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SUMMARY

The direct flight muscles of *Drosophila melanogaster* are innervated by the anterior dorsal mesothoracic (*ADM*) nerve and the mesothoracic accessory (*MAC*) nerve. Each of the four conspicuously large axons in the *ADM* nerve serves one of the muscles designated *pa1*, *pa3*, *pa4* and *pa5*. Muscle *pa4* is additionally innervated by a very small neurosecretory axon. Muscle *pa6*, also innervated by the *ADM* nerve, receives at least one small nerve fibre but no large axon. Muscle *pa2* is innervated by a large axon from the *MAC* nerve. Large motor axons, identified by serial section tracing from their respective muscles, are consistent among different individuals in both relative positions and relative diameters within the *ADM* nerve.

INTRODUCTION

Several neurones involved in escape behaviour and flight have been relatively well-studied in *Drosophila*. The giant fibre pathway, which triggers the initial events of escape, has been described anatomically (King & Wyman, 1980; Koto *et al.* 1981) and physiologically (Tanouye & Wyman, 1980). Motor neurones to the indirect flight muscles, which provide the main power for flight, are also known both anatomically (Coggshall, 1978; Ikeda, Koenig & Tsuruhara, 1980) and physiologically (Harcombe & Wyman, 1977, 1978; Koenig & Ikeda, 1980; Tanouye & Wyman, 1981). Recent experiments have examined the genetics of these interconnected motor systems (Thomas, 1980, 1981; Tanouye, Ferrus & Fujita, 1981; Thomas, Costello & King, 1982; Thomas & Wyman, 1982). Genetic study has been facilitated by the large size and simple synaptic relationships of neurones in the giant fibre and flight motor pathways.

This report and the one that follows (Tanouye & King, 1983) extend the analysis of associated motor systems in *Drosophila* by providing initial anatomical and physiological descriptions of neurones controlling direct flight muscles. In contrast to the large fibrillar muscles, which indirectly generate powerful wing beats by distorting the

thoracic walls, the direct flight muscles are small tubular muscles which act directly on wing-base sclerites to generate wing movements. The direct flight muscles control wing position for wing opening and closing and for directional guidance during flight. The present report establishes that the motor axons of several direct flight muscles are among the largest and most easily identified nerve fibres in the fly. The motor neurones are accessible for indirect recording of their electrical activity. At least one of these neurones is activated by the giant fibre pathway (Tanouye & King, 1983). This information provides a basis for further physiological and neurogenetic investigation into the neuromuscular mechanisms controlling flight in *Drosophila*.

MATERIALS AND METHODS

Adult female *Drosophila melanogaster* (wild-type strain Hochi, from the collection of Dr R. Wyman, Department of Biology, Yale University, New Haven, Connecticut, U.S.A.), aged 4–10 days post-eclosion, were immersed in cacodylate-buffered glutaraldehyde. Fixative could penetrate the thorax through holes cut by removing the head, abdomen, legs and wings. Specimens were then post-fixed in osmium tetroxide and dehydrated in ethanol. Thoraces embedded in Epon were cut into serial sections (0·5–5 μm) for light microscopy or ultrathin sections for electron microscopy. Ultrathin sections could be cut at selected locations by re-embedding thick sections that had previously been studied with the light microscope (King, Kammlade & Murphy, 1982). Individual motor axons within peripheral nerves were identified by tracing the axons through serial sections to the target muscles. Specimens for scanning electron microscopy were fixed, critical-point dried, and then dissected. For additional details on the histological processing, see King & Wyman (1980).

OBSERVATIONS

Our studies have confirmed Zalokar's (1947) gross anatomical description of direct flight muscles in the thorax of *Drosophila melanogaster*. The present account of motor innervation focuses on muscles which originate on the pleural body wall anterior to the pleural apophysis and insert *via* tendons or apodemes directly onto wing base sclerites, muscles which Zalokar called anterior pleural muscles (Fig. 1A). Zalokar's

