

MOTOR UNIT DISTRIBUTION OF THE DORSAL LONGITUDINAL FLIGHT MUSCLES IN LOCUSTS

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I. INTRODUCTION

That locust flight muscles conform to the arthropod pattern in containing only a small number of motor units, has recently been shown by recordings of muscle action potentials in flying locusts (Wilson & Weis-Fogh, 1962). Records of the dorsal longitudinal muscles (hereafter abbreviated to D.L.M.) were difficult to analyse, however, since the number of fluctuating steps in the height of the recorded action potentials were too many to be counted with certainty, especially when the record was further complicated by double firing. Thus a study of the pattern of motor innervation of these muscles seems not inapposite, particularly since previous work on the mechanics and physiology of contraction in the *locust metathoracic* D.L.M. has tended to make it the type example of a neurogenic insect flight muscle (Buchthal & Weis-Fogh, 1956; Weis-Fogh, 1956; Buchthal, Weis-Fogh & Rosenfalck, 1957). Moreover, this muscle was particularly convenient for analysis into motor units using simple techniques, because the units proved to be arranged as discrete bundles of fibres.

Details of motor innervation have previously been described for only three insect flight muscles, namely the basalar muscle of a lamellicorn beetle (Darwin & Pringle, 1959), and the two dorsal longitudinals of a silk moth (Nüesch, 1957). So far, however, none of these muscles has been described at the single-unit level. Apart from its comparative anatomical interest, a precise knowledge of the motor fibre circuitry of the locust flight muscles is necessary for detailed analysis of flight on the motor-unit level, such as that started by Wilson & Weis-Fogh (1962). Also, as was hoped, the investigation of the *peripheral* features of the flight motor system has provided some clues for future work on *central* inter-relationships of motor units. The results are discussed with this in mind and are compared with those of Nüesch (1957) on motor innervation of moth muscles, and of Wilson (1961) who analysed the effects of strategic nerve transections on coordination within the locust flight system.

II. MATERIALS AND METHODS

Adults of the desert locust (*Schistocerca gregaria*, Forskål, *phasis gregaria*) have been used, and were supplied by the Anti-Locust Research Centre, London. Because the muscles (Weis-Fogh, 1952) and their apophyses of origin and insertion (Neville, unpublished) continue to grow for 2-3 weeks after the final moult, the details given below refer specifically to fully grown adults. The number and distribution of motor

units in the dorsal longitudinal muscles of the desert locust has been established by recording mechanical and electrical responses in stimulated nerve-muscle preparations which were observed throughout with the aid of a binocular microscope. The preparation of the bisected thorax was essentially the same as that used by Weis-Fogh (1956). These muscles make convenient preparations, since they are suspended elastically in the thorax, so that the mechanical response can be recorded *in situ* with an RCA 5734 mechano-electrical transducer, in response to any kind of stimulation (Neville & Weis-Fogh, 1963).

The routes of the motor fibres from the ganglia to the muscles have been determined from frozen-sectioned material fixed for 48 hr. in 4 % neutral buffered formaldehyde at 4° C., with subsequent examination by phase-contrast microscopy. The results were checked experimentally by electrical stimulation at strategic points, and by transection of nerves in various sequences in living animals, when the triggering of an action-potential in the severed axons elicited a twitch in the relevant muscle units.

III. RESULTS

(a) *Number of motor units*

The following observations show without doubt that there are five distinct motor units present in each D.L.M. There are:

(i) Five steps in height of muscle force (Text-fig. 1) in response to graded stimulation of the motor nerve *IBa* (notation of Ewer, 1953), Pl. 1.

(ii) Five corresponding steps in height of muscle action-potential (Text fig. 2).

(iii) Five steps in height of motor nerve action-potential recorded with a monopolar electrode, and correlated with (i) and (ii).

(iv) Sections of the motor nerve taken near to the muscle showed five large axons (Pl. 1).

(v) Five potentials have been recorded in flight from metathoracic nerve *IBa* in flying locusts (E. Gettrup, personal communication).

(vi) Five anatomically distinct mechanical units were visible in active muscles and were numbered 1 to 5 as in Text-fig. 3. This was best observed when the stimulus repetition frequency was set low (5 cyc./sec.), and the preparation was observed through a binocular microscope. When a particular unit was active, its fibres were clearly seen to move alongside the adjacent stationary units or cuticle. The transducer output was amplified and monitored aurally through a loudspeaker. The twitches of the various units could then be associated with changes in the strength of the beats in the loudspeaker while the preparation was continuously under observation.

(b) *Routes of motor axons*

The routes of the motor axons from the ganglia to the muscles were traced from nerve sections of fixed material and from nerve transections in live material, and were confirmed electrophysiologically whenever possible.

