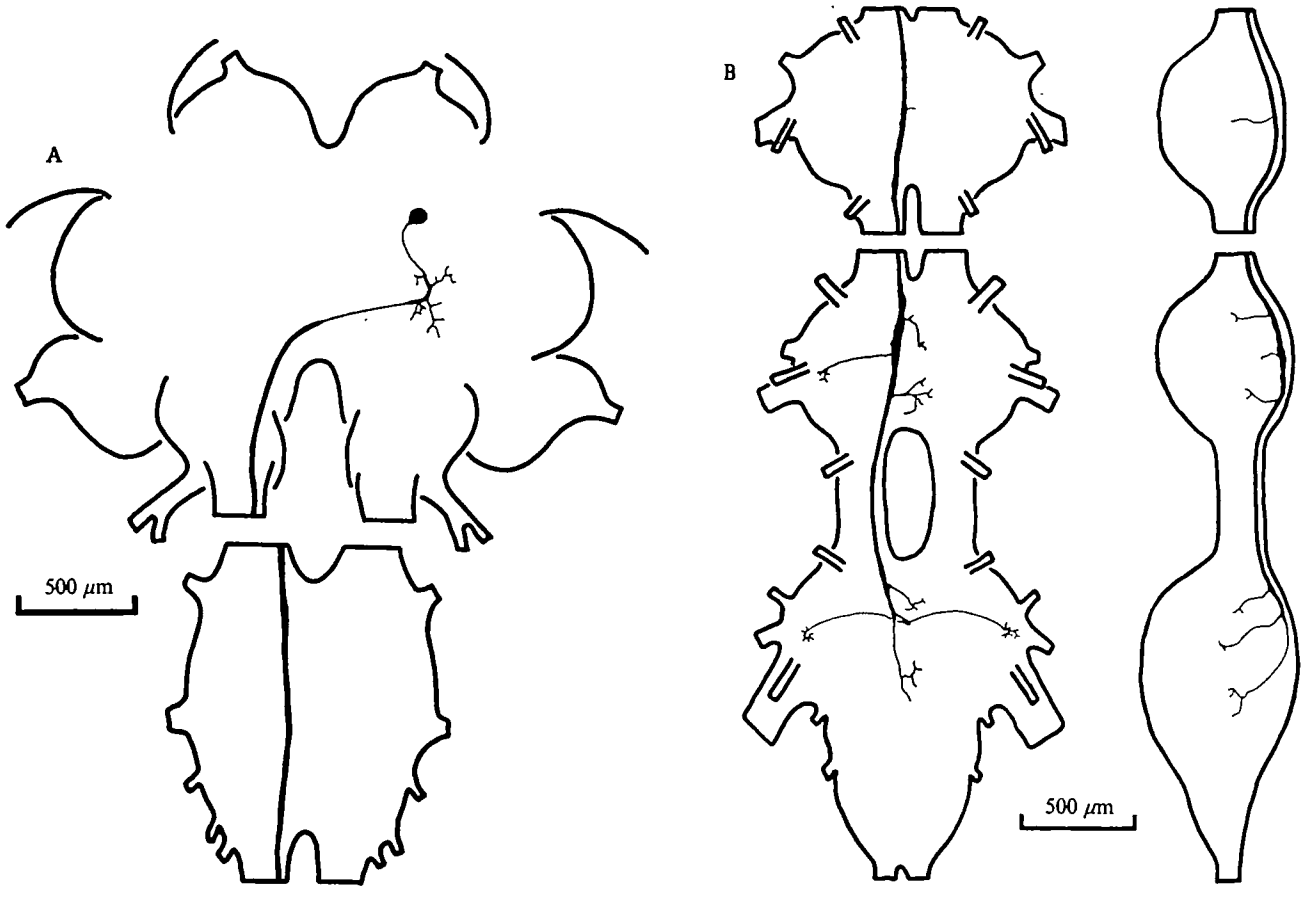
As a final approach, we filled the thoracic projections of the DCMD by axonal iontophoresis from the brain. To do this, a lesion was made in the brain which would sever the DCMD axon; the surface of this lesion was then brought into contact with cobalt solution, and the usual procedure was followed. This method was necessary for good visualization of the thoracic projection because it proved difficult to inject enough cobalt into an impaled axon to fill the adjacent ganglia; further, iontophoresis along a severed thoracic connective normally fills too many axons for good resolution of any single one. The technique of using a lesion in the brain gave a highly selective fill of the contralateral DCMD, as very few large axons, other than it, cross the brain and descend the contralateral cord.