Fig. 1. (A) Scanning electron micrograph illustrating the anterior pleural direct flight muscles. This view shows the medial aspect of the right pleural wall. The several branches of the *ADM* nerve were broken proximally during specimen preparation. The unlabelled muscle just posterior to the spiracle is *pp7*, which inserts on the prothoracic coxa (Zalokar, 1947). (B) Diagram of the anterior pleural muscles summarizing observations from six specimens prepared like the one above, together with information from serial sections. The branching pattern of the *ADM* and *MAC* nerves is reconstructed from sections since these nerves were always broken by dissection for scanning electron microscopy. Sites of axon entry into muscle are consistent among specimens. Note that, in contrast to other nerve branches, the nerve to muscle *pa6* distributes across the medial surface of the muscle. *ADMN* = anterior dorsal mesothoracic nerve, *MACN* = mesothoracic accessory nerve, *WN* = wing nerve, a sensory branch of the *ADM* nerve, *pADM* = the posterior branch of the *ADM* nerve, *NB* = nervenbrucke containing axons from both *ADM* and *MAC* nerves coursing in opposite directions, *pp* = axons from both *ADM* and *MAC* nerves continue to innervate posterior pleural muscles, *SP* = spiracle. Individual anterior pleural muscles (*pa*) are numbered according to Zalokar's (1947) system of nomenclature. Scale bar = 100 μm.
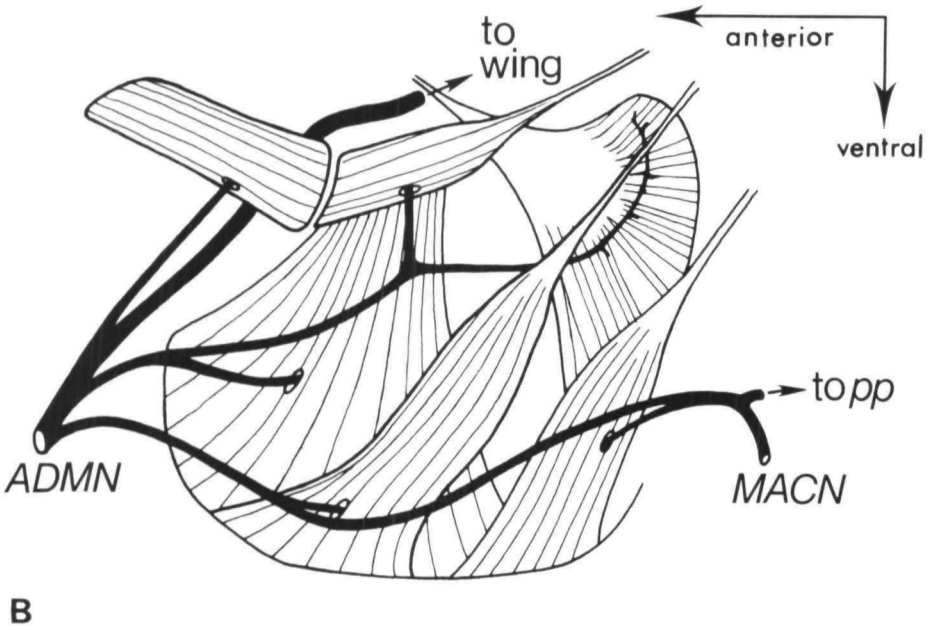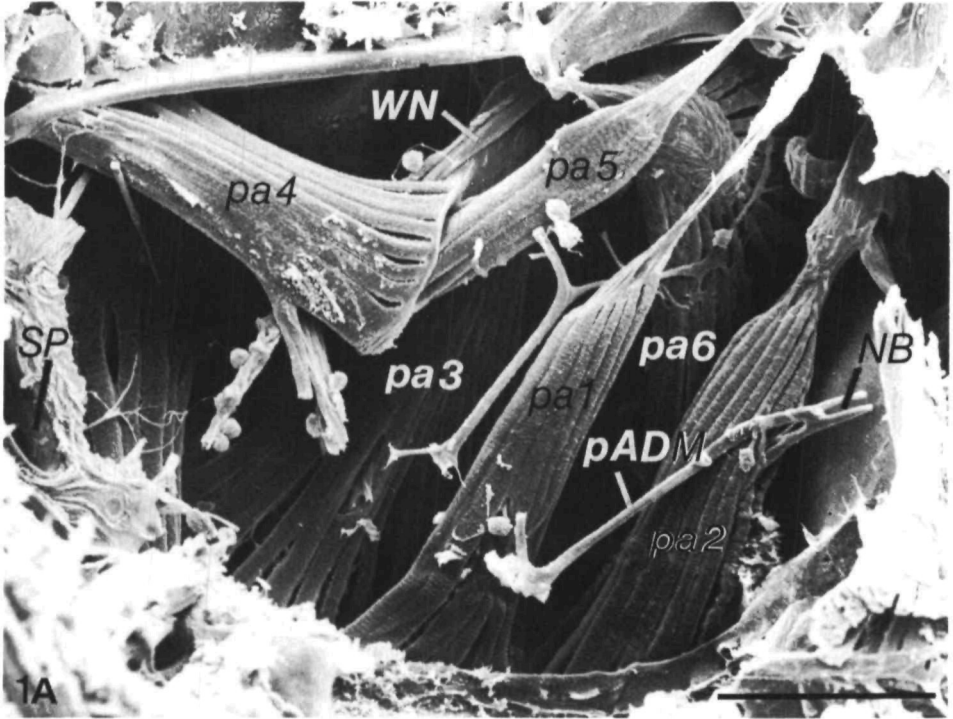
Table 1. *Synonyms for dipteran anterior pleural muscles*