Records from a D.L.M. showed that cutting a motor axon triggered one action-potential which elicited a *single* twitch in the motor unit of the relevant muscle (Text-fig. 7). A second and equal twitch could be evoked by transecting the axon again a little further distad. Aided by the conveniently discrete layout of the muscle units

this transection method was used for the analysis of the precise routes of motor axons from ganglia to muscles. Nerve transections were in fact preferable to electrical stimulation particularly in the metathorax, since the small lengths of the nerve branches involved near to the ganglia lessened the chances of specific stimulation of a desired nerve; electrotonic spread of stimulus current could produce indiscriminate and therefore unreliable excitation. Accordingly, each thorax used in the experiments was sagittally bisected, a little to one side of the mid-line, leaving the ganglia intact.

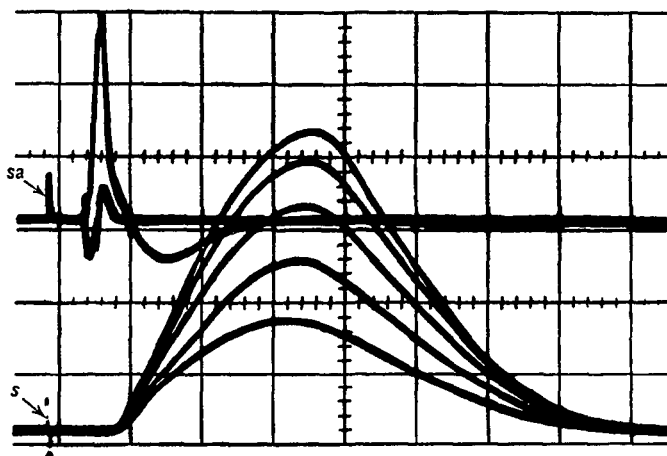


Fig. 1. Five superimposed oscilloscope sweeps showing the effect of graded electrical stimulation of the motor nerve *IBA* on electrical (upper trace) and mechanical (lower trace) activity in the locust metathoracic D.L.M. The five steps in twitch height indicate that the muscle comprises five motor units. The twitch duration is constant for all five levels of activity and the five units are synchronous. *s*, Stimulus; *sa*, stimulus artifact; time base, 5 msec./cm. grid; electrical trace, 2 mV./cm. grid; mechanical trace, 8 g./cm. grid. The experimental conditions resemble those during flight in an intact animal (36° C.; muscle shortening, 3–4 % of the muscle length in the animal).

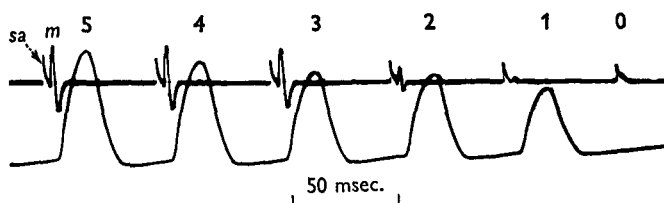


Fig. 2. Five consecutive twitches (lower trace) in a metathoracic D.L.M., resulting from decreasing intensity of electrical stimulation at a typical flight frequency (18 cyc./sec.). The number of units contributing to each twitch is indicated on the record. Correlated with the five levels of mechanical activity can be seen five steps in height of muscle action-potential, compounded from the five synchronous units (upper trace). *sa*, stimulus artifact; *m*, muscle action-potential.

Isolating either D.L.M. down to the nerve trunk network shown and labelled in Text-fig. 5 produced no twitches in either muscle. The longitudinal connectives on the side irrelevant to the experiment were then severed, again with no response in either D.L.M. The neurons and axons supplying a D.L.M. must therefore be contained within the network of ganglia and nerve trunks shown in Text-fig. 4, which thus formed the starting point for further analysis of the routes of axons and sites of neurons. It was

