

ON CERTAIN PROPERTIES OF THE FLIGHT MUSCLES  
OF THE ORTHOPTERA

BY D. W. EWER AND S. H. RIPLEY

*Department of Zoology, University of Natal, Pietermaritzburg*

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(With Plate 9)

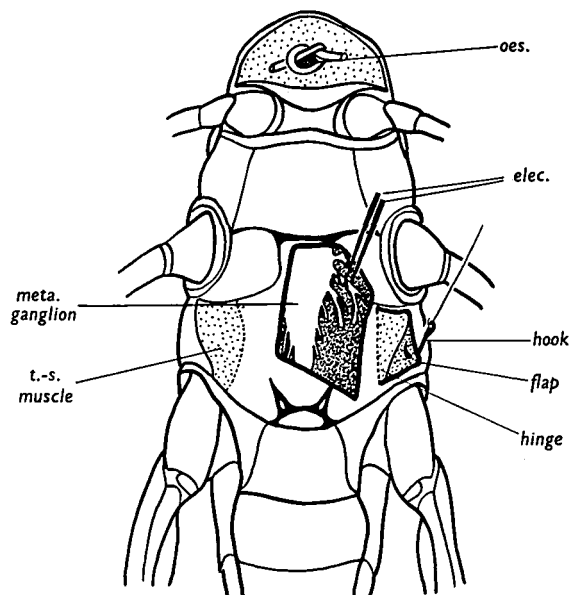
The physiological characteristics of the wing musculature of insects have been but little investigated. Solf (1931) has given a brief account of the responses of the dorsal longitudinal muscles of *Grylotalpa vulgaris* (L.) to direct electrical stimulation. A more extensive study has been made by Heidermanns (1931) of the responses to direct electrical stimulation of the wing muscles of *Aeschna coerulea* (Ström). The latter author found that at a stimulation frequency comparable with that of the normal wing beat of the dragonfly, the muscles were in a partial tetanus. He concluded that this was their normal condition in flight. More recently, Pringle (1949) has found that potentials recorded from the wing muscles of *Calliphora* occur at a lower frequency than the wing movements. He concluded that these flight muscles were highly specialized, and that impulses from the nervous system activated rather than excited the muscles. Similar observations have been made by Roeder (1950, 1951) on *Vespa*, *Lucilia* and *Tabanus*. In *Periplaneta* and the moth *Agrotis*, however, Roeder recorded a single muscle potential for each cycle of the wing beat. He concludes that in the latter two species the wing-beat frequency was such as to permit a conventional neuromuscular system to move the wings.

In 1947, Voskresenskaya published an account of the responses of the tergo-sternal and associated vertical direct-flight muscles of *Locusta migratoria* L. when either the nerve supplying the tergo-sternal muscle or the metathoracic ganglion was stimulated electrically. The nerve supply to these muscles was in all cases left attached to the ganglion. The muscular responses were recorded from the movements of the hind wing. With this preparation she was able to show that at stimulation frequencies below 20 cyc./sec. the wing muscles respond with discrete contractions. At higher frequencies the response of the muscle depends upon the intensity of stimulation; at low intensities the muscles respond with discrete contractions at 12-18 beats/sec., but at higher intensities a clonus or a smooth tetanus can be obtained. It will be appreciated that this preparation is complicated both by the large number of muscles concerned in the movement of the wing and by the connexion of the nerves to the metathoracic ganglion. It is, moreover, not clear whether in removing the dorsal longitudinal muscles the sensory nerves from the basalar and subalar regions which join with this motor nerve were cut.

In ignorance of Voskresenskaya's investigations we have performed essentially similar experiments, also using *L. migratoria*. These are briefly described below. The preparation we have employed records the movement of the tergo-sternal muscle only (muscle 113; Snodgrass, 1929), although the nerve stimulated supplies muscles 118, 125, 126, 127 and 128, some of which are concerned in the movement of the wing. The results we have obtained agree well with those of Voskresenskaya, but the mechanical records alone are insufficient to give a definite interpretation of the effects observed. We have now had the opportunity to make electrical recordings from these muscles which permit a decision between the various interpretations which can be offered. The second half of this paper is concerned with these recordings.