| Reference	Organism	Muscles					
		pa1	*pa2*	*pa3*	*pa4*	*pa5*	*pa6*
Zalokar (1947)	*Drosophila melanogaster* (Drosophilidae)						
Ritter (1911)	*Calliphora vomitoria* (Calliphoridae)	Musculus levator alae primus (meI)	Musculus levator alae secundus (meII)	Musculus abductor alae secundus (mbII) and Musculus pronator alae (mp)	Musculus abductor alae primus (mbI)	Musculus gracilis (mg)	Musculus anonymus (ma)
Mihalyi (1935)	'Stubenfliege' (Muscidae)	Direktor D3	Retractor R2	Extensor E2	Extensor E1	Retractor R1	Direktor D1 and D2
Maki (1938)	*Orthellia claripennis* (Muscidae)	Pleuro-axillary muscle of 1st axillary sclerite	Pleuro-axillary muscle of 3rd axillary sclerite	Sterno-basalar muscle	2nd tergo-pleural muscle	1st tergo-pleural muscle	3rd tergo-pleural muscle
Williams & Williams (1943)	*Drosophila repleta* (Drosophilidae)	Anterior muscle of 1st axillary sclerite	Anterior muscle of 3rd axillary sclerite	Sternobasalar muscle	2nd basalar muscle	1st basalar muscle	3rd and 4th basalar muscles
Bonhag (1949)	*Tabanus* spp. (Tabanidae)	Anterior head, pleural muscle of the 1st axillary sclerite	Anterior head, pleural muscle of the 3rd axillary sclerite	Pleural muscle of the basalare	Anterior tergal muscle of the basalare	Posterior tergal muscle of the basalare	Tergal muscle of the pleural wing process
Miller (1950)	*Drosophila melanogaster* (Drosophilidae)	Muscle of the 1st axillary sclerite (53)	Muscle of the 3rd axillary sclerite (54)	Basalar muscle (51)	Muscle of prealar apophysis (49)	Muscle of prealar apophysis (50)	Basalar muscle (52)
Smart (1959)	*Anisopus fenestralis* (Anisopodidae)	Sternopleural branch of the axillary muscle of the 1st sclerite (9ai)	Episternal branch of the axillary muscle of the 3rd sclerite (9aii)	Inferior episternal basalar muscle (8b)	Anterior episternal basalar muscle (8a)	Tergobasalar muscle (7a)	Tergopleurosulcal muscle (7b)
Nachtigall & Wilson (1967)	*Muscoid* spp.	m.le.1	m.le.2	m.ab.2	m.ab.1	m.gr.	m.an.
Heide (1971)	*Calliphora erythrocephala* (Calliphoridae)	I1	III1	b2	b1	b3	tp

nomenclature, in which anterior pleural muscles are designated by the abbreviation *pa* followed by a number (e.g. *pa2, pa3*) will be followed in this report. Synonyms for these anterior pleural muscles and for homologous muscles in other species are listed in Table 1.

The anterior pleural muscles are innervated by two separate nerves named in Power's (1948) description: the anterior dorsal mesothoracic nerve (*ADM* nerve) and the mesothoracic accessory nerve (*MAC* nerve).

The *ADM* nerve is a large mixed nerve arising from the dorsolateral aspect of the mesothoracic neuromere. This nerve contains about 500 fibres, including four axons which are much larger than the others and are easily resolved at low power by light microscopy. These large axons, with diameters of 3 μm or greater, are motor fibres innervating muscles *pa1, pa3, pa4* and *pa5*. Among the smaller fibres are additional axons innervating *pa4*; at least one of the muscles *pa3, pa5* and *pa6*; and several posterior pleural muscles. Other small axons form a large and coherent bundle of sensory fibres originating from receptors on or near the wing. After leaving the ganglion the *ADM* nerve passes laterally across the front of the anterior dorso-ventral (fibrillar) muscle. Here the large motor axons begin to separate from the nerve trunk to form three separate branches, as indicated in Fig. 1B. Cross sections of these nerve branches are shown beside the anterior dorso-ventral muscle on the left side of Fig. 2A, and enlarged in Fig. 2B, C and D. The first of these branches (Fig. 2D) arises along the ventral aspect of the *ADM* nerve and curves posteriorly. This posterior branch contains one large motor axon that innervates muscle *pa1*, and five smaller axons which continue posteriorly, eventually crossing the pleural apophysis to innervate posterior pleural muscles. The next branch (Fig. 2C) also arises from the ventral aspect of the *ADM* nerve; it contains two large axons and three small axons. The more ventral of the two large axons soon separates to innervate muscle *pa3*. The other large axon innervates muscle *pa5*. The three small axons are difficult to trace reliably through serial sections; at least one must continue to innervate muscle *pa6* since no other nerve approaches this muscle. The third branch (Fig. 2B) arises dorsally; it contains one large axon and one very small axon, both of which innervate muscle *pa4*. The remainder of the *ADM* nerve, called the wing nerve, consists of sensory axons