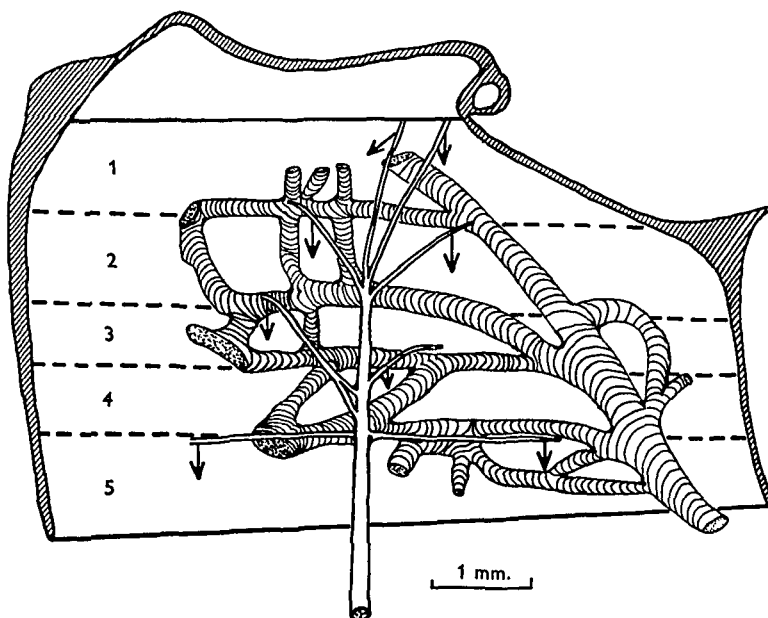


Fig. 3. A camera lucida drawing showing the five topographically discrete muscle motor units of the locust metathoracic D.L.M., together with a typical layout of the first-order branches of the motor nerve *IBA*, and the main tracheal trunks which serve the muscle. The arrows indicate the muscle units supplied by the branches of the various axons: this has been functionally confirmed by cutting the branches and eliciting a twitch in the relevant muscle unit.

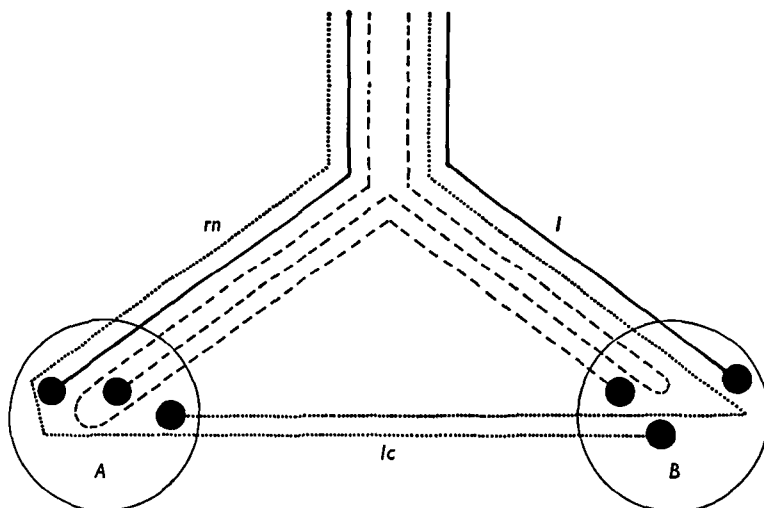


Fig. 4. A diagram illustrating the number of possible routes of a single motor axon from its neuron to a muscle, when it is known that one of two possible ganglia may contain the neuron. *rn*, recurrent nerve; *I*, nerve base I; *lc*, longitudinal connective; *A*, prothoracic ganglion; *B*, mesothoracic ganglion. The black circles represent the neuron. *Direct type* of motor route is indicated by unbroken lines; *diverted type* by closely dotted lines; *recurrent type* by dashed lines.

Established above that there exist five motor axons to each D.L.M. Text-fig. 4 shows that there are six possible routes for each of those axons, from neuron to muscle. The transection method of analysis given below sought to eliminate some of the possible routes and so to establish the real ones. In each of the following experiments, two possible routes in the two sides of the triangle in Text-fig. 4 were eliminated, so that a positive response in the only remaining side could be interpreted. The results of various sequences of cuts, performed upon the triangular nerve network, are presented in Text-fig. 5. Some specimens had a longer recurrent nerve and a longer metathoracic nerve base *I* than others, and were consequently selected for transection experiments.

Experiments concerning the *mesothoracic* muscle are described first. To facilitate description, it is assumed that all of the motor routes are of the direct type, running straight from ganglion to muscle (indicated by unbroken lines in Text-fig. 4). This assumption will later be shown to be valid. Experiment *a* (Text-fig. 5*a*) showed that

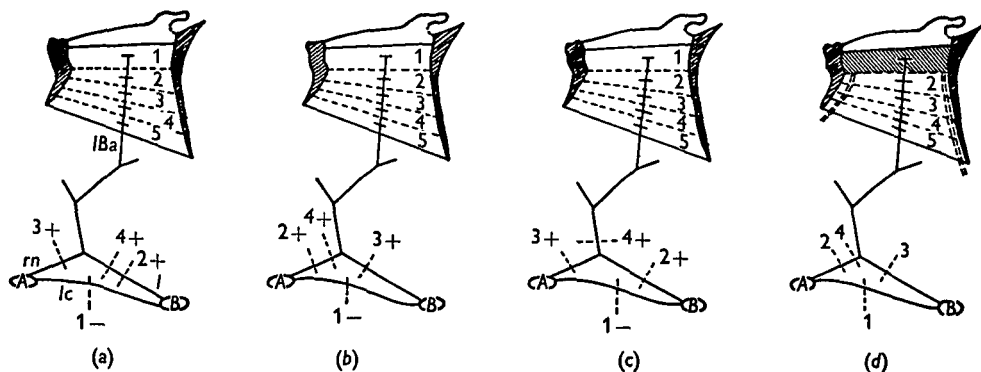


Fig. 5. Four experiments to trace the routes of motor axons by strategic nerve transections in sagittally bisected thorax preparations. Each cut, indicated by a broken line, triggered an action potential in the severed axons, causing a twitch in the relevant part of the muscle. The cuts were performed in the order indicated, and the result of each cut (positive or negative) is shown. In experiment (*d*), muscle motor units 2-5 were severed at both origin and insertion by cuts, indicated by double broken lines. Cut (3) produced a twitch in motor unit 1 (hatched), which was the only twitch to move the phragmata. *rn*, recurrent nerve; *lc*, longitudinal connective; *I*, nerve base *I*; *IBa*, nerve carrying motor axons to D.L.M.; *A*, prothoracic ganglion; *B*, mesothoracic ganglion.