RESULTS

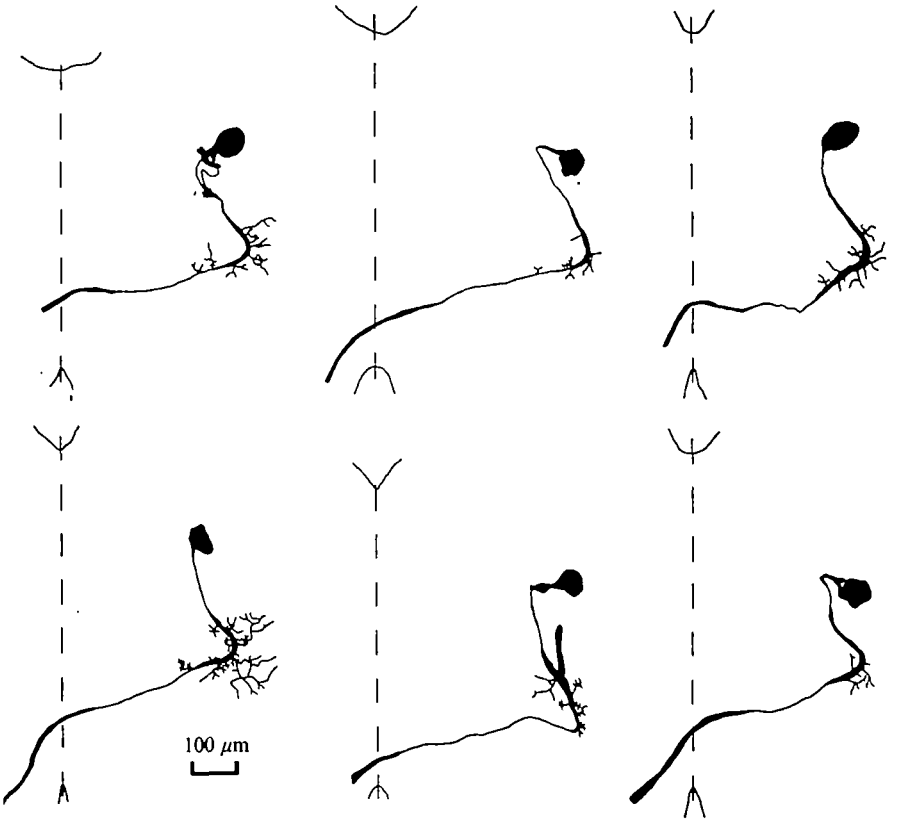
The results are summarized in Text-figs. 3A, B; specific aspects are presented in more detail below..

(*a*) *In the nerve cord*

The axon of the DCMD is located peripherally in the dorso-medial segment of the thoracic nerve cord (Plate 1 A), and is in a similar position in the circumoesophageal connectives. It is the largest axon in the cord, having a diameter of 15–17 μm, and it can easily be identified with good stereo optics in a trans-illuminated connective. The size and peripheral location of the axon account for the large (1–3 mV) amplitude of the DCMD spike in extracellular recordings.



Text-fig. 3 A, B. Schematic representation of the anatomy of the DCMD neurone in the brain and suboesophageal ganglion (A) and in the thoracic ganglia (B). The brain is viewed from the posterior aspect and the thoracic ganglia are viewed from above (dorsally). Note that in the mesothoracic ganglion there is a thickening of the DCMD axon. A lateral view of the ganglia is shown to the right of B.



Text-fig. 4. Camera lucida drawings of the DCMD in the brain of six different animals. All viewed in identical perspective. Note individual variation in position of neurone relative to the midline of the brain (dashed line); position of soma; course of neurite; curvature and form of integrating segment; and point of thickening of main axon. All these features are presumably independent of the degree of success in staining the cell. In addition, considerable variation is seen in the points of departure of the dendrites from the integrating segment, though this feature could be an artifact of staining, i.e., a totally impregnated cell might show all the branches seen in the various preparations here figured.

(*b*) *Brain and suboesophageal ganglion*

The soma of the DCMD is possibly the largest in the brain (45–50 μm diameter) and is located on the posterior face of the protocerebrum, contralateral to its projection to the thoracic nervous system. It lies on average (n = 8) 350 μm lateral to the midline of the brain, and 216 μm below the horizontal drawn through the lowest point between the cerebral hemispheres. Deviations of up to 30% from these means were recorded in individual cases. This variation is not appreciably reduced if the measurements are expressed as percentages of the absolute dimensions of the brain, which show much less variation (absolute range about $\pm 6\%$).

A fine neurite emerges from the anterior face of the soma and loops anteriorly and ventrally to a point where it expands into what, on the basis of its structure and unpublished electrophysiological data, appears to be an integrating segment (cf. Sandeman, 1969). From the integrating segment, which has a maximum diameter of some 20 μm, a fine axon crosses the brain and expands to about 15 μm diameter just

before crossing the midline and descending the ventral nerve cord (see Plates 1 B and 2). In most preparations at least six major dendrites can be seen leaving the integrating segment, and their fine branches effectively fill a sphere of *ca.* 200 μm diameter around the integrating segment. The shape of the integrating segment and the positions of even major dendritic branches show considerable variation from individual to individual (Text-fig. 4). It has not been possible to show a direct projection of the DCMD to the optic lobe by filling the cell with cobalt from the axon or the soma. Attempts to fill the DCMD from the cut proximal stump of the optic lobe have also failed, even though many fine fibres can be filled in this way. We think it unlikely, therefore, that a dendrite extends to the optic lobe. We return to this topic in the Discussion.

No branches of the DCMD have been observed in the suboesophageal ganglion; the axon passes through in a dorso-medial position and varies little in diameter.

(*c*) *The thoracic ganglia*

The descending axon maintains its dorsal and medial position in the thoracic ganglia. It shows no narrowing in the ganglia, unlike the giant fibres of the cockroach (Roeder, 1948 and subsequent workers) or cricket (R. Murphey, personal communication), and in many preparations seems to widen appreciably (see Text-fig. 3 B). In the metathoracic ganglion it tapers to its termination.