FIG. 2. Motor nerves innervating the anterior pleural muscles, with axons identified by tracing through serial sections to their target muscles. (A) Parasagittal section showing the positions of motor nerves to the anterior pleural muscles. The nerve branches labelled B, C, D, E are enlarged below. DVM = dorsoventral muscle, TTM = tergotrochanteral muscle, WN = wing nerve, the sensory branch of the *ADM* nerve, HN = haltere nerve. Scale bar = 100 μm. (B) This branch of the *ADM* nerve innervates muscle *pa4* and contains one large axon and one very small axon. The small axon (arrowhead) is probably the source of neurosecretory terminals found in muscle *pa4*. The large motor axon is somewhat collapsed in this cross section, presumably due to some problem with preparation. The true diameter of this axon, as determined in more proximal sections where a more normal profile was seen, is approximated by the long axis of the profile in this micrograph. Scale bar = 5 μm. (C) This branch of the *ADM* nerve contains axons to muscles *pa3, pa5* and *pa6*. The two large axons go to muscles *pa3* and *pa5*. Of these two, the *pa3* axon is larger and (in most specimens) more ventral. The three smaller axons (arrowheads) have not been reliably traced in serial sections. At least one must be destined for muscle *pa6*, the only other muscle innervated by this branch of the *ADM* nerve. Scale bar = 5 μm. (D) This posterior branch of the *ADM* nerve contains one large axon innervating muscle *pa1* and five smaller axons which travel posteriorly across the pleural apophysis to innervate posterior pleural muscles. Scale bar = 5 μm. (E) This distal branch of the *MAC* nerve contains one axon which passes anteriorly across the pleural apophysis to innervate muscle *pa2* and three additional axons going to posterior pleural muscles. Scale bar = 5 μm.
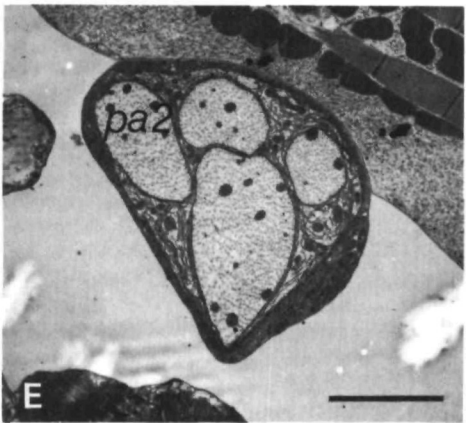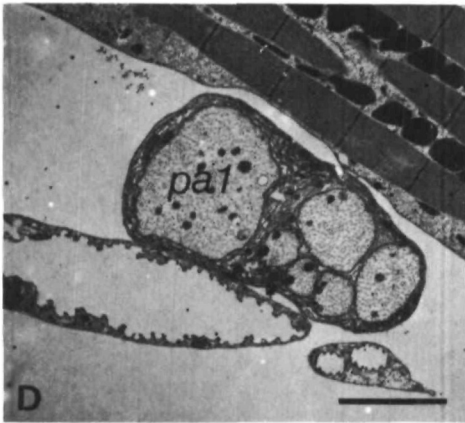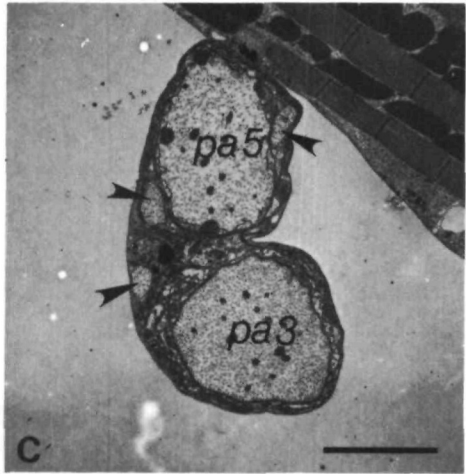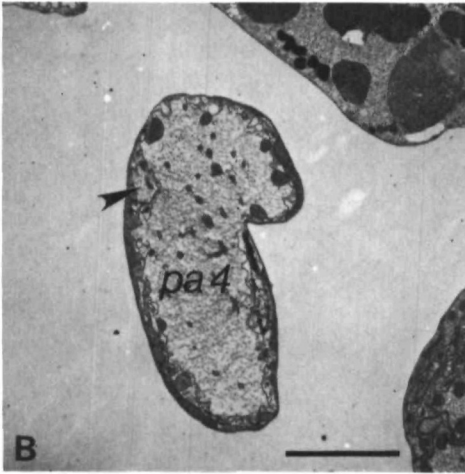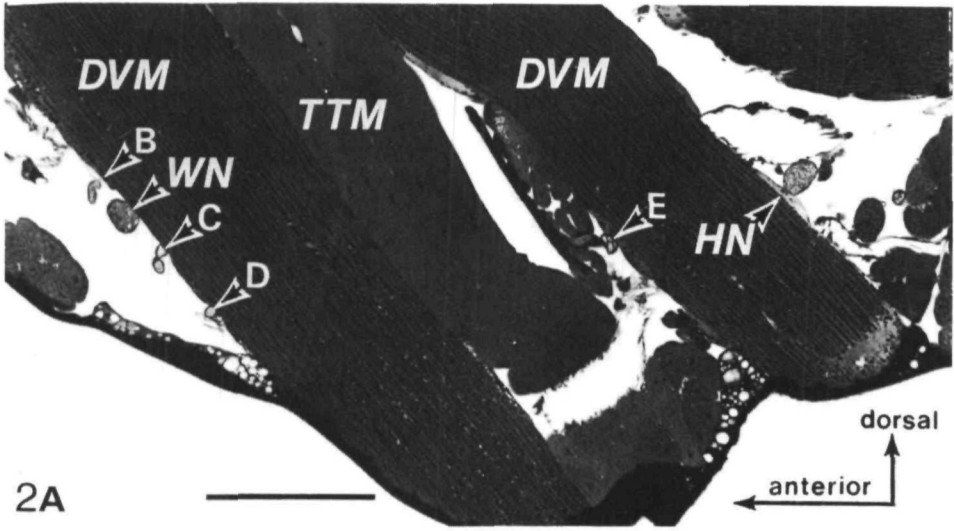
from the wing. The arrangement of large motor axons in the distal *ADM* nerve is quite consistent from individual to individual. The conspicuous components of the nerve are arrayed, from dorsal to ventral, as follows (Fig. 2A; also see Fig. 2B in Tanouye & King, 1983): *pa4* axon, wing nerve sensory axons, *pa5* axon, *pa3* axon and *pal* axon. The axons to muscles *pa3* and *pa5* may occasionally be reversed; but may be distinguished since the *pa3* axon is the larger of the two. The motor axon to muscle *pa4* is most readily identified, since it is separated from the other motor axons by the sensory bundle and is also consistently the largest axon in the nerve.