at least one of the five motor axons is routed through the recurrent nerve from the prothoracic ganglion (*A*). Experiment *b* (Text-fig. 5*b*) indicated that at least one of the motor axons is routed through nerve base *I* from the mesothoracic ganglion (*B*). From observation of the muscle during the cutting of mesothoracic nerve base *I*, only one motor unit appeared to be active, and that the most dorsal one (unit 1). In experiment *b*, cut (4) controlled that the recurrent nerve had not been damaged by cut (3). Cuts as in Text-fig. 5*c* resulted in (1) negative, (2) a twitch, (3) a larger twitch, and (4) an even larger twitch. Since experiments using stimuli graded in intensity showed that the muscle units are approximately equal in capacity for doing work (see discussion, §*a*), the evidence from experiments *b* and *c* suggested that more axons are routed through the recurrent nerve than through the base of nerve *I*. The allocation of the five motor fibres might be 4:1 or 3:2, with the evidence in favour of 4:1.

Experiment *d* was designed to check which muscle unit is innervated from the

ganglion in its own segment, and to determine the presence or absence of overlap between the two routes of innervation. Motor units 2-5 were severed at both origin and insertion; this operation did not interfere with the innervation to the muscle, which spreads from the centre towards both ends of the fibres (Text-fig. 3). Cut (1) was as usual negative; cut (2) produced a twitch in units 2-5 *without* moving the phragmata. Cut (3) caused unit 1 to twitch, moving the phragmata, while cut (4) had the same effect as cut (2). The control cut (4) was only made possible because of the pronounced inherent elasticity of the muscle, which permits fast relaxation following a twitch even after disconnection from both origin and insertion; such a mutilated muscle could respond to more than one stimulus. Cut (3) could then be interpreted as producing a specific effect in unit 1; had there been any overlap of units, this would have been discernible. This experiment was repeated after the origin and insertion of the uppermost unit alone had been cut. The relevant results of the previous experiment were reversed. It is concluded (*a*) that unit 1 is exclusively innervated by a motor fibre from the ganglion in its own segment and (*b*) that it is *not* doubly innervated by fast axons; there is no overlap between units innervated from separate ganglia.

It remains to prove the assumption that the motor routes are all of the direct type (as shown by unbroken lines in Text-fig. 4). The fact that no response was observed in either D.L.M. when any of the longitudinal connectives between the ganglia were transected indicates that there is no diverted route for motor fibres from one ganglion to the next via these connectives (close dotted lines in Text-fig. 4).

That the motor routes are not of the recurrent type (shown by dashed lines in Text-fig. 4) is easily established in the metathorax from the number of large axons in the relevant sections (Pl. 1), which total only the required 5. In the mesothorax, although there is a sufficient number of large axons near to the ganglia to allow for one motor fibre following such a recurrent course, Text-fig. 5*d* proved otherwise, since the response to cutting nerve base *I* was restricted to unit 1, while cutting of the recurrent nerve gave response only in units 2-5. Because the motor routes are all of the simple direct type, the neurons are tentatively indicated in Pl. 1.

The transection experiments were repeated in several locusts with constant results, both in the meso- and metathorax.

The presence of four axons in the mesothoracic recurrent nerve was confirmed electrophysiologically by stimulating its proximal end, after it had been disconnected from the prothoracic ganglion by a cut to avoid possible spontaneous discharge. Nerve base *I* was severed from the mesothoracic ganglion for the same reason. A monopolar recording electrode placed a little further along the recurrent nerve checked that the mechanical twitches recorded in the muscle were in fact due to excitation of that nerve. Graded stimulation produced four steps in mechanical tension, and only units 2-5 were active. When the base of mesothoracic nerve *I* was stimulated electrically only unit 1 twitched, as expected. The experiment could not be performed in the metathorax, owing to the smaller lengths of nerve involved.

The nerves were frozen-sectioned at various levels and the large motor axons were counted. When combined with the evidence from nerve-transection experiments the results gave the motor axon distribution presented in Pl. 1.

