J. exp. Biol. **105**, 241–251 (1983)
Printed in Great Britain © The Company of Biologists Limited 1983

GIANT FIBRE ACTIVATION OF DIRECT FLIGHT MUSCLES IN *DROSOPHILA*

BY MARK A. TANOUYE

Division of Biology, California Institute of Technology, Pasadena, CA 91125, U.S.A.

AND DAVID G. KING

Department of Anatomy and Department of Zoology, Southern Illinois University, Carbondale, IL 62901, U.S.A.

(*Received 2 September 1982 – Accepted 14 March 1983*)

SUMMARY

1. Electrical stimuli delivered to the brain were used to activate the giant fibre of *Drosophila*.

2. The giant fibre drove a prominent wing opening movement.

3. Intracellular microelectrode recordings from direct wing opener muscle fibres showed that giant fibre activation of an anterior pleural muscle, *pa3*, was responsible for the wing opening movement.

4. The giant fibre drove a slight wing elevation movement.

5. Intracellular recordings from direct wing elevator muscle fibres showed that these muscles were not activated by the giant fibre.

6. It is suggested that giant fibre-driven wing elevation movements were mediated by the tergotrochanter muscle (*TTM*).

INTRODUCTION

A number of behavioural events act to initiate escape in many flies including *Drosophila* (Nachtigall, 1966; Nachtigall & Wilson, 1967; Kaplan & Trout, 1974). The most prominent events involve the mesothoracic legs and the wings. The mesothoracic legs drive a powerful escape jump, lifting the animal into the air. At the same time the wings are quickly elevated (Kaplan & Trout, 1974). The wings also move horizontally forward from the closed rest position into the opened flight position. Full wing opening is achieved over the course of the first six wingbeats (Nachtigall, 1966; Nachtigall & Wilson, 1967).

Escape may apparently be initiated by any of several neural pathways (Thomas, 1981). These pathways are, for the most part uncharacterized. However, recent studies have shown that the cervical giant fibres of *Drosophila* mediate a visually induced escape response by driving potentials in several escape muscles (Tanouye & Wyman, 1980; Thomas, 1980, 1981). The cervical giant fibres are a bilaterally symmetrical pair of large axons which run from the brain to the thoracic ganglion where their motor outputs occur (Power, 1948; Coggshall, Boschek & Buchner, 1973;

Key words: *Drosophila* giant fibre system, direct flight muscles, escape initiation.

King & Wyman, 1980; Koto *et al.* 1981). Giant fibre activation of the tergotrochanteral muscle (*TTM*) appears to account for several of the behaviour patterns observed in escape initiation (Tanouye & Wyman, 1980). The *TTM* is a large tubular muscle which functions as the main extensor of the middle leg and is responsible for the escape jump (Williams & Williams, 1943; Nachtigall & Wilson, 1967; Mulloney, 1969). Anatomically, the *TTM* could function as an indirect wing elevator (Williams & Williams, 1943; Nachtigall & Wilson, 1967). Thus, its activation might be sufficient to explain the first wing elevation seen in escape initiation. The giant fibre also activates the dorsal longitudinal muscle (*DLM*) and dorsoventral muscles (*DVM*s) which respectively provide the main power for wing depression and wing elevation during flight (Tanouye & Wyman, 1980). Since these muscles are fibrillar muscles, providing powerful contractions only when stretched (Pringle, 1949; Machin & Pringle, 1959), they do not make substantial contributions to wing position during escape initiation (Nachtigall & Wilson, 1967).

Previous studies on the giant fibres of *Drosophila* have focused on the *TTM*, *DLM* and *DVM*s, muscles which control wing position indirectly through distortions of the thoracic walls (Tanouye & Wyman, 1980). The present study extends the earlier analyses by describing giant fibre activation of direct flight muscles, tubular muscles which attach directly to the wing base. In particular, all direct wing elevators and wing openers were examined for giant fibre activation. The relationship between these giant fibre-driven wing movements and movements seen during escape initiation is discussed.

MATERIALS AND METHODS

Preparation

Wild type *Drosophila melanogaster* of the Canton-Special strain were obtained from the collection of Dr S. Benzer, Division of Biology, California Institute of Technology, Pasadena, CA, U.S.A. Adult females (aged 4–6 days posteclosion) were used.

For examining wing and leg movements, the tip of an insect pin was glued to the dorsal surface of the head and thorax with Eastman 910 adhesive. The pin was held with the fly suspended in the flight position. For experiments requiring dissection, flies were mounted so that the left side, facing up, was exposed to Ringer's solution while the right side, including all right side spiracles, was in contact with air circulating beneath the fly. Softseal tackiwax (Cenco Scientific) was used to keep the animal stationary and maintain the air/water interface. To facilitate placement of stimulating electrodes, the head was rotated 90° about the cervix so that the ventral surface of the head was facing up. The position of the head was stabilized with tackiwax.