The *MAC* nerve appears to contain motor axons only. This nerve originates from the posterior aspect of the mesothoracic neuromere, passes beneath the arm of the sternal apophysis (mesofurca), then curves laterally behind the sternopleural muscle. Proximal branches of the *MAC* nerve innervate the sternopleural muscle as well as more posterior muscles not identified in this study. Distal to these branches the *MAC* nerve contains four large axons only, one noticeably larger than the rest (Fig. 2E). One of the three similar-sized axons passes anteriorly across the pleural apophysis to innervate muscle *pa2*; muscle *pa2* is the only anterior pleural muscle not innervated by the *ADM* nerve. The other *MAC* axons innervate posterior pleural muscles.

It may be noted from this description that both the *ADM* nerve and the *MAC* nerve innervate both anterior and posterior pleural muscles. Five small axons from the posterior branch of the *ADM* nerve cross the pleural apophysis posteriorly while one axon from the *MAC* nerve crosses the pleural apophysis anteriorly, with the curious result that at this point a single nerve bundle includes motor axons coursing in opposite directions. Heide (1971) first pointed out this 'nervenbrucke' connecting the *ADM* nerve and the *MAC* nerve in *Calliphora*, whose gross pattern of flight motor nerve branches appears virtually identical to that described here.

The axons innervating muscles *pal*, *pa2*, *pa3*, *pa4* and *pa5* are all relatively large for *Drosophila*, with diameters typically ranging from 3–10 μm (Fig. 2). The motor axon to muscle *pa4* is consistently the largest of these, occasionally reaching a diameter of 15 μm. The *pa4* axon is thus one of the largest nerve fibres in the fly, comparable only to the cervical giant fibre and the giant motor axon of the tergotrochanteral muscle, both elements in a specialized giant fibre pathway (King & Wyman, 1980). The axon to muscle *pa3* is noticeably smaller than that to *pa4*; the *pa5* and *pal* axons are slightly smaller still; and the *pa2* axon is the smallest of these identified nerve fibres. Absolute axon diameters vary rather widely, perhaps in relation to size or age of the specimen or to specific location along the nerve; these factors were not carefully controlled. It is the *relative* size among these large motor axons that is consistent among individual flies. The large diameters of these direct flight muscle axons together with the small size of *Drosophila* greatly facilitates serial section tracing of individual axons from ganglion to muscle. This should allow routine identification of motor processes within the thoracic ganglion and permit future analysis of synaptic contacts involving direct flight muscle motor neurones.

Each of the anterior pleural muscles (with one exception) receives one large motor axon. The motor axon enters its target muscle at a consistent location (Fig. 1B) by passing between adjacent muscle fibres. From within the muscle the axon sends fine terminal branches between muscle fibres where neuromuscular junctions are

established (Fig. 3A, B). The only exception to this pattern is muscle *pa6*. This muscle receives no large axons; the small axon(s) apparently destined for this muscle (Fig. 2C) have not yet been reliably traced through serial sections. Since muscle *pa6* is basically a planar fan of muscle fibres rather than a solid mass, its motor nerve cannot penetrate into the muscle but rather is distributed across its medial surface (Fig. 1).

Neuromuscular junctions have been observed in muscles *pa1*, *pa3* and *pa4*. The presynaptic terminals contain synaptic vesicles, some of which are intimately associated with specialized T-bar membrane structures (Fig. 3B). These endings thus display morphology typical of dipteran chemical synapses (see Toh & Kuwabara, 1975; Osborne, 1975). The subsynaptic cytoplasm of the muscle fibres appears denser beneath each junctional contact than in surrounding regions (Fig. 3B). Neuromuscular junctions are frequently encountered in most sections of these muscles, but their size, distribution and ultrastructure have not yet been quantitatively studied. In addition to such standard neuromuscular junctions, muscle *pa4* was found to include small neural processes, distinct from the motor axon terminals, which contain large dense vesicles (Fig. 3C). These processes resemble neurosecretory endings found in other insects. They probably arise from a very small axon that accompanies the large *pa4* motor axon (Fig. 2B). Whether any other direct flight muscles receive similar neurosecretory endings has not been determined. Since five axons (two large and three small) are associated with the three muscles *pa3*, *pa5* and *pa6*, at least one of these muscles must also be multiply innervated, although this has not yet been directly confirmed. Muscle *pa1* appears to receive one large axon only, since the other five axons in the posterior branch of the *ADM* nerve continue across the pleural apophysis.

DISCUSSION

The identified motor axons described above are consistent not only in their peripheral nerve courses and destinations but also in their relative positions and diameters within the peripheral nerve. These observations extend the results of Ikeda *et al.* (1980) who found consistent relative positions of dorsal longitudinal muscle motor axons in the posterior dorsal mesothoracic nerve of *Drosophila*. These consistent morphological features offer an opportunity to identify specific motor neurones without the laborious tracing of axons from muscles or the use of intracellular labels