The first-order branching of motor nerve *IBa* was traced after intravital staining

with methylene blue in locust saline, by specific cuts with fine scissors in preparations with nerve *IBa* centrally disconnected. Observation of the medial surface of the muscle during these cuts indicated no overlap of innervation routes. The branches were variable, but a typical scheme is drawn in Text-fig. 3. A branch to unit 5 was dissected out and sectioned, and proved to contain a single large 'fast' axon (Pl. 1). This was confirmed by high-power observation in phase contrast for all of the first-order branches. There is thus neither morphological nor physiological evidence for multiple innervation of units by large (fast) axons. The presence of slow innervation in these muscles seems unlikely, but, although there is neither physiological nor morphological evidence for it, its absence cannot be established with certainty, since nerve *IBa* carries many unknown small axons.

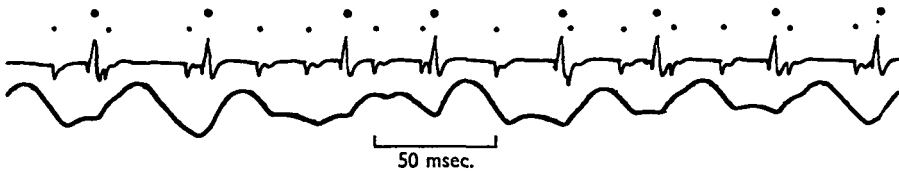


Fig. 6. Electrical record (upper trace) and mechanical record (lower trace) from a sagittally bisected thorax, of a 'spontaneously' firing D.L.M. Two motor units are active within the muscle and fire at independent frequencies (indicated by the two sizes of dots above the record). They are driven by the flight oscillators within the C.N.S. Observation of the preparation confirmed that no other fast muscles were active in the thorax. Note that the frequencies are not related in a simple submultiple way.

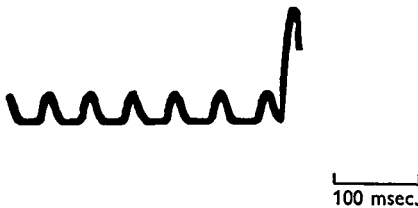


Fig. 7. A mechanical recording of a 'spontaneously' firing unit in the metathoracic D.L.M., in which only the mesothoracic ganglion and recurrent nerve were left intact. A final cut made through the recurrent nerve caused a *single* twitch in all four of the motor units which are normally excited via that nerve.

(c) '*Spontaneous*' motor activity in sagittally bisected thoracic preparations

'Spontaneous' rhythmical firing, perhaps induced by the dissection, occurred in one or more motor units of each D.L.M. in many sagittally bisected thorax preparations in which all three ganglia were still intact. Even within a single muscle, any one of the five motor units was capable of firing rhythmically by itself; the observations also gave further confirmation of the anatomical distribution of the motor units. The units could fire at independent frequencies even within a single muscle. Text-fig. 6 shows both electrical and mechanical recordings from a D.L.M. in which two motor units were directly seen to vibrate. The recorded frequencies do *not* bear a simple sub-multiple relationship to each other, showing the independence phenomenon not to be due merely to refractoriness in one of the units, which can cause it to miss alternate cycles (Neville & Weis-Fogh, 1963). The lability of such outputs raises interesting questions with regard to central integration during flight.

A 'spontaneously' firing *metathoracic* ganglion-nerve-muscle preparation was isolated down to *mesothoracic* ganglion, recurrent nerve, nerve *IBa* and metathoracic D.L.M., and a force-recording transducer was inserted for recording from the muscle. The small height of the mechanical contractions (Text-fig. 7), together with observation of the muscle, indicated a rhythmically firing single unit. A cut was made through the recurrent nerve, eliciting a single larger contraction, caused by the release of an action potential in all four motor fibres within that nerve. Thereafter the preparation remained silent (Text-fig. 7). It is concluded (*a*) that, as already noted, transecting an axon produces a single twitch in the relevant muscle unit, and (*b*) that the rhythmically firing unit must have been triggered by some oscillator within the C.N.S. At the same time, this type of record offers rigid proof that the metathoracic D.L.M. is innervated via the mesothoracic recurrent nerve. Finally, it is worth emphasizing that the inherent spontaneity of neurons in semi-intact preparations makes it essential to disconnect all insect nerve-muscle preparations centrally by *clean cuts* through all possible innervation routes. The literature is unfortunately filled with examples in which the ganglia have been left connected or nerves merely locally crushed.



Fig. 8. A tonic motor output along mesothoracic nerve *IBa* recorded from a sagittally bisected thorax, and containing three units firing at different frequencies. It is suggested that the output is to the small lateral dorsal muscles.