#### KYMOGRAPHIC RECORDINGS

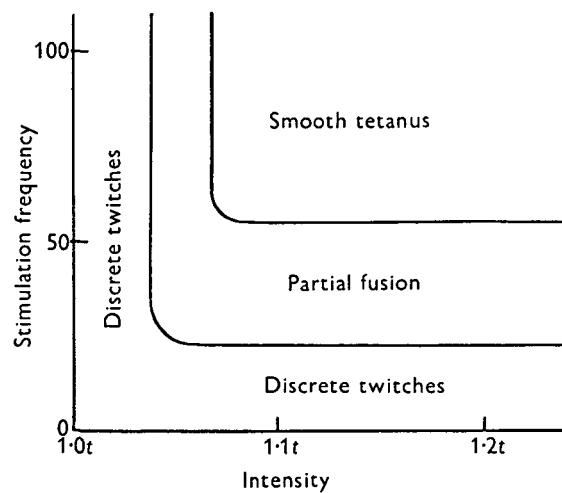
The preparation used was made as follows. The animal was decapitated. An approximately rectangular portion of the metathoracic sternite was removed to expose the ganglion. The nerve trunk supplying the tergo-sternal and other muscles was crushed at its point of emergence from the ganglion, and all other



Text-fig. 1. Ventral view of *Locusta* prepared for recording of tergo-sternal muscle movement. *elec.* electrodes. The *flap* of exoskeleton with an inserted *hook* and mesial *hinge* line may be seen. *meta. ganglion*, metathoracic ganglion. *oes.* cut and ligatured oesophagus. *t.-s. muscle*, origin of the tergo-sternal muscle.

homolateral nerves severed close to the ganglion. Care was taken not to damage the ventral air sacs, tracheae or salivary glands. Incisions were then made through the lateral extensions of the metathoracic basisternite (Text-fig. 1). These incisions freed three sides of a small flap of exoskeleton which carries only the origin of the tergo-sternal muscle. The remaining muscles innervated by the nerve take their

origins more laterally. The flap remained attached along its mesial side which acted as a hinge. A small metal hook was inserted into the free edge of this flap, clear of the muscle attachment; a thread from the hook was attached to a light isometric lever. The flap was reflected outward from the body so that the muscle was under a slight initial tension. The nerve was stimulated with a square wave oscillator (Leisegang & Ripley, 1949) using platinum electrodes. The normal period of stimulation was 5 sec.; the preparation was allowed 1 min. rest after each stimulation. The preparation continued to make respiratory movements and the neuromuscular system being studied remained excitable for 3-6 hr. All experiments were conducted at 28° C.

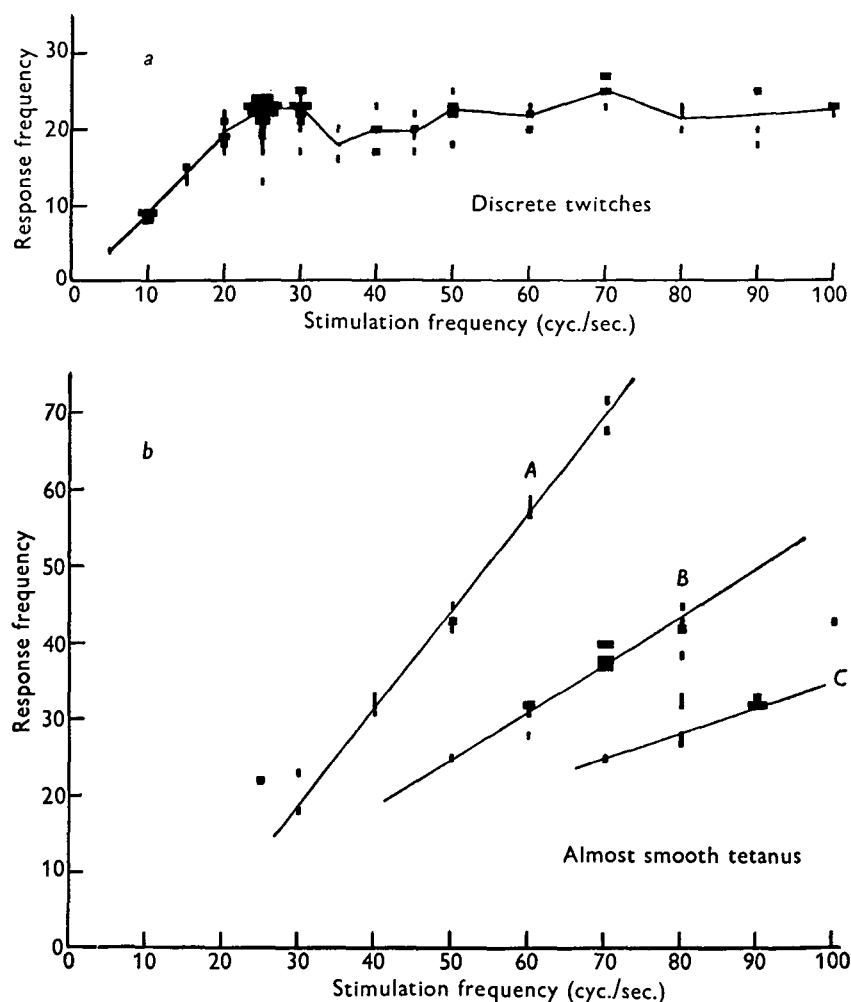


Text-fig. 2. Diagrammatic representation of the relationship between frequency and intensity of stimulation and the character of the muscular response.  $t$  = threshold voltage.