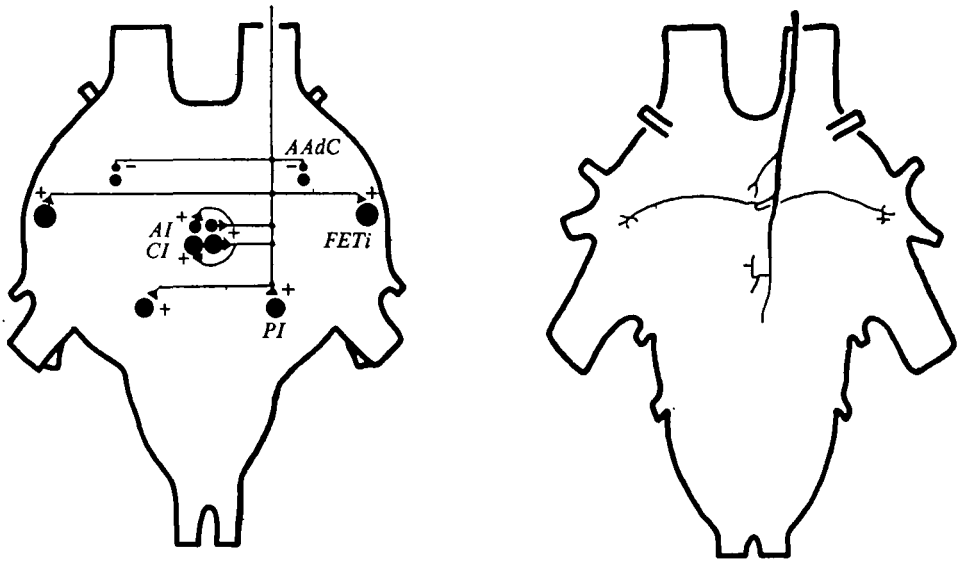
Only a single branch has been observed in the prothoracic ganglion. It arises centrally from the main axon and plunges deep into the neuropile (Text-fig. 3 B). In both the meso and metathoracic ganglia there are three main branches, and these give rise to several subsidiary ones (Text-fig. 3 B and Plates 3 A, B). Other very fine branches are sometimes seen arising from the descending axon; these are, however, very difficult to visualize or trace in the neuropile and our description will be limited therefore to those larger branches which are clearly seen in all preparations.

The most anterior and the most posterior branches of the DCMD in the mesothoracic ganglion pass ventrally and medially towards the midline. The small terminal arborization is approximately medial and extends into both hemiganglia. The central branch dips ventrally and projects ipsilaterally; it has a small zone of arborization in the lateral neuropile. In the metathoracic ganglion the most anterior branch dips ventrally and medially; its arborization, as in the mesothoracic ganglion, is medial. The second branch projects ventrally and towards the midline and then sends two fine axons to the ipsi, and contralateral margins of the ganglion. These terminate laterally, just anterior to the exit of the main leg nerve (Nerve 5), in a small zone arborization. The DCMD axon continues posteriorly, tapers, curves ventrally and branches again. We have been unable to observe any projection of the DCMD into the abdominal cord.

DISCUSSION

(1) *Output connexions in the thorax*

Output connexions of the DCMD and its ipsilaterally projecting counterpart (DIMD) with metathoracic motoneurones have been ascertained by electrophysiological techniques (Burrows & Rowell, 1973). Each of the four interneurones makes bilateral connexions with the following motoneurones: excitatory connexions with the



Text-fig. 5. Comparison of the course of the DCMD in the metathoracic ganglion, as visualized by cobalt injection, with that deduced from electrophysiological recordings from motoneurones by Burrows & Rowell (1973).

Note that the latter experiments were performed on *S. gregaria*, and the ganglion is viewed ventrally (drawing A). In the present work, *S. vaga* was used and the ganglion is viewed dorsally (drawing B); these differences account for the different outlines of the ganglia. Burrows and Rowell constructed the connectivity diagram by drawing straight lines to the sites of known motoneurones. The abbreviations used are: *AAdC*, anterior coxal adductor motoneurone; *FETi*, fast extensor tibiae motoneurone; *AI*, anterior inhibitory flexor tibiae motoneurone; *PI*, posterior inhibitory flexor tibiae motoneurone; *CI*, common inhibitory motoneurone.

fast extensors of the tibia (FETi), excitatory connexions with the posterior and anterior inhibitors of the antagonistic flexors (PI, AI), and with the common inhibitor (CI), which among other functions inhibits the slow fibres of the extensor tibiae muscle and of the anterior coxal adductor muscle. Finally, they make inhibitory connexions with the anterior adductor of the coxa (AAdC). Burrows (1973) and Burrows & Hoyle (1973) have determined the anatomy of these motoneurones. All of them are ipsilateral, and all have extensive dendritic trees. The FETi soma lies on the lateral periphery of the ganglion, about half way down its length; the AAdC nearer the midline and more anteriorly; and the various inhibitors lie near the midline and in the posterior half of the ganglion. The major portions of the dendritic trees of all these cells lie relatively near to the somata.