(*d*) *Tonic motor output to mesothoracic lateral dorsal muscles*

In the mesothorax two small muscles, the lateral dorsals (muscles 82*b* and 82*c* of Wilson & Weis-Fogh, 1962), receive innervation from a branch of nerve *IBa*. The branch has previously been described by Ewer (1954) in *Acanthacris ruficornis* (Fab.) and by Campbell (1961) in *Locusta migratoria migratorioides* R. & F. Since these small muscles are the only other ones served by nerve *IBa*, the following results seem appropriate to this paper. In a half-thorax preparation with intact ganglia a recording was made with a monopolar electrode placed upon mesothoracic nerve *IBa*. Nerve *IBb* was severed to eliminate the possibility of interference from the tonic sensory input of the wing-base stretch receptor (Gettrup, 1962). A tonic nerve response was recorded, unaccompanied by either mechanical or electrical activity in the D.L.M. In order to check that the signal was not a sensory input, nerve *IBa* was dissected free over the surface of the D.L.M. and severed at the furthest possible point, near to the uppermost muscle unit. A monopolar recording from the end of the nerve still showed the signal, proving it to be a motor output. As a control that it was not a motor output to the D.L.M., carbon tetrachloride was applied to the pro- and mesothoracic ganglia, when bursts of motor nerve spikes of about 10 times the amplitude appeared on the record; these large spikes originated in the very much larger D.L.M. motor fibres. Finally, the fact that the response could be picked up so far out along

nerve *IBa* ruled out the possibility that it was an output to a spiracular muscle, whose nerve leaves *IB* much further ventrally. It is thus suggested that the record (Text-fig. 8), which contains at least three units firing at different frequencies, represents a motor output to the very small lateral dorsal muscles. The absence of a similar output from the metathorax is correlated with the absence of these muscles from that segment. The evidence suggests that the lateral dorsals are tonic muscles; their small size perhaps indicates that they are vestigial in locusts.

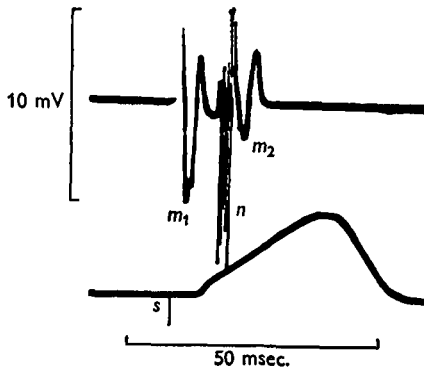


Fig. 9

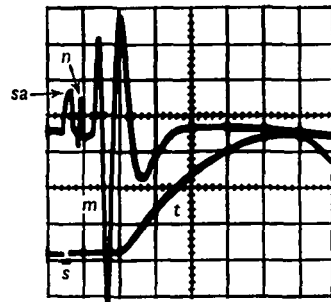


Fig. 10

Fig. 9. A record from a metathoracic D.L.M. nerve-muscle preparation in which a single supraliminal stimulus forced the motor nerve *IBa* to fire twice. The 5 second firing nerve action-potentials are spread in time. Upper trace, electrical record: lower trace, mechanical record: *s*, single supraliminal stimulus; *m*₁, first firing of muscle action-potential; *m*₂, second firing of muscle action-potential; *n*, second firing of nerve-action potentials.

Fig. 10. A typical nerve-muscle-force record from the metathoracic D.L.M. *s*, stimulus; *sa*, stimulus artifact; *n*, nerve potential recorded near to the muscle, and compounded from five synchronous motor axon spikes; *m*, muscle action potential; *t*, auxotonically recorded mechanical twitch. Time base, 4.5 msec./cm. grid; electrical trace, 1 mV./cm. grid; mechanical trace, 3.3 g./cm. grid.

IV. DISCUSSION

(a) The motor units

Each D.L.M. consists of five anatomically discrete motor units, arranged in layers from dorsal to ventral. In the metathorax the main trunks of the tracheae are allocated in close concordance with the mechanical layout of the muscle motor units, and proved useful as positional reference points (Text-fig. 3). Since they remained full of air during experiments when immersed under saline, they stood out as prominent silver tubes upon the muscle surface.

Five bundles of fibres can be distinguished in some of the homologous dorsal musculature of the locust abdomen. Furthermore, Tiegs (1955) found five bundles of fibres in each developing D.L.M. of the pterothorax of grasshopper nymphs. The number may even prove to be constant throughout the order Orthoptera, with reduction in a few non-flying forms. There is some evidence for five units in the mesothoracic D.L.M. of Lepidoptera (§d below).

In previous work on the metathoracic D.L.M. (Buchthal *et al.* 1957; Weis-Fogh, 1956), the dorsal part of the muscle was cut away, leaving a bundle of fibres of almost

equal length. Reference to figures 1 and 4 of Buchthal & Weis-Fogh (1956) shows that they cut away unit 1 and worked only with motor units 2-5. In good agreement with their estimate, units 2-5 together produce about 0.5 g. cm. of work per twitch at flight temperature (Table 1).