The general form of the responses obtained at different frequencies and intensities of stimulation is summarized diagrammatically in Text-fig. 2. At low intensities of stimulation the muscle responds with a series of discrete twitches regardless of the frequency of stimulation. At moderate intensities a partial fusion of the response is obtained with all frequencies of 25 cyc./sec. and greater, but no smooth tetanus is developed. At still higher intensities partial fusion is observed between 25 and 60 cyc./sec.; above this frequency the muscle responds with a smooth tetanus.

A detailed study of the response frequency of the preparation shows that at low frequencies of stimulation the discrete twitches closely follow the stimulation frequency; the slight differences between the stimulation and response frequency are probably due to errors in calibration. At frequencies above 20 cyc./sec. and at intensities producing discrete twitches only the frequency of response becomes remarkably constant. This may be seen in Text-fig. 3*a*, in which the response frequency is plotted against stimulation frequency. The mean frequency for all such cases recorded between stimulation frequencies of 25 and 100 cyc./sec. is

$22.4 \pm 0.25$  beats/sec. This appears to accord well with the wing-beat frequency of 20 beats/sec. for intact locusts reported by Kennedy, Ainsworth & Toms (1948).



Text-fig. 3. Relationship between the stimulation and response frequencies of the tergo-sternal muscle. The width of the blocks indicates the number of observations; for example, at a stimulation frequency of 10 cyc./sec., five responses were recorded at 9 cyc./sec. and three at 8 cyc./sec. *a*, responses are discrete twitches. The continuous line joins the means of the response frequencies at different stimulation frequencies. *b*, responses are almost smooth tetanus. *A*, *B* and *C* are explained in the text.

A very different picture is obtained from responses in which the tetanus is almost smooth (Text-fig. 3*b*). Here there are suggestions of various modes of response tentatively indicated by the three lines *A*, *B* and *C*.

These results are in complete agreement with the findings of Voskresenskaya. We are able further to confirm her statement that the tergo-sternal muscles require far higher frequencies of stimulation than do those of the legs to produce a partial

fusion of contractions and a smooth tetanus. Results obtained from a study of different metathoracic leg muscles stimulated by way of the main nerve trunk are shown for comparison in Table 1.

There appear to be two possible interpretations of the results which we have obtained from the tergo-sternal muscle. The first is that there exists in these muscles a mechanism somewhat similar to that found by Pringle in *Calliphora*. The relative constancy of the response frequency found in Text-fig. 3*a* would then be regarded as due to an inherent myogenic rhythm. The smooth tetanus obtained at higher intensities of stimulation could be attributed to a second system of 'tonic' fibres in the nerve trunk with a higher threshold than those stimulating the muscle to contract with discrete twitches. Complicating assumptions would have to be introduced to explain the type of results which are shown in Text-fig. 3*b*.

Table 1. *Frequencies at which fusion of contractions and smooth tetanus first appear in different muscles of Locusta*

Muscle	Fusion (cyc./sec.)	Smooth tetanus (cyc./sec.)
Depressor tibiae	8	25
Levator tibiae	19	25
Depressor tarsi	11	20
Levator tarsi	16	20
Tergo-sternal	25	60

*Note.* The first four values refer to the muscles of the metathoracic leg of *pharus gegeraria*.

The alternative explanation lies in the assumption that while the muscle itself has the normal properties of striped muscle, the nerve supplying it is characterized by the fact that following stimulation there is a long period of subnormal excitability. At low intensities and high frequencies of stimulation the stimuli will be counted down by some approximate submultiple of the stimulation frequency. Increasing intensity of stimulation will result in more nerve impulses reaching the muscle, which will pass through varying degrees of fusion to a smooth tetanus when both stimulation frequency and intensity are high enough. Text-fig. 3*a* then merely reflects the fact that a frequency of 20–25 beats/sec. is about the fastest frequency at which the muscle can contract without the development of residual tension. The variations between the mean frequency of response at different stimulation frequencies may be regarded as a product of this counting down. This explanation also allows an interpretation of Text-fig. 3*b*. The line *A* represents a response from the muscle to almost every shock, *B* to almost every second shock and *C* to almost every third.