The direct flight muscles were exposed by dissecting free and removing the mesopleurum (episternum) and part of the pteropleurite (Ferris, 1950). Particular care was taken not to damage air sacs and tracheoles. The *Drosophila* Ringer's solution (Jan & Jan, 1976) contained 128 mM-NaCl, 2 mM-KCl, 4 mM-$MgCl_2$, 35·5 mM-sucrose and 1·8 mM-$CaCl_2$. Solutions were buffered to pH 7·1 with 5 mM-Hepes (Sigma).

Electrodes and electronics

Electrical stimuli were delivered to the brain using insulated tungsten electrodes. Intracellular recordings of direct flight muscle potentials were made using glass micropipettes filled with a 4% aqueous solution of the dye Pontamine sky blue 6BX (G. T. Gurr Ltd, London). Pipettes were pulled on a Brown and Flaming puller (Sutter Instruments) and had resistances of 80–120 MΩ. Similar electrodes had resistances of 40–60 MΩ when filled with 3 M-KCl. Dorsal longitudinal muscle (*DLM*) potentials were recorded with insulated tungsten electrodes. Potentials were recorded through M4-A preamplifiers (WP Instruments) and displayed on a storage oscilloscope. Indifferent electrodes were Ag-AgCl in dissected preparations. In undissected preparations, indifferent electrodes were uninsulated tungsten electrodes located in the abdomen.

Response latencies were measured according to Tanouye & Wyman (1980). Measurements were made from the stimulus artifact (0·01 ms duration) to the first detectable voltage deflection of the evoked response. To facilitate determination of the latter, measurements were made using threshold stimuli and ineffective stimulus traces were used as baselines. Measurements were made directly from the storage oscilloscope screen or from a Polaroid photograph of the screen.

Giant fibre activation

Giant fibre activation was identified physiologically as described previously (Tanouye & Wyman, 1980; Koto *et al.* 1981; Tanouye, Ferrus & Fujita, 1981). Identification relies on the fact that the giant fibre drives the *DLM* in a one-to-one manner. The muscle apparently is not driven one-to-one by any other axon descending from the brain (Tanouye & Wyman, 1980; Koto *et al.* 1981; Tanouye *et al.* 1981). At the start of each experiment, an electrical stimulus delivered to the brain was used to drive the *DLM*. Threshold voltage to this stimulus was determined. A similar threshold voltage for each penetrated direct flight muscle fibre was determined and compared with the *DLM* threshold. Results from muscle fibres which could not be driven by brain stimulation were discarded. Ten to twenty stimuli were usually used to determine threshold for each direct flight muscle fibre examined. An additional 10–20 stimuli were used to measure latency relationships between the evoked direct flight muscle potential and the *DLM* potential. After threshold voltage and latency measurements had been made, stimulus polarity was reversed and the values were remeasured. The change in polarity controlled, to a certain extent, for differences in stimulating electrode position.

Evoked direct flight muscle potentials with the same threshold as the *DLM* were considered to be driven by the giant fibre, and hence part of the giant fibre system. Previous studies on the giant fibre with dye-filled intracellular microelectrodes have confirmed that this physiological identification is sufficient for reliable identification of giant fibre activation (Tanouye & Wyman, 1980; Koto *et al.* 1981).

Muscle terminology and identification of recording sites

Fig. 1 shows a schematic representation of the muscles referred to in this report.