The metathoracic anatomy of the DCMD shows a striking correspondence with what one might expect on the basis of the above description. If the anatomical data are compared with the schematic diagram from Burrows & Rowell (1973), which was made before detailed anatomical knowledge of the DCMD projections was available, a remarkable similarity can be seen (Text-fig. 5). There are no major branches of the DCMD to be seen in the metathoracic ganglion which cannot be accounted for by the known connexions with motoneurones involved in the jump. Although this is negative evidence, it may indicate that the innervation of the jump motoneurones is the only, or at least the most important, function of the DMD neurones.

The situation with regard to the DCMD in the other thoracic ganglia, and the

DIMD in all of them, is less certain. We have preparations in which is seen what is presumably the DIMD running more or less parallel with the DCMD in the metathoracic ganglion, duplicating all its ramifications. This is in accord with the electrophysiological results, which found identical connexions between the two pairs of neurones and the same population of motoneurones. We have, however, no adequate preparations of the DIMD in the other thoracic ganglia. Electrophysiological measurements of the DIMD (Burrows & Rowell, 1973) show that its axonal conduction velocity, and thus its diameter, is approximately identical with that of the DCMD, but the small amplitude of its spike, recorded extracellularly, shows that it is not located peripherally in the cord. Cross-sections of the cord show a very large axon profile in the centre of the cord, and we assume that this is the DIMD, but without as yet more definitive proof. Our fills suggest that the DCMD has three branches in the mesothoracic ganglion, but that there is no extensive contralateral projection, whereas in the prothoracic ganglion only one branch can be seen. If one makes the assumption that here too, as in the metathoracic ganglion, the output must be interpreted functionally in relation to the jump, no great improvement in understanding follows; the action of the front two pairs of legs in the jump is not known, and the position of the various motoneurones in the anterior two thoracic ganglia is not known either. On the basis of present knowledge, it seems probable that the DCMD connexions in the mesothorax are homologous with those in the metathorax, but probably only unilateral, and that those in the prothoracic ganglion are different. Electrophysiological data from the two anterior ganglia would now be welcome.

The relative simplicity of the DCMD structure in the metathoracic ganglion is in marked contrast with the complicated dendritic tree of the motoneurones. The difference between the two classes of neurones is probably directly related to the finding (Burrows & Rowell, 1973) that the motoneurones are acting as final common pathways, integrating input from many different sensory modalities which converge upon them.

(2) *Input connexions in the brain*

The implications of the structure of the integrating segment and dendrites in the brain can only be discussed in conjunction with the results of intracellular recording from these structures and from the soma, and these will be presented elsewhere (O'Shea & Rowell, 1973). One of the major questions still unresolved by electrophysiological experiments concerns the nature of the input from the optic lobe which generates the spikes recorded in the DCMD. It is possible to record, with an extracellular tungsten electrode in the base of the optic lobe, a large action potential, which precedes 1:1 the DCMD axon spike (R. Northrop, personal communication, and our own observations). It would be tempting to conclude that the spike recorded in the optic lobe is a DCMD dendrite spike, and that therefore the dendrite in question is of large diameter and runs into the optic lobe. Unfortunately, our anatomical results do not support this hypothesis. On a very few occasions we have observed an exceedingly fine process which extends from the integrating segment in the direction of the optic lobe, which it may even reach, but the diameter of this process is such that it is most unlikely that a spike in it could be recorded extracellularly. We therefore tend to believe that the optic-lobe spike derives from a relatively large axon belonging to a unit pre-synaptic to the DCMD, to which it is connected by an electrical synapse

or by a very reliable chemical synapse. There is, in fact, a large axon in the brain which runs from the lobula and comes so close to the integrating segment of the DCMD that they can in some histological preparations appear to be continuous (J. L. D. Williams, unpublished observations).

The variation seen in the geometry of the neurone in the brain is comparable with that reported from motoneurones in other parts of the arthropod CNS (e.g. Burrows, 1973; Stretton & Kravitz, 1973), and its explanation must be sought in the ontogeny of the individual. It impresses us, however, that neural variation is relatively great, in so far as it can be intuitively judged; that is, the structure of the DCMD, were it immediately apparent on inspection of the animal, would not be a good taxonomic character compared with many other morphological features. We make three assumptions, all of which are at least partially supported by evidence: that there is a physiological correlate of the neuronal variation, that the resultant physiological variation is at least partially expressed in the behaviour of the individual animal, and that neuronal variation has a major genetic component. Granting these assumptions, it is tempting to suppose that variation in individual neurones is the morphological correlate of the behavioural variance of the population, and that such a range of behavioural capacity is a significant evolutionary asset, which selection could act to preserve and increase.

(3) *Cobalt staining techniques for neuro-anatomists*

We would like to suggest that electrophysiology could benefit greatly by anatomical studies on the larger arthropod neurones, even if these are carried out in total ignorance of the physiological function of the units involved. The methylene blue preparations of Retzius and Zawarin, which were so produced, are only now being superseded by modern techniques of silver impregnation and dye injection, and have long given service to physiologists. When the anatomy of a large cell is known in sufficient detail to allow the electrophysiologist to locate soma and integrating segment with microelectrodes a functional description of the unit will soon follow. In recent years the tendency has been for dye-injection techniques to be applied secondarily by neurophysiologists in the course of their experiments, and the anatomy is elucidated after the functional attribution is made. With the introduction of the cobalt modification of Iles and Mulloney's axonal iontophoresis technique this need no longer be the case, and anatomists could, without expensive equipment, uncover rich new fields for physiological investigation.