Electrical recordings from the muscles should permit a distinction between these alternatives. If the first interpretation is correct the contractions of the muscles may be expected to be independent of the stimulating shocks at high frequencies and low intensities of stimulation; if the latter, such contractions as occur should follow particular stimuli.

## ELECTRICAL RECORDINGS

The responses of the muscles were recorded with fine platinum electrodes inserted directly into the muscles being studied. The responses were amplified with a Grass pre-amplifier and the amplifiers of a Cossor oscillograph. The relevant nerve was stimulated with a simple condenser discharge circuit.

Using a preparation essentially similar to that in the preceding section a study was made of the responses of the tergo-sternal muscles and also of the direct depressor-extensor of the hind wing (muscle 129) which lies beneath the epimeron of the metathorax. To be able directly to observe the behaviour of the indirect flight muscles when stimulated, a simple preparation of the thorax cut parasagittally was used. The musculature of one side was removed in this manner, while the metathoracic ganglion and the musculature of the opposite side remained in position. By suitable dissection the relevant nerves could be exposed and direct observations made on the musculature. Electrical recordings from the dorsal longitudinal muscle (muscle 112) were also made with this preparation. Using *Periplaneta americana* (L.) a few recordings were made from a muscle which runs from the base of the wing to the meron of the coxa (muscle 169; Carbonell, 1947); this muscle is homologous with the depressor extensor of the hind wing of the locust. All experiments were performed at room temperature (c. 18° C.).

The results obtained clearly indicate that the various muscles studied respond to nervous stimulation, and there is no suggestion of any myogenic rhythm. Pl. 9 shows a series of records from the dorsal longitudinal muscle of a single preparation stimulated at 62 cyc./sec. The three records, *a*, *b* and *c*, were taken at different intensities of stimulation, and it can be seen that the muscle is responding to every fourth, every third and every second shock as the intensity of stimulation is increased. Such regularity of response as is shown by these records is not always found. Text-fig. 4*d* shows a record from the tergo-sternal muscles at the same stimulation frequency. The very irregular character of the response, producing an average frequency of 18 contractions/sec., can be seen. It would thus appear that the explanation of the effects described in the previous section lies in a prolonged period of subnormal excitability in the nerve.

From a study of the minimal response frequency an estimate can be made of the duration of this period of subnormal excitability. For the two indirect muscles and the depressor extensor of the locust a value of about 75 msec. is found. For the depressor extensor of the cockroach a smaller value of 25–30 msec. appears. The long period of subnormal excitability of the nerves of *Locusta* corresponds to a response frequency of 12–14 contractions/sec. This condition will only be found when the stimulus intensity is just supra-liminal, and in practice with stimulation frequencies within the range of 35–65 cyc./sec. the response frequency usually found at low stimulation intensities is 18–24. This agrees well with the results shown in Text-fig. 3*a*, using kymographic recording.

Although in the preparation of the tergo-sternal muscle which we have employed to record mechanical contractions there is every reason to believe that the pre-

paration records the movement of this muscle alone, it seemed desirable to check by visual observation that the indirect flight muscles did contract tetanically at high intensities and frequencies of stimulation of their nerve supply and thus to eliminate finally the possibility that the records of a smooth tetanus might be due to the tonic contractions of some direct muscles. Direct observations of the behaviour of the two sets of indirect muscles were made using the parasagittally cut thorax. Both muscles were observed to contract into a smooth tetanus when their nerves were strongly stimulated at about 60 cyc./sec.