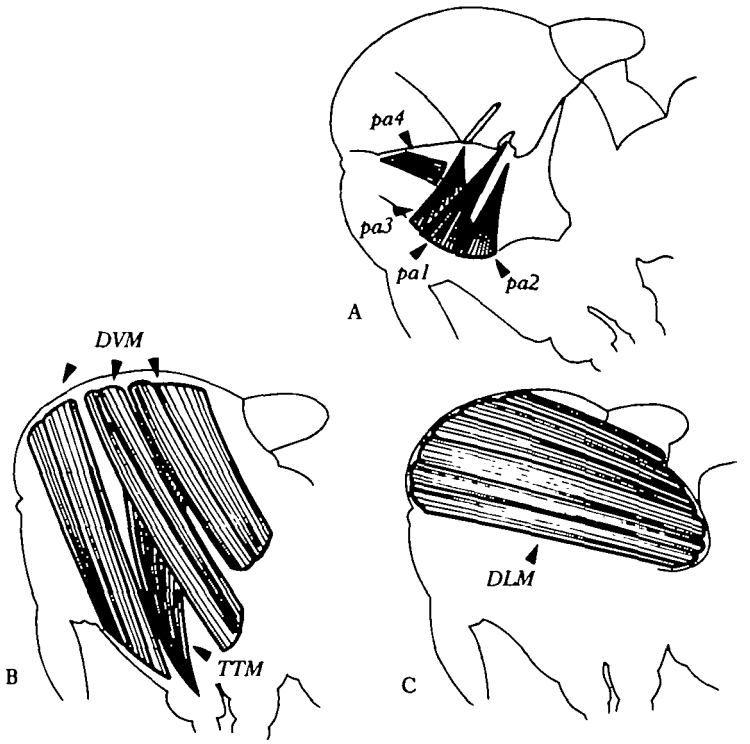
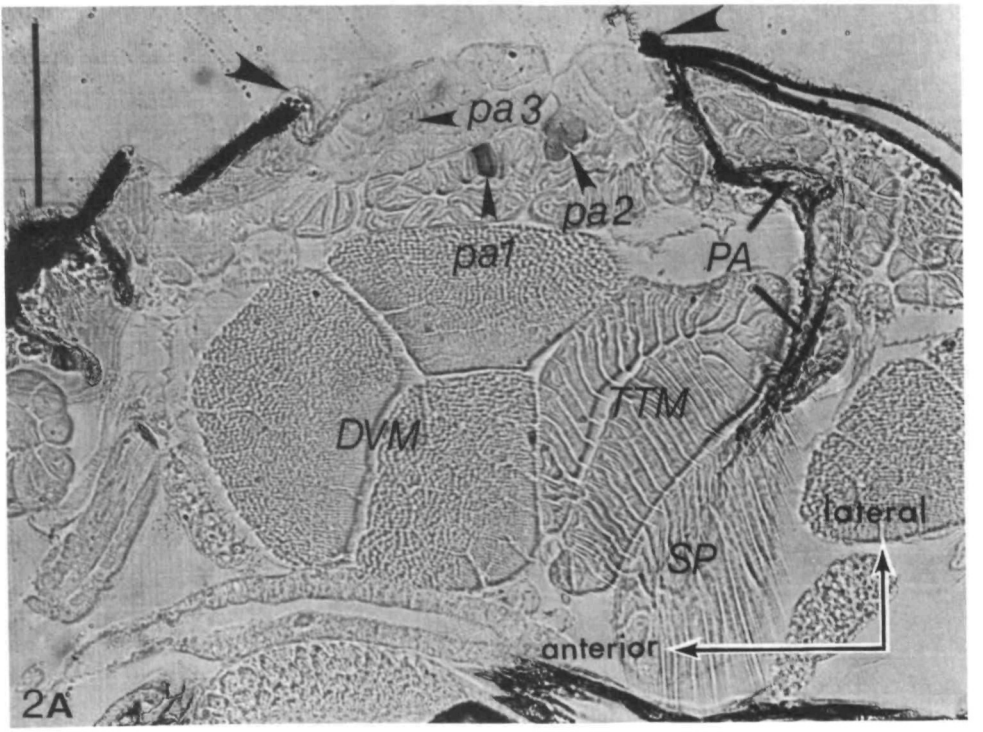
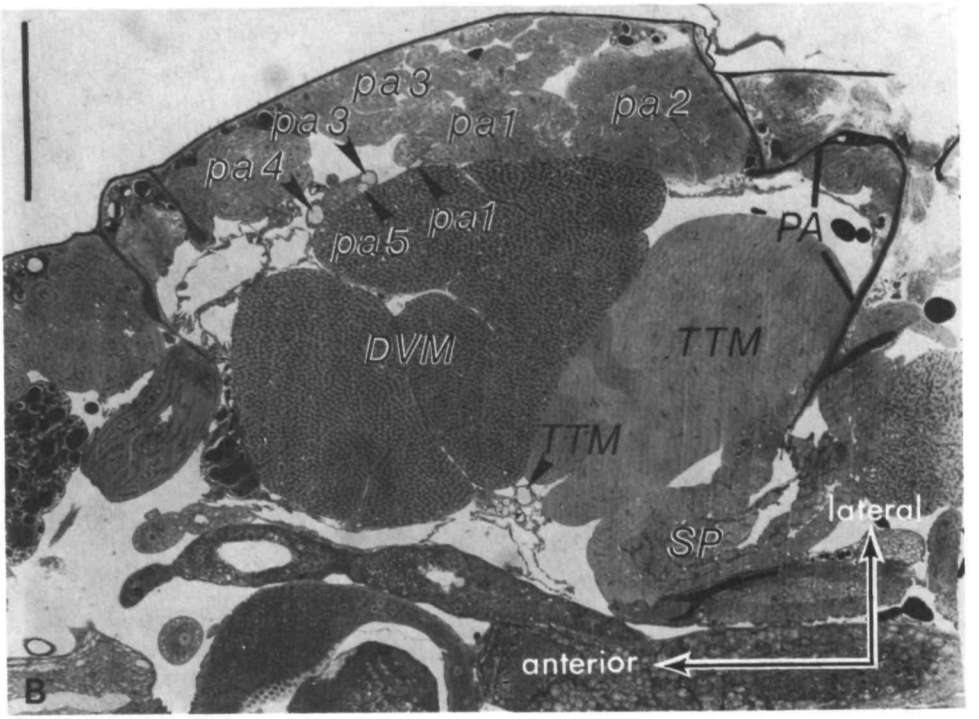