SUMMARY

1. The DCMD neurone is physiologically well-known and runs from the brain to the metathoracic ganglion. It responds to novel movement of small contrasting objects in the visual field and synapses on metathoracic motoneurones which mediate the jump of the locust. Its anatomy, here reported, has been visualized by intracellular cobalt staining.

2. The soma is 50 μm in diameter and lies on the upper posterior face of the protocerebrum, lateral to the midline. A neurite runs to a thickened integrating segment 20 μm in diameter, which bears numerous dendrites; none of these extends to the optic lobe. An axon leaves the integrating segment, crosses the brain, thickens to about 17 μm and descends the contralateral nerve cord.

3. The descending axon terminates in the metathoracic ganglion, where it has three major branches both ipsi- and contralateral. Its branching in the mesothoracic ganglion is similar, but extends only ipsilaterally; in the prothoracic ganglion there is reduced branching, and in the suboesophageal ganglion none at all.

4. The branching pattern in the metathorax is compatible with, and entirely explicable by, the known synaptic connexions with motoneurones.

5. The morphological description of the cell has made possible intracellular recording from axon, integrating segment and soma.

We are grateful to Dr R. Northrop for permission to quote his unpublished results, to Dr S. B. Kater for the use of his microscope, to Mr A. Blaker for advice on photographic technique. Ms B. Bacher, Dr Kater, Dr R. Murphey and Dr D. Bentley kindly criticized the manuscript. The work was supported in part by Grant 1 Ro1 NS09404 to C.H.F.R., and an S.R.C./N.A.T.O. postdoctoral fellowship to M. O'Shea.

REFERENCES

BURROWS, M. (1973). Physiological and morphological properties of the common inhibitory neuron of the locust. *J. cell. comp. Physiol.* **82**, 59–78.

BURROWS, M. & HOYLE, G. (1973). Neural mechanisms underlying behavior in the locust *Schistocerca gregaria*. III. Topology of limb motoneurones in the metathoracic ganglion. *J. Neurobiol.* **4**, 167–86.

BURROWS, M. & ROWELL, C. H. F. (1973). Connections between visual interneurons and metathoracic motoneurons in the locust. *J. comp. Physiol.* (in the Press).

HORN, G. & ROWELL, C. H. F. (1968). Medium and long-term changes in the behaviour of visual neurones in the tritocerebrum of locusts. *J. exp. Biol.* **49**, 143–69.

ILES, J. F. & MULLONEY, B. (1971). Procion yellow staining of cockroach motor neurons without the use of microelectrodes. *Brain Res.* **30**, 397–400.

NICHOLSON, C. & KATER, S. B. (eds.) (1973). *Intracellular Staining Techniques in Neurobiology*. Springer-Verlag.

O'SHEA, M. & ROWELL, C. H. F. (1973). Intracellular recording from the locust DMD neurones. (In preparation.)

PALKA, J. (1967*a*). An inhibitory process influencing visual responses in a fibre of the ventral nerve cord of locusts. *J. Insect Physiol.* **13**, 235–48.

PALKA, J. (1967*b*). Head movement inhibits locust visual unit's response to target movement. *Am. Zool.* **7**, 728.

PALKA, J. (1972). Moving movement detectors. *Am. Zool.* **12**, 497–505.

PITMAN, R. M., TWEEDLE, C. D. & COHEN, M. J. (1972). Branching of central neurons: intracellular cobalt injection for light and electron microscopy. *Science, N.Y.* **176**, 412–14.

REMLER, M., SELVERSTON, A. & KENNEDY, D. (1968). Lateral giant fiber of crayfish: location of somata by dye injection. *Science, N.Y.* **162**, 281–83.

ROEDER, K. D. (1948). Organization of the ascending giant fibre system in the cockroach (*Periplaneta americana*). *J. exp. Biol.* **108**, 243–61.

ROWELL, C. H. F. (1971*a*). The orthopteran descending movement detector (DMD) neurones: a characterisation and review. *Z. vergl. Physiol.* **73**, 167–94.

ROWELL, C. H. F. (1971*b*). Variable responsiveness of a visual interneurone in the free-moving locust, and its relation to behaviour and arousal. *J. exp. Biol.* **55**, 727–47.

ROWELL, C. H. F. (1971*c*). Antennal cleaning, arousal, and visual interneurone responsiveness in a locust. *J. exp. Biol.* **55**, 749–61.

ROWELL, C. H. F. & HORN, G. (1967). Response characteristics of neurones in an insect brain. *Nature, Lond.* **216**, 702–3.

ROWELL, C. H. F. & HORN, G. (1968). Dishabituation and arousal in the response of single nerve cells in an insect brain. *J. exp. Biol.* **49**, 171–83.

SANDEMAN, D. C. (1969). Integrative properties of a reflex motoneuron in the brain of the crab *Carcinus maenas*. *Z. vergl. Physiol.* **64**, 450–69.