In each twitch the muscle shortened against a spring with a linear force/displacement characteristic. Thus, knowing the stiffness of the transducer ($= 0.00083$ cm./g.), it is possible to calculate the division of work between the five motor units from records like that in Text-fig. 1, in which the conditions for work approached those during flight. The muscle works partly against a constant force (the resting tension), and partly against a variable force (the changing tension due to the muscle twitch itself). Thus,

$$\begin{aligned}\text{twitch work} &= \text{resting tension } (r) \times \text{total shortening } (s) \\ &\quad + \frac{1}{2} \text{ active tension } (a) \times \text{total shortening} \\ &= s(r + \frac{1}{2}a).\end{aligned}$$

In order of ascending stimulation threshold, the five units perform the amount of work in Table 1, in single twitches at 36°C . In accordance with their relative sizes (Pl. 1), the five units have approximately the same capacity for work.

Table 1. *Absolute and relative twitch work of the five motor units of the metathoracic D.L.M. measured under conditions close to those during flight in the animal*

36°C .: muscle shortening, 3-4 % of the length of the muscle in the animal, corresponding to optimal working conditions; total force from all five units, 33 g., giving a total work output of 0.66 g. cm.

| Motor units in order of ascending stimu- lation threshold | Work contribution (g. cm.) | Work contributed (%) |
|---|----------------------------------|----------------------------|
| First unit | 0.14 | 21.2 |
| Second unit | 0.12 | 18.2 |
| Third unit | 0.16 | 24.2 |
| Fourth unit | 0.14 | 21.2 |
| Fifth unit | 0.10 | 15.2 |

(b) *Large size of motor axons*

Fast processes are a feature of insect flight systems. It is therefore not surprising to find that the D.L.M. motor axons are among the largest so far found in insects ($15\text{--}25\ \mu$ in average diameter; Pl. 1). Although these fibres are larger than the so-called giant axons in the nerve cord of *Locusta* (Cook, 1951), they should not be referred to as giant axons. Their large size explains the very large nerve potentials recorded, up to 15 mV. from a single axon with *external* electrodes (Text-fig. 9). The sections show that both the motor axons and the large sensory axon from the stretch receptor taper appreciably, increasing diameter in the direction of propagation, so that both sensory and motor fibres presumably propagate impulses towards their destination with increasing velocity. The significance of this is unknown but intriguing.

(c) *Synchrony of motor units*

Most muscles in fast-working repetitive systems such as the insect flight system need to operate, at least at times, as synchronous entities. Text-fig 1 shows the perfect synchrony between the five units of the metathoracic D.L.M. at a typical flight

temperature, when the common stimulus occurs via 1 cm. length of motor nerve. Similar synchrony was noted between the fast motor units in the coxal muscles of the cockroach (Usherwood, 1962). In the D.L.M. several peripheral factors make synchronous contraction possible. (1) The motor axons are approximately equal in diameter at any one level of section (Pl. 1), so that they have similar propagation speeds. The nerve spike recorded externally at the point where the nerve reaches the muscle is a synchronous potential compounded from the five motor axons (Text-fig. 10). (2) The large motor axons have a high propagation speed at typical flight temperatures (an average of 8 m./sec. at 36° C. from ganglia to muscle; Neville & Weis-Fogh, 1963), reducing minor differences in relative timing of contraction. (3) The rise in internal thoracic temperature of about 7° C. above the external temperature (Weis-Fogh & Muus, unpublished) contributes towards this high propagation speed.

Synchrony of contraction depends not only on peripheral features however, but mainly on unison between the motor neurons at the central level. Wilson & Weis-Fogh (1962) found that the degree of synchronization between units within multi-unit muscles could change over a number of wingstroke cycles. Thus, although the peripheral design of the motor units makes possible fast synchronous contractions, the degree of synchrony is centrally determined.

(d) Comparison with *Lepidoptera*

Making use of the necessity of motor innervation for the development of insect muscles, Nüesch (1957) checked the thoracic muscles of adult silk moths (*Telea polyphemus*) emerging from pupae whose nerves had been selectively cut. While not including detail at the muscle unit level, his results nevertheless indicate that the four ventral bundles of the *mesothoracic* D.L.M. are innervated from the prothoracic ganglion via the recurrent nerve, while the uppermost bundle is innervated from the mesothoracic ganglion. Because of the exact morphological and numerical homology with the corresponding locust D.L.M., it is tempting to suggest that the five muscle bundles in the silk moth do in fact represent muscle motor units.

The only difference between the locust and the silk moth in mesothoracic D.L.M. innervation is that two of the muscle bundles of the moth apparently receive a double supply from the two ganglia involved, i.e. there is some overlap of innervation routes. In the locust D.L.M. there is no evidence for anything except single fast axon innervation of each unit. That this innervation is perhaps exclusively of the 'fast' type is indicated by the absence of facilitation, shown by the maximal response in any unit to a single liminal stimulus.

The silk moth *metathoracic* D.L.M. consists of two anatomically distinct bundles, with the dorsal one innervated from the metathoracic ganglion and the ventral one innervated from the mesothoracic ganglion (Nüesch, 1957). The similarity to the basic pattern in the locust is again evident.