Fig. 1. Schematic representation of muscles referred to in this report. Redrawn from Zalokar (1947). Medial aspect. (A) Parasagittal view of the lateral thorax showing the anterior pleural direct flight muscles *pa1*, *pa2*, *pa3* and *pa4*. For clarity, other direct flight muscles are not included. (B) Parasagittal view of the thorax slightly medial to that in (A) showing the dorsoventral muscles (*DVM*) and the tergotrochanter muscle (*TTM*). (C) Parasagittal view of the thorax medial to that in (B) showing the dorsal longitudinal muscle (*DLM*).

The dorsal longitudinal muscle (*DLM*) and the dorsoventral muscles (*DVM*s) are the indirect fibrillar muscles which respectively depress and elevate the wing during flight (Harcombe & Wyman, 1977, 1978; Tanouye & Wyman, 1981). The tergotrochanter muscle (*TTM*) is a large tubular muscle used to extend the mesothoracic leg, providing the main power for jumping (Williams & Williams, 1943; Nachtigall & Wilson, 1967; Mulloney, 1969). The *TTM* may also act as an indirect wing elevator (Williams & Williams, 1943; Nachtigall & Wilson, 1967). The nomenclature of Zalokar (1947) is used for the direct flight muscles. Equivalent names for anterior pleural muscles *pa1*, *pa2*, *pa3* and *pa4* may be found in King & Tanouye (1983).

Electrode placement in the *DLM* was guided by its insertion site on the dorsal surface of the thorax (Tanouye & Wyman, 1980, 1981). Recording sites in direct flight muscle fibres were marked by a small amount of dye iontophoresed from the recording electrode. If more than one recording site was injected in a single specimen, the relative positions of these sites were mapped. At the end of each experiment, the thorax was fixed in Kahle's fixative, dehydrated in an ethanol series and embedded in Epon *via* propylene oxide. Each thorax was serially sectioned at 5 μm. Recording sites were identified by the iontophoresed dye, which appeared on unstained sections as faint blue spots confined within single muscle fibres (Fig. 2).


pa3
pa2
pa1
PA
TTM
DVM
lateral
SP
anterior
2A


pa3
pa3
pa1
pa2
pa4
pa5
pa1
PA
DVM
TTM
TTM
lateral
SP
anterior
B

RESULTS

Giant fibre system-driven wing movements

The giant fibre was stimulated electrically on the brain, and wing movements were observed through the dissecting microscope. Prior to stimulation, the wings were folded over the abdomen in the normal resting position. Following a single stimulus, the wings quickly moved laterally from the closed position to a partially opened position, making an angle of about 45° with respect to the long axis of the fly. The wings did not remain open, but closed back to the resting position. The closing occurred in two phases. A rapid initial closing brought the wings to an intermediate position about halfway back to resting. A final movement back to the resting position was slower and in some cases took several seconds. In response to high frequency (10 Hz) stimulation of the giant fibre, the wings remained tonically open (45° relative to the body axis) for several seconds. In spite of continued stimulation, however, the wings did not remain open but closed to an intermediate position similar to that described above. This intermediate position was maintained throughout the duration of the high frequency stimulation. After cessation of the stimulation, the wings moved slowly back to the resting position.

In addition to wing opening, giant fibre activation caused a very slight wing elevation. This movement was not as prominent as wing opening and was best observed during high frequency (10 Hz) stimulation of the giant fibre. Unlike wing opening which occurred tonically during high frequency stimulation, wing elevations occurred as small discrete movements correlated with the delivery of each stimulus. Interestingly, mesothoracic leg extensions were similarly discrete movements during high frequency stimulation (see below). Wing elevations were only observed while the wing was opened about 45° (above). They were not observed when the wing had closed to the intermediate position.

The remainder of this paper deals with the problem of how the giant fibre system generates wing elevation and opening movements. In particular, intracellular microelectrode recordings of direct wing elevator muscle fibres and direct wing opener muscle fibres were made during giant fibre activation. These recordings defined which of the direct flight muscles were driven by the giant fibre system. An earlier study described giant fibre activation of indirect wing elevators (Tanouye & Wyman, 1980). Thus, taken together, all major muscles responsible for wing elevation and opening