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Fig. 3. Axon terminals in anterior pleural muscle *pa4*. (A) Low power electron micrograph showing the large motor axon as it enters muscle *pa4* (large arrowhead). Small terminal branches (small arrowheads) establish neuromuscular contact between muscle fibres. Continuity of these small processes with the large motor axon was confirmed by examination of serial ultrathin sections. Scale bar = 5 μm. (B) An axon terminal (A) filled with synaptic vesicles forms several specialized junctions (arrowheads) with the adjacent muscle fibre. At one of these junctions a dense T-bar (double arrowheads), attached to the presynaptic membrane, appears to be organizing the synaptic vesicles between the axon membrane and the cap of the T-bar. Dense subsynaptic cytoplasm (asterisks) can be seen in the muscle fibre beneath each junction. *mf* = myofilaments, *mit* = mitochondrion. Scale bar = 1 μm. (C) Associated with specialized motor axon terminals (A) are small profiles (large arrowheads) containing large dense vesicles. These apparently neurosecretory endings arise from small fibres (such as that indicated by the small arrowheads) distinct from the motor axon terminals. Scale bar = 1 μm.
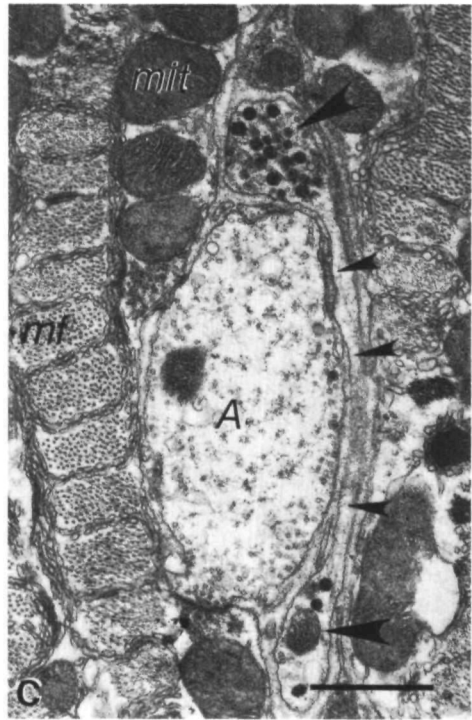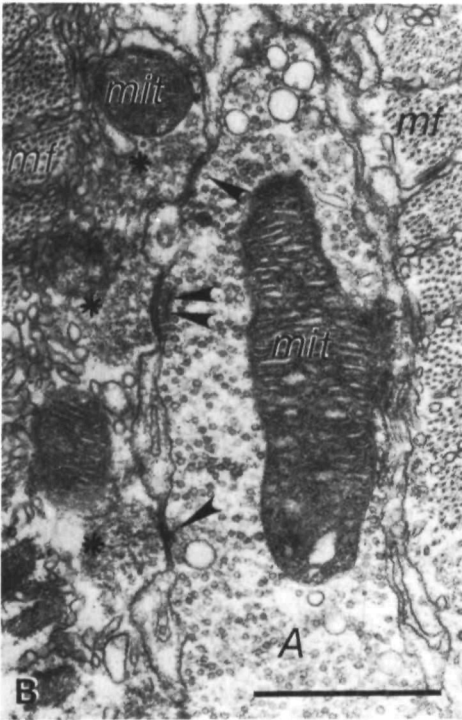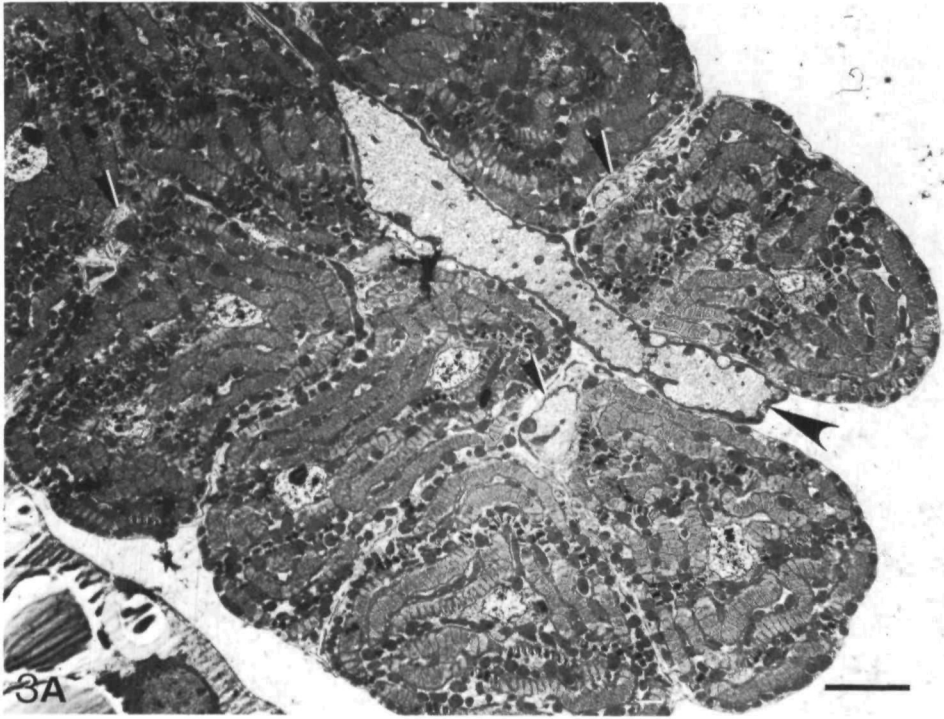
injected into somata. Preliminary observations within the central nervous system (D. G. King, unpublished) suggest that here also these motor axons display consistency in the positions of their major postsynaptic processes. Thus, these identified motor neurones may become routinely accessible for histological analysis of both synaptic morphology and peripheral axonal form.