SATIJA, R. C. (1957). Studies on the course and function of the giant nerve fibres from the eye and the tegumentary nerve to the ventral nerve cord in *Locusta migratoria*. *Res. Bull. Panjab. Univ. Sci.* **132**, 511–19.

SATIJA, R. C. (1958). A histological and experimental study of nervous pathways in the brain and thoracic nerve cord of *Locusta migratoria migratorioides*. *Res. Bull. Panjab Univ. Sci.* **137**, 13–22.

STRETTON, A. O. W. & KRAVITZ, E. A. (1968). Neuronal geometry: determination with a technique of intracellular dye injection. *Science, N.Y.* **162**, 132–34.

STRETTON, A. O. W. & KRAVITZ, E. A. (1973). Intracellular dye injection: the selection of Procion Yellow and its application in preliminary studies of neuronal geometry in the lobster nervous system. In *Intracellular Staining Techniques in Neurobiology* (ed. C. Nicholson and S. B. Kater), chapter 11. Springer-Verlag.

WILLIAMS, J. L. D. (1972). Some observations on the neuronal organisation of the supra-oesophageal ganglion in *Schistocerca gregaria* Forskål with particular reference to the central complex. Ph.D. thesis, University of Wales, Cardiff, Wales.

EXPLANATION OF PLATES

PLATE 1

A. Transverse section of cervical connectives of *S. vaga*. The arrows indicate the DCMD axon profiles in the dorso-medial segments of the cords.

B. Integrating segment, soma and neurite of the DCMD, cobalt injected. Stereoscopic pair, to be viewed with standard stereo viewers with 65 mm. separation.

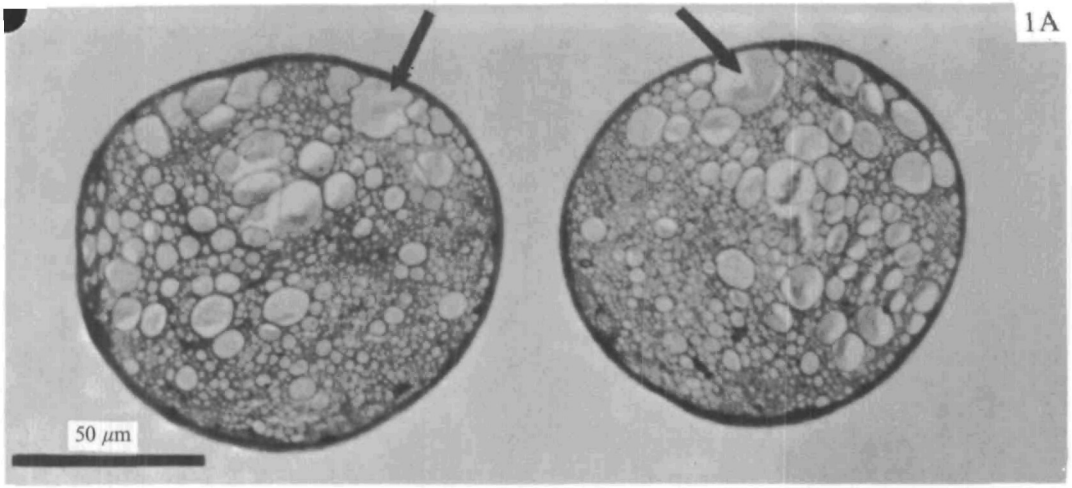
PLATE 2

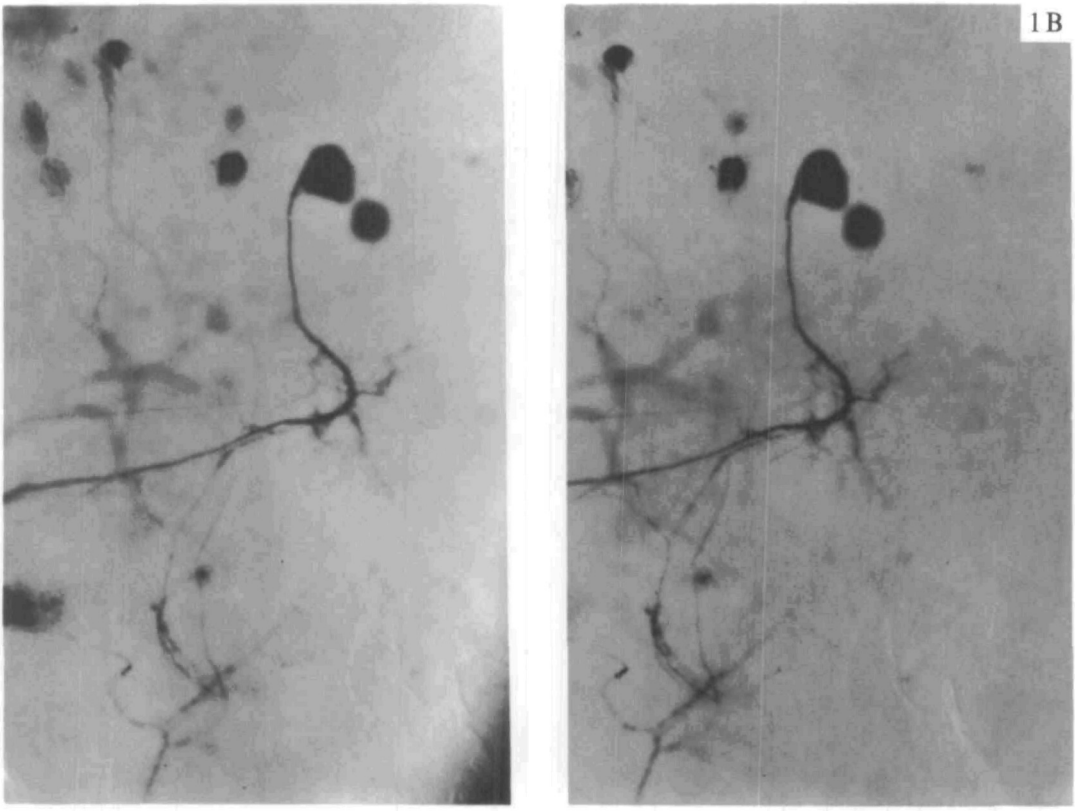
Whole mount of brain impregnated with cobalt by axonal iontophoresis from the neck connective. The DCMD axon is clearly seen crossing the brain to its integrating segment and soma. Most of the cells filled in this way are ipsilateral to the filled connective. Note the absence of projection of DCMD to the optic lobe.