---

Fig. 2. Horizontal sections (anterior is to the right) of the *Drosophila* thorax showing recording sites in fibres of the anterior pleural muscles. (A) Unstained section of a specimen with Pontamine sky blue iontophoresed at three separate recording sites. Dye is visible in one labelled fibre of muscle *pa1*. A fainter label is also visible in a fibre of muscle *pa2*. Dye was also visible in a labelled fibre of muscle *pa3* in another section from the same specimen (not shown). Small arrowheads indicate labelled muscle fibres. Cuticle in the region between large arrowheads was cut away to allow penetration of individual muscle fibres by glass microelectrodes. (B) Stained section from another specimen to display more clearly the relative positions of thoracic muscles and motor nerves. Note the large motor axons (small arrowheads) in the anterior and posterior mesothoracic nerves. The identified axons are labelled to match the muscles they innervate. The relative positions and diameters of these axons are consistent among different individuals (see King & Tanouye, 1983); the *pa4* axon is similar in diameter to the *TTM*. The symbols are: *DVM* = dorsoventral fibrillar muscle, *TTM* = tergotrochanter muscle, *pa* = anterior pleural muscles (numbered *1–4*), *SP* = sternopleural muscle, *PA* = pleural apophysis. Scale bar = 100 μm.

have been tested and a complete description of giant fibre system contributions to these movements is presented here.

Intracellular recordings from direct flight muscle fibres

Intracellular microelectrode recordings from direct flight muscle fibres gave resting potential values of −80 to −90 mV and evoked potentials of 70–90 mV amplitude. Spontaneous activity was rarely observed. Action potential and resting potential amplitudes in direct flight muscle fibres declined rapidly by 20–40 mV following the delivery of three-to-five effective stimuli. This was probably due to damage at the microelectrode recording site caused by contraction of the muscle fibre. The changes in amplitude had no effect on the voltage thresholds required to evoke muscle potentials and the latencies of the potentials. Thus, the amplitude changes do not alter the main conclusions presented in this report and no attempt was made to minimize muscle fibre contractions.

Direct wing elevators are not driven by the giant fibre system

The anterior pleural muscles *pa1* and *pa2* (Fig. 1) are the direct flight muscles responsible for wing elevation (Williams & Williams, 1943; Nachtigall & Wilson, 1967). Fig. 3 shows evoked potentials recorded in the *DLM* and *pa1*. The *DLM* and *pa1* have different thresholds to brain stimulation suggesting that *pa1* is not driven by the giant fibre. In Fig. 3A stimulus voltage was at threshold for the *DLM* response (9 V). Two stimuli were delivered. One stimulus was effective and evoked a potential in the *DLM*. A second stimulus of the same intensity was ineffective and failed to evoke the *DLM* response. The ineffective trace was superimposed on the effective trace and serves as a baseline. Note that neither stimulus evoked a response in the *pa1* fibre.

Fig. 3B shows responses for the same two fibres where stimulus voltage was at threshold for the *pa1* response (17 V). One stimulus was effective in evoking a *pa1* potential. A second stimulus was ineffective. Since the stimuli were above threshold for the *DLM* response, both stimuli evoked *DLM* potentials. These results indicate that the *pa1* has a different threshold to brain stimulation than the *DLM*. The suggestion is that *pa1* is not driven by the giant fibre. Similar results were obtained for seven

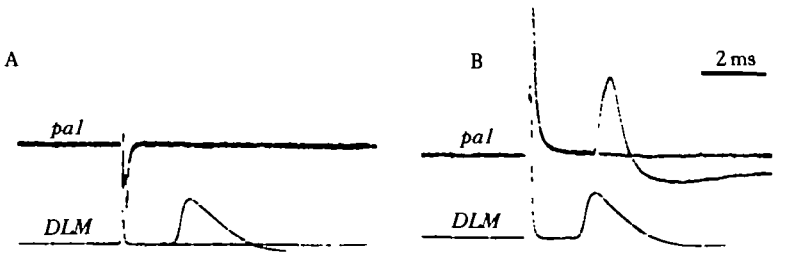


Fig. 3. Evoked potentials in a *DLM* and *pa1* fibre following brain stimulation. (A) Stimulus voltage at threshold for the *DLM* response (9 V, 0·01 ms duration). *DLM* responses to an effective (suprathreshold) stimulus and an ineffective (subthreshold) stimulus are shown superimposed. Note that neither stimulus evokes a *pa1* response. (B) Stimulus voltage at threshold for the *pa1* response (17 V, 0·01 ms duration). *pa1* responses to an effective stimulus and an ineffective stimulus are shown superimposed. Note that both stimuli evoke *DLM* responses.

pa1 fibres in five animals. The *pa1* is, however, activated one-to-one by another, higher threshold, descending pathway which can be activated by brain stimulation. This descending pathway was not identified in the present analysis.

Similar results were obtained for the other direct wing elevator muscle, *pa2*. Like *pa1*, *pa2* was activated by brain stimulation but at a threshold of stimulation different from *DLM* activation. Thus, *pa2* is likewise not driven by the giant fibre but rather is activated one-to-one by another, non-giant fibre, descending pathway. *pa1* and *pa2* are the only direct wing muscles thought to subserve wing elevation (Nachtigall & Wilson, 1967). Thus, the results presented here indicate that giant fibre-driven wing elevation movements are not mediated by direct wing muscles.