One anterior pleural muscle (*pa4*) receives a fine neurosecretory fibre in addition to its single large motor axon. This is apparently the first report of neurosecretory endings in *Drosophila* flight muscles, although similar endings are found in a variety of insect and vertebrate muscles (Osborne, Finlayson & Rice, 1971). A previous description of neuromuscular endings in *Drosophila* did not indicate such neurosecretory terminals in the dorsal longitudinal muscle (Shafiq, 1964). The function of neurosecretory axons in muscle is still obscure. Even the distribution of such axons to different muscles within an organism has never been determined. The direct flight muscles of *Drosophila* may provide a good model for studying the distribution of such fibres, since several distinct muscles can be included in single sections prepared for electron microscopy. The existence of small midline neurones with fine axons to flight motor nerves (Coggshall, 1978), together with the current observation of neurosecretory endings in flight muscle, suggests that *Drosophila* may possess neurones similar to the neurosecretory DUM (dorsal, unpaired, median) neurones found in locusts (Hoyle, Colquhoun & Williams, 1980). Genetic manipulation of muscle development in *Drosophila* (Costello & Thomas, 1981) may eventually offer some clues to the functional role of neurosecretion in muscle.

A major challenge for developmental biology is presented by the morphogenesis of specific features that characterize identifiable neurones. The motor neurones described in this report are characterized by consistent diameters and positions within their respective nerves as well as by specific peripheral pathways and target muscles. A growing axon may reach its destination along pathways established by pioneer fibres early in development when the distances to be traversed are still quite small (Goodman & Bate, 1981). This of course does not explain how a growing motor axon recognizes its appropriate target muscle, nor why axons destined for adjacent muscles follow divergent nerve courses in the *ADM* and *MAC* nerves. The anatomical site from which a muscle originates (Lawrence, 1982) does not seem to determine the course of its motor axon, since both *ADM* and *MAC* nerves innervate muscles from both dorsal and ventral regions.

Mechanisms that determine consistent axon diameter are also unknown. The hypothesis that axon diameter is precisely adapted to provide a behaviourally appropriate conduction velocity is appealing. If this were true, the ontogenetic mechanisms which determine axon diameter must allow for differential modification of individual axons through evolution. This hypothesis is supported by the existence of giant-axon pathways mediating rapid escape reflexes in a variety of invertebrate and vertebrate animals. Whether this concept will include axons which are not so extremely specialized is not yet clear. It was somewhat surprising that the largest axon to a direct flight muscle (*pa4*) in *Drosophila* is not linked to the giant fibre pathway while a smaller axon (to muscle *pa3*) within the same nerve is associated with this pathway (Tanouye & King, 1983). Perhaps muscle *pa3*, although driven by the cervical giant fibre, need not be activated immediately in the reflex initiation of flight. By contrast,

the tergotrochanteral muscle, also activated by the cervical giant fibre (Tanouye & Wyman, 1980) receives one of the shortest and largest-diameter axons in the fly (King & Wyman, 1980). The large axon to muscle *pa4*, although apparently not needed in the giant fibre reflex, may participate in some other very rapid reflex, perhaps involved in flight guidance. The occurrence of motor axons as small as those apparently innervating muscle *pa6* is difficult to interpret in view of a total lack of information about the function of this muscle. (See Tanouye & King, 1983 for additional discussion of the functions for direct flight muscles in *Drosophila*.) Such functional rationalization for specific axon diameter is unconvincing, however, without additional supporting data. An alternative explanation for consistent differences in diameter among different identified axons is that such differences reflect incidental consequences of developmental processes and have no specific adaptive significance with respect to conduction velocity.

Additional physiological investigation together with comparative study of homologous axons in other flies, whose relative axonal size distribution can differ from that in *Drosophila* (D. G. King, unpublished observations), may help resolve the significance of specific determination of motor axon size. Such studies, which complement and extend those analysing genetically modified motor pathways in *Drosophila* (Thomas & Wyman, 1982; Thomas, 1980, 1981; Thomas *et al.* 1982), can contribute toward understanding the mechanisms that regulate cellular morphogenesis in the nervous system.

REFERENCES

BONHAG, P. F. (1949). The thoracic mechanism of the adult horsefly (Diptera: Tabanidae). Cornell University Agricultural Experiment Station, Memoir 285.

COGGSHALL, J. C. (1978). Neurons associated with the dorsal longitudinal flight muscle of *Drosophila melanogaster*. *J. comp. Neurol.* 177, 707–720.

COSTELLO, W. J. & THOMAS, J. B. (1981). Development of thoracic muscles in muscle-specific mutant and normal *Drosophila melanogaster*. *Soc. Neurosci. Abstr.* 7, 543.

GOODMAN, C. S. & BATE, M. (1981). Neuronal development in the grasshopper. *Trends in Neurosciences* 4, 163–169.

HARCOMBE, E. S. & WYMAN, R. J. (1977). Output pattern generation by *Drosophila* flight motoneurons. *J. Neurophysiol.* 40, 1066–1077.

HARCOMBE, E. S. & WYMAN, R. J. (1978). The cyclically repetitive firing sequences of identified *Drosophila* flight motoneurons. *J. comp. Physiol.* 123A, 271–279.