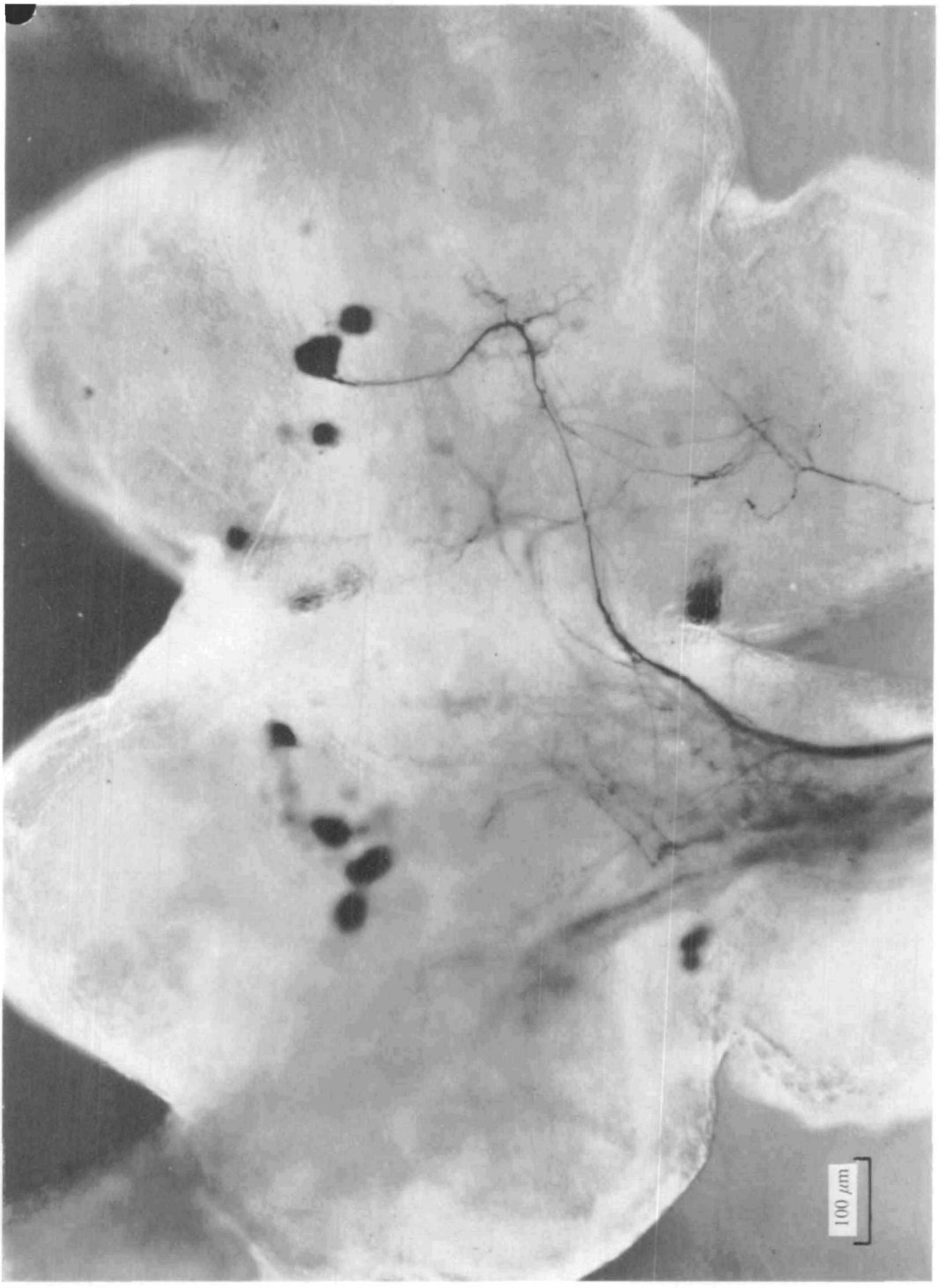
PLATE 3

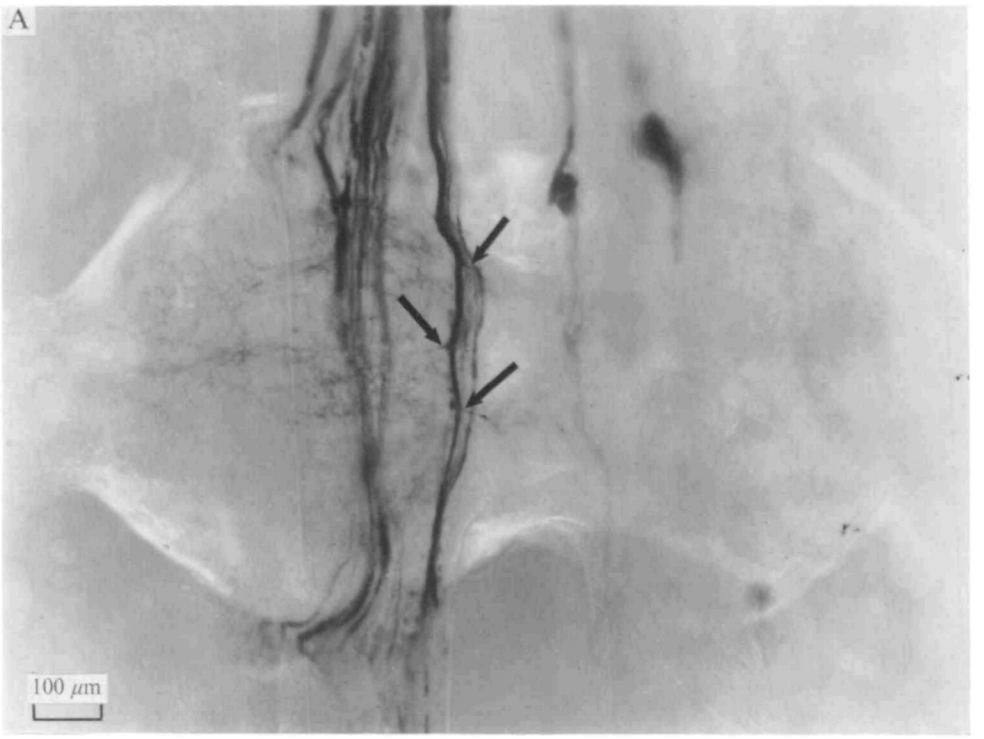
A. Whole mount of the mesothoracic ganglion. The DCMD, along with other neurones, filled with cobalt by axonal iontophoresis from the cervical connective. The arrows indicate the major branches of the DCMD.