The direct wing opener pa3 *is driven by the giant fibre system*

The anterior pleural muscles *pa3* and *pa4* (Fig. 1) are the direct wing muscles responsible for wing opening (Williams & Williams, 1943; Nachtigall & Wilson, 1967; Heide, 1971). Results presented in this section show that *pa3* is activated by the giant fibre; *pa4* is not. Thus, giant fibre-driven wing opening movements appear to be mediated solely *via* activation of *pa3*.

Fig. 4 shows evoked potentials recorded in the *DLM* and a direct flight muscle fibre of *pa3*. The evoked muscle potentials had the same threshold and were never evoked independently. In Fig. 4, two threshold stimuli were delivered to the brain. One stimulus was effective and evoked potentials in both the *DLM* and the *pa3* fibre. One stimulus of the same intensity was ineffective and failed to evoke potentials in either fibre. The ineffective trace was superimposed on the effective trace and appears as a baseline. Similar results were obtained for five *pa3* fibres in four animals. Thus, in response to electrical stimulation of the brain, *pa3* and the *DLM* share the same threshold. Since the *DLM* is activated *via* stimulation of the giant fibre (Tanouye & Wyman, 1980; Koto *et al.* 1981), the suggestion is that *pa3* muscle potentials are also activated by the giant fibre and *pa3* is part of the giant fibre system of escape initiation.

As well as having identical thresholds, evoked muscle potentials in the *DLM* and *pa3* showed characteristic latency relationships. In experiments directly comparing

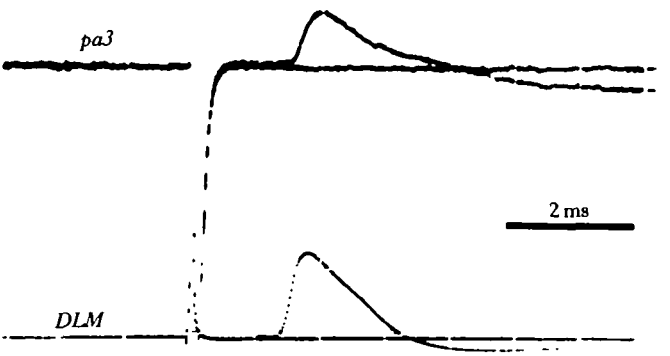


Fig. 4. Evoked potentials in a *DLM* and *pa3* fibre following brain stimulation. Responses to an effective (suprathreshold) stimulus and an ineffective (subthreshold) stimulus are shown superimposed. The effective stimulus evokes potentials in both recording electrodes. The ineffective stimulus fails to evoke a potential in either trace and serves as a baseline. Stimulus: 11 V, 0·01 ms duration.

responses in the *DLM* and *pa3*, the average latency of the evoked *DLM* response w[illegible] 1·13 ± 0·05 ms (S.D.) (five recordings). This value was similar to those reported by Tanouye & Wyman (1980). The average latency of the evoked *pa3* response was 1·28 ± 0·10 ms (S.D.). The average latency difference recorded between evoked *DLM* and *pa3* responses was 0·15 ± 0·10 ms (S.D.). Based on anatomical (King & Wyman, 1980; Koto *et al.* 1981) and physiological (Tanouye & Wyman, 1980) arguments, only one chemical synapse appears to be interposed between the giant fibre and the *DLM* motoneurone. The similarity in latencies between the *pa3* and *DLM* responses suggests that only one, or possibly two chemical synapses, might be interposed between the giant fibre and the *pa3* motoneurone.

The results for the direct wing opener *pa4* were similar to those reported earlier for the direct wing elevators. That is, *pa4* was activated by brain stimulation but at a threshold of stimulation different from *DLM* activation. Thus, *pa4* is not driven by the giant fibre but is activated one-to-one by another, non-giant fibre, descending pathway. *pa3* and *pa4* are the only muscles thought to subserve wing opening (Nachtigall & Wilson, 1967). Taken together, the results presented here suggest that giant fibre-driven wing opening movements during escape initiation are mediated solely through activation of *pa3*.

DISCUSSION

The giant fibre system has proved to be an ideal subject for physiological and genetic study of motor pathways in *Drosophila*. Several studies have shown that the giant fibre system is responsible for rapid activation of the fibrillar muscles and the *TTM*. However, these muscles alone are not capable of producing the wing opening movements that accompany giant fibre stimulation. It has also not been clear whether the wing elevation that occurs upon giant fibre stimulation is produced by the *TTM* (an indirect wing elevator) or by direct flight muscles. Our observations were designed to give a more complete understanding of the role of the giant fibre system in escape behaviour. We have reached the following conclusions: (1) wing opening during giant fibre stimulation is due to activation of muscle *pa3* alone. No other wing opener is closely linked to the giant fibre pathway. (2) Since no direct wing elevators are activated by giant fibre stimulation, the slight wing elevation which occurs must be powered by an indirect muscle. The *TTM* is the only candidate with appropriate stimulus-response properties. (3) Other muscles served by other descending pathways may participate in escape-flight initiation under natural conditions.