HEIDE, G. (1971). Die Funktion der nicht-fibrillären Flugmuskeln von *Calliphora*. Teil I. Lage, Insertionsstellen und Innervierungsmuster der Muskeln. *Zool. Jb. (Physiol.)* 76, 87–98.

HOYLE, G., COLQUHOUN, W. & WILLIAMS, M. (1980). Fine structure of an octopaminergic neuron and its terminals. *J. Neurobiol.* 11, 103–126.

IKEDA, K., KOENIG, J. H. & TSURUHARA, T. (1980). Organization of identified axons innervating the dorsal longitudinal flight muscle of *Drosophila melanogaster*. *J. Neurocytol.* 9, 799–823.

KING, D. G., KAMMLADE, N. & MURPHY, J. (1982). A simple device for assisting with reembedding thick plastic sections. *Stain Technol.* 57, 307.

KING, D. G. & WYMAN, R. J. (1980). Anatomy of the giant fiber pathway in *Drosophila*. I. Three thoracic components of the pathway. *J. Neurocytol.* 9, 753–770.

KOENIG, J. H. & IKEDA, K. (1980). Neural interactions controlling timing of flight muscle activity in *Drosophila. J. exp. Biol.* **87**, 121–136.

KOTO, M., TANOUYE, M. A., FERRUS, A., THOMAS, J. B. & WYMAN, R. J. (1981). The morphology of the cervical giant fiber neuron of *Drosophila. Brain Research* **221**, 213–217.

LAWRENCE, P. A. (1982). Cell lineage of the thoracic muscles of *Drosophila. Cell* **29**, 493–503.

MAKI, T. (1938). Studies on the thoracic musculature of insects. *Mem. Fac. Sci. Agric. Taihoku Imp. Univ.* **24**, 1–343.

MIHALYI, F. (1935/1936). Untersuchungen über Anatomie and Mechanik der Flugorgane an der Stubenfliege. *Arb. ung. biol. Forsch Inst.* **8**, 106–119.

MILLER, A. (1950). The internal anatomy and histology of the imago of *Drosophila melanogaster.* In *Biology of* Drosophila, (ed. M. Demerec). New York: John Wiley & Sons, Inc.

NACHTIGALL, W. & WILSON, D. M. (1967). Neuro-muscular control of dipteran flight. *J. exp. Biol.* **47**, 77–97.

OSBORNE, M. P. (1975). The ultrastructure of nerve-muscle synapses. In *Insect Muscle*, (ed. P. N. R. Usherwood). New York: Academic Press.

OSBORNE, M. P., FINLAYSON, L. H. & RICE, M. J. (1971). Neurosecretory endings associated with striated muscles in three insects (*Schistocerca, Carausius*, and *Phormia*) and a frog (*Rana*). *Z. Zellforsch. mikrosk. Anat.* **116**, 391–404.

POWER, M. E. (1948). The thoracico-abdominal nervous system of an adult insect, *Drosophila melanogaster. J. comp. Neurol.* **88**, 347–409.

RITTER, W. (1911). The flying apparatus of the blow fly. A contribution to the morphology and physiology of the organs of flight in insects. *Smithson. misc. Collns* **56**, 1–77.

SHAFIQ, S. A. (1964). An electron microscopical study of the innervation and sarcoplasmic reticulum of the fibrillar flight muscle of *Drosophila melanogaster. Q. Jl microsc. Sci.* **105**, 1–6.

SMART, J. (1959). Notes on the mesothoracic musculature of diptera. *Smithson. misc. Collns* **137**, 331–364.

TANOUYE, M. A., FERRUS, A. & FUJITA, S. C. (1981). Abnormal action potentials associated with the *Shaker* complex locus of *Drosophila. Proc. Natn. Acad. Sci. U.S.A.* **78**, 6548–6552.

TANOUYE, M. A. & KING, D. G. (1983). Giant fiber activation of direct flight muscles in *Drosophila. J. exp. Biol.* **105**, 241–251.

TANOUYE, M. A. & WYMAN, R. J. (1980). Motor outputs of giant nerve fiber in *Drosophila. J. Neurophysiol.* **44**, 405–421.

TANOUYE, M. A. & WYMAN, R. J. (1981). Inhibition between flight motoneurons in *Drosophila. J. comp. Physiol.* **144A**, 345–355.

THOMAS, J. B. (1980). Mutations affecting the giant fiber system of *Drosophila. Soc. Neurosci. Abstr.* **6**, 742.

THOMAS, J. B. (1981). A single gene mutation alters the morphology of the giant fiber in *Drosophila. Soc. Neurosci. Abstr.* **7**, 543.

THOMAS, J. B., COSTELLO, W. J. & KING, D. G. (1982). A genetic mutation may affect the growth rate of an identified neuron in a *Drosophila* motor system. *Soc. Neurosci. Abstr.* **8**, 702.

THOMAS, J. B. & WYMAN, R. J. (1982). A mutation in *Drosophila* alters normal connectivity between two identified neurons. *Nature, Lond.* **298**, 650–652.

TOH, Y. & KUWABARA, M. (1975). Synaptic organization of the flesh fly ocellus. *J. Neurocytol.* **4**, 271–287.

WILLIAMS, C. M. & WILLIAMS, M. V. (1943). The flight muscles of *Drosophila repleta. J. Morph.* **72**, 589–597.

ZALOKAR, M. (1947). Anatomie du thorax de *Drosophila melanogaster. Revue suisse Zool.* **54**, 17–53.