(e) Comments on flight co-ordination

Wilson (1961) investigated the effect of strategic nerve cuts on locust flight. The present results substantiate his finding that the prothoracic ganglion is necessary for perfect flight, since disconnecting it would remove four-fifths of the innervation to the

mesothoracic D.L.M. For similar reasons, cutting the recurrent nerve to the metathoracic D.L.M. also reduced the flight performance.

Evidence from 'spontaneously' firing preparations supports Wilson's (1961) hypothesis that the motor neurons are themselves the oscillators, the existence of which was demonstrated by his experiments. The possibility of independent firing of single motor units at independent frequencies even within a single muscle, taken together with the fact that the units of each D.L.M. are divided between two discrete ganglia, only serves to emphasize the degree of central integrative coupling which must occur between the units during normal flight. From a comparison of the neural coupling of bifunctional muscles with the same motor units involved both in flight and walking, Wilson (1962) has demonstrated that muscles which are synergists in flight may act reciprocally during walking. He concludes that the mechanisms of coupling between units clearly cannot be either structurally or functionally rigid. The above finding that the units of a single muscle can vibrate at independent frequencies strongly supports this conclusion. The motor neurons of the flight system and of the individual muscles are independent both in regard to quantity (i.e. many or few units may fire during a particular wingstroke, Wilson & Weis-Fogh, 1962), and also to timing (i.e. they may fire at different frequencies). For the purpose of functional analysis it becomes necessary to regard each motor unit of the flight system as a potentially separate muscle. During flight there must be a facultative coupling which assures concerted action of synergists. Locust locomotion is provided by a markedly flexible motor system with a reduced number of components, which are coordinated in varying combinations according to functional needs.

SUMMARY

1. The peripheral pathways of the 'fast' motor fibres to the locust dorsal longitudinal flight muscles are described at the single unit level, from electrophysiological and histological studies. This is summarized in a diagram on Pl. 1.

2. Both pterothoracic dorsal longitudinal muscles consist of five anatomically distinct motor units, arranged in layers from dorsal to ventral. Each of the four more ventral units of both muscles receives a motor axon from the segment in front via the recurrent nerve, whereas the uppermost motor unit is innervated in each case from the segment containing the muscle. The motor units are nearly equal both in size and capability for work.

3. Each of the five 'fast' motor axons innervates one topographically distinct bundle of muscle fibres. There is no overlap between muscle motor units.

4. Even within a single muscle, motor units are capable of vibrating at independent frequencies. This indicates that the coupling of units which occurs during flight is neither structurally nor functionally rigid.

5. With respect to peripheral features, the motor units within each dorsal longitudinal muscle are designed for fast response which improves the synchronization when the relevant neurons fire simultaneously (large motor axons, 15–25 μ in average diameter with high propagation velocity, 8 m./sec. at flight temperature).

6. It is suggested that a tonic motor output, containing at least three units, which was recorded from mesothoracic nerve *IBa*, travels to the small lateral dorsal muscles.

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APPENDIX

Suitability of locust dorsal longitudinal nerve-muscle preparation for student practicals

The locust dorsal longitudinal nerve-muscle system is, for teaching purposes, probably the most accessible and informative preparation of its kind in the class Insecta and some of its special features are therefore listed below:

- (i) The preparation can be made to work in 10 minutes by sagittally bisecting a thorax and pinning it down in a dish. Records such as those in Text-figs. 1 and 10 are relatively easy to obtain.
- (ii) The nerve is long (8-9 mm.) and easy to locate because of its prominent yellow fat flanges.
- (iii) The muscle shows a typical 'fast' response.
- (iv) A good preparation can pull 35 g.
- (v) Large motor nerve spikes (up to 15 mV.) can be recorded with *external* electrodes.
- (vi) The five motor units are topographically discrete, and the active units can be

directly observed, and correlated with five corresponding steps in the mechanical tension. This is convincingly demonstrated by sound-monitoring of the twitches.

(vii) A single-unit preparation can be made by stimulating the base of mesothoracic nerve *I*.

(viii) Nerve *I* can be used for measurements of propagation velocity. The high speed (8 m./sec. at 36° C.) can be associated with the very large diameter of the motor axons (15–25 μ).

EXPLANATION OF PLATE I

A map of the peripheral pathways of the motor fibres to the locust dorsal longitudinal muscles, drawn approximately to scale. Photographs (all at the same magnification) of sections of the nerves in phase contrast are inset, with guide lines indicating the levels of section. The interpretation incorporates the results of confirmatory electrophysiological experiments. Note that units 2–5 of each muscle are innervated from the ganglion of the next segment in front. The neurons are tentatively indicated. *ph*₁, 2 and 3, first, second and third phragmata respectively. Nerve nomenclature follows Ewer (1953, 1954). *A*, *B* and *C*, pro-, meso- and metathoracic ganglia respectively. The vertical dotted lines indicate segmental boundaries.