Electrical stimuli delivered to the brain evoke potentials in the *DLM* at a sharply defined threshold of stimulation. The present experiments examined several phenomena which had the same threshold. Specifically, the electrical stimuli drove wing opening movements, wing elevation movements, and potentials in the *pa3* wing opener muscle. Since the *DLM* is activated *via* the giant fibre (Tanouye & Wyman, 1980; Koto *et al.* 1981), the suggestion is that the wing movements and *pa3* muscle potentials are also activated by the giant fibre. In contrast, the muscles *pa1*, *pa2* and *pa4* were activated at different thresholds of stimulation from the *DLM*. Thus activation of these muscles was *via* non-giant fibre descending pathways. All thoracic muscles previously examined (i.e. *TTM*, *DLM*, *DVM*s) have been found to be driv[illegible]

by the giant fibre. The muscles *pa1*, *pa2* and *pa4* are the first thoracic muscles analysed which are not activated by giant fibre stimulation.

The above conclusions are dependent on the reliability of *DLM* activation threshold to brain stimulation as an accurate predictor of giant fibre activation. As argued by Tanouye & Wyman (1980), an electrical stimulus grossly applied to the brain might not be able to discriminate between pathways with slightly different thresholds. Such a problem would seriously alter the conclusions presented in this report. Arguing against this possibility is the finding that different thresholds were always observed for *pa1*, *pa2* and *pa4* activation, but were never observed for *pa3* and wing movement activation. Thus, electrical stimulation of the brain can, apparently, distinguish between different neural pathways descending to the thorax. Also, a number of recent investigations examining the giant fibre with intracellular microelectrodes (Tanouye & Wyman, 1980; Koto *et al.* 1981; Tanouye *et al.* 1981) have shown that *DLM* activation is a reliable indication of giant fibre activation. Thus, it is suggested here that *pa3* and the wing movements are driven by the giant fibre and thus, part of the giant fibre system of escape initiation. The muscles *pa1*, *pa2* and *pa4* are not part of the giant fibre system and any role they play in escape initiation must be *via* other, non-giant fibre neural pathways.

The most prominent wing movement driven by the giant fibre was an opening of the wings. Only two muscles in the fly, *pa3* and *pa4*, subserve wing opening (Williams & Williams, 1943; Nachtigall & Wilson, 1967; Heide, 1971). Giant fibre stimulation was found to drive *pa3* but not *pa4*. Thus, giant fibre-driven wing opening movements appear to be generated solely by *pa3*.

pa3 activity in flight has been best described for larger flies (Nachtigall, 1966; Nachtigall & Wilson, 1967; Heide, 1971). *pa3* is most prominently active during extreme turning movements (Nachtigall & Wilson, 1967; Heide, 1971). *pa3* activity and wing opening movements are also observed at the start of flight (Nachtigall, 1966). However, considerable variability was observed, and *pa3* was not active in all starts. Since giant fibre activity was not monitored in these studies, it is not known if *pa3* is not driven by giant fibres in larger Diptera, or if only a small fraction of starts examined were giant fibre-driven. The present study shows that for *Drosophila*, the giant fibre always drives *pa3*, and thus must participate in all starts initiated by giant fibre activity.

The failure of the giant fibre to drive *pa4* is somewhat surprising since anatomical investigation (King & Tanouye, 1983) has shown that the motor axon innervating *pa4* is one of the largest in the *Drosophila* nervous system. The *pa4* motor axon is comparable in size to the giant fibre and *TTM* motor axon, both integral parts of the giant fibre system (King & Wyman, 1980; Tanouye & Wyman, 1980). The *pa4* motor axon is the only large calibre fibre thus far examined which is not part of the giant fibre system. In contrast, the motor axon innervating *pa3*, which is driven by the giant fibre, is of relatively modest diameter (King & Tanouye, 1983).

In addition to wing opening movements, the giant fibre also drove wing elevation. Giant fibre-driven wing elevation was not as prominent as wing opening and was only reliably observed during high frequency giant fibre stimulation experiments. Only two direct wing muscles, *pa1* and *pa2*, are known to be wing elevators (Nachtigall & Wilson, 1967). Neither was found in the present study to be driven by giant fibre

stimulation. The only elevators known to be driven by the giant fibre are indirect wing elevators, the *TTM* and the three *DVM* muscles (Tanouye & Wyman, 1980). The *DVM*s are fibrillar muscles and contract strongly only when stretched (Pringle, 1965). Thus, the likely candidate to explain wing elevation movements due to giant fibre stimulation is the *TTM*.