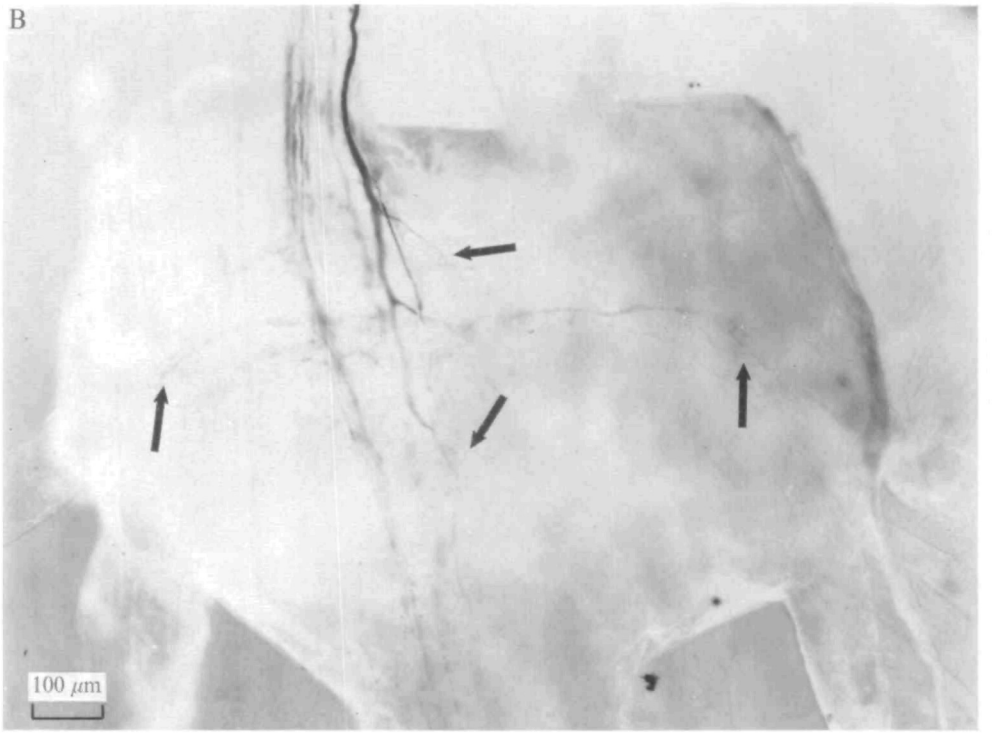
B. Whole mount of the metathoracic ganglion, filled from the pro/mesothoracic connective. Arrows indicate the major output arborizations. Compare with Text-fig. 3B.


1A
50 μm


1B


100 μm


A
100 μm


B
100 μm