The results of Kaplan & Trout (1974) showed a very prominent wing elevation at the start of escape initiation in *Drosophila*. In contrast, only slight wing elevation movements were found to be driven by the giant fibre in the present study. Direct comparisons between the present results and those of Kaplan & Trout (1974) are difficult, however, since giant fibre activation was not monitored in the earlier study. Also, it is possible that the giant fibre drives a larger wing elevation under more natural conditions than in the present report. In particular, observations in larger flies (Nachtigall, 1966; Nachtigall & Wilson, 1967) have shown that 15–30 ms prior to escape initiation, the pleurosternal muscle is often activated tonically. The pleurosternal muscle improves the effectiveness of indirect flight muscle contractions by controlling the lateral stiffness of the thorax. Since pleurosternal muscle activity was not monitored in the present experiments, the influence of its activation on these observations is not known.

The present paper provides an initial physiological examination of direct flight muscles in *Drosophila*. To our knowledge, such recordings have not been presented previously. Electrical activity in each of the muscles is easily monitored since they are all activated by inputs descending from the brain. In the case of *pa3*, activation is *via* the giant fibre, thus extending our understanding of the giant fibre system of escape initiation. The short and characteristic latency of the response suggests a relatively simple pathway between the giant fibre and the *pa3* motoneurone. Subsequent analysis will provide a description of the pathway similar to that provided for the giant fibre-*DLM* and giant fibre-*TTM* pathways (King & Wyman, 1980; Tanouye & Wyman, 1980). *pa1*, *pa2* and *pa4* are activated *via* unknown pathways descending from the brain. This activation, however, should facilitate identification and characterization of these pathways and is an important next step toward the long range goal of providing a complete understanding of escape in *Drosophila*.

We thank Dr Seymour Benzer for the use of his facilities and his support during these experiments. We thank our colleagues Drs L. Kauvar, S. Shotwell, B. Tempel, T. Venkatesh, C.-F. Wu and L. Zipursky for discussion. This research was supported by National Science Foundation Grant PCM 7911771 to S. Benzer and the Pew Memorial Trust. M. A. T. was supported by National Institutes of Health Training Grant GM 7401-02.

REFERENCES

COGGSHALL, J. C., BOSCHEK, B. & BUCHNER, S. (1973). Preliminary investigations on a pair of giant fibres in the central nervous system of dipteran flies. *Z. Naturf.* **78C**, 783–784.

FERRIS, G. F. (1950). External morphology of the adult. In *Biology of Drosophila*, (ed. M. Demerec), pp. 368–419. New York: Hafner.

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

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

HEIDE, G. (1971). Die Funktion der nicht-fibrillären Flugmuskeln von *Calliphora*. Teil II. Muskuläre Mechanismen der Flugsteuerung und ihre nervöse Kontrolle. *Zool. Jb. (Physiol.)* **76**, 99–137.

JAN, L. Y. & JAN, Y. N. (1976). Properties of the larval neuromuscular junction in *Drosophila melanogaster*. *J. Physiol., Lond.* **262**, 189–214.

KAPLAN, W. D. & TROUT, W. E. (1974). Genetic manipulation of an abnormal jump response in *Drosophila*. *Genetics* **77**, 721–739.

KING, D. G. & TANOUYE, M. A. (1983). Anatomy of motor axons to direct flight muscles in *Drosophila*. *J. exp. Biol.* **105**, 231–239.

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.

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.

MACHIN, D. E. & PRINGLE, J. W. S. (1959). The physiology of insect fibrillar muscle. II. Mechanical properties of a beetle flight muscle. *Proc. R. Soc. B* **151**, 204–255.

MULLONEY, B. (1969). Interneurons in the central nervous system of flies and the start of flight. *Z. vergl. Physiol.* **64**, 243–253.

NACHTIGALL, W. (1966). Die Kinematik der Schlagflugelbewegungen von Dipteran. Methodische und analytische Grundlagen zur Biophysik des Insektenflugs. *Z. vergl. Physiol.* **52**, 155–211.

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

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

PRINGLE, J. W. S. (1949). The excitation and contraction of the flight muscles of insects. *J. Physiol., Lond.* **108**, 226–232.

PRINGLE, J. W. S. (1965). Locomotion: flight. In *The Physiology of Insects*, Vol. II, (ed. M. Rockstein), pp. 283–329. New York: Academic Press.

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. & 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.* **144**, 345–355.

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

THOMAS, J. B. (1981). Mutations altering the connectivity between identified neurons in *Drosophila melanogaster*. (Ph.D. dissertation). New Haven, CT: Department of Biology, Yale University.

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*. *Rev. suisse Zool.* **54**, 17–53.