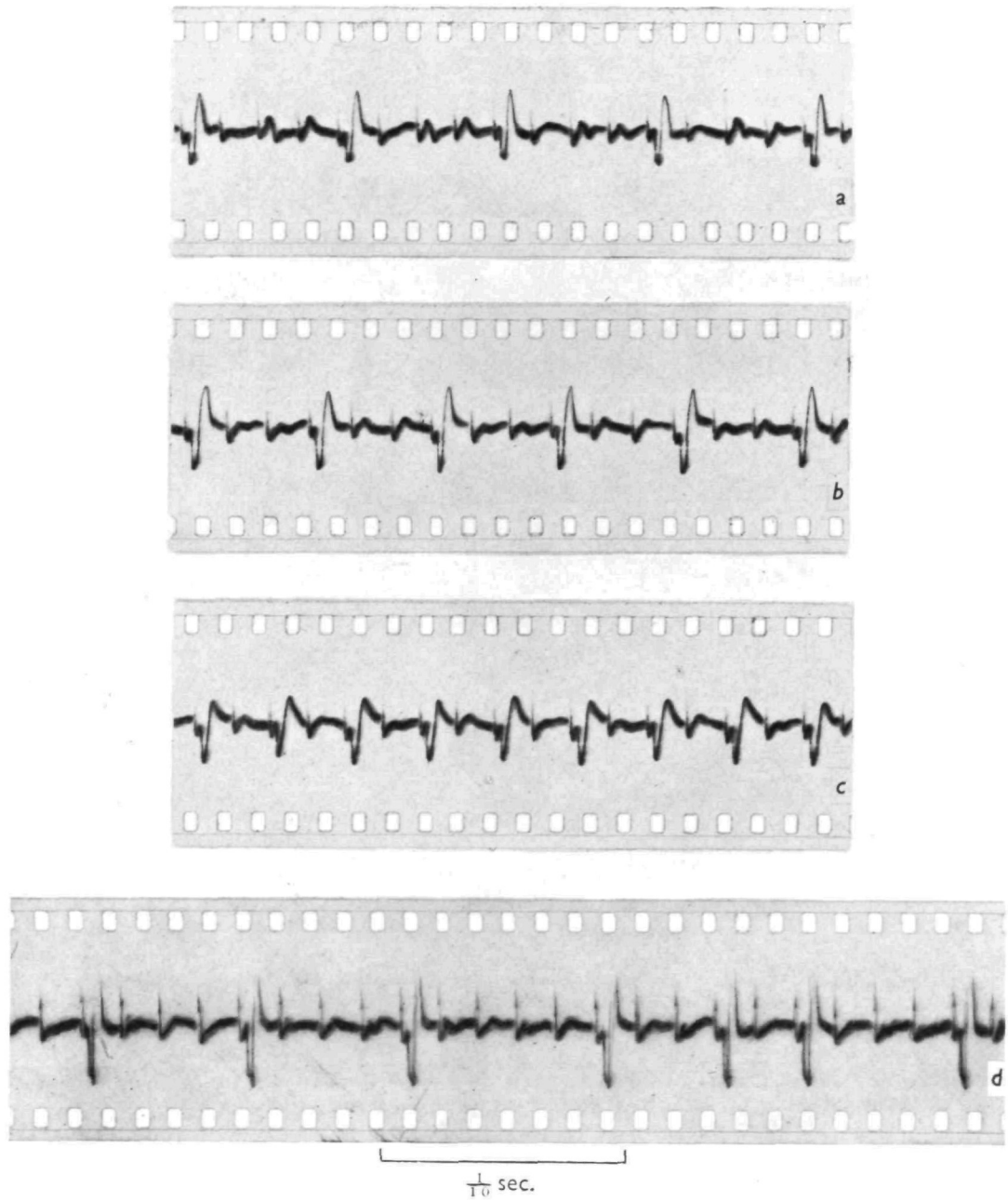
#### CONCLUSION

From these results it first appears that the indirect flight muscle of the locust thorax can contract without fusion at a frequency comparable to the normal wing beat of the animal. It is noteworthy that in the leg muscles of *Periplaneta* fusion of contractions commences at a higher frequency than in those of *Locusta*; the frequency characteristics found in *Periplaneta* resemble those which we have found in the tergo-sternal muscles of *Locusta*. Thus Pringle (1939), using the extensor tibiae, found that with the quick fibre fusion of contractions started at about 30 cyc./sec. and a smooth tetanus developed above 70 cyc./sec. The same effect, but still more pronounced, has been found by Roeder & Weiant (1950) with the tergal muscles of the trochantin of *Periplaneta* where fusion of contractions does not begin until the motor nerve is stimulated at a frequency above 40 cyc./sec., and complete fusion has still not been attained at 100 cyc./sec. These results may be compared with the values found for the leg muscles of *Locusta* shown in Table 1. They suggest that the performance of the tergo-sternal muscles may well be typical of orthopteran skeletal muscle, but that the muscles of the hind leg of *Locusta* are specialized. This may possibly be a reflexion of the specialization of the meta-thoracic limb of *Locusta* for jumping. It is, however, clear that there is no need to assume that in the locust the flight muscles are in a state of partial tetanus during flight, as has been suggested for the flight muscles of *Aeschna* by Heidermanns (1931).

The second result is that there is no evidence to be found of any specialization leading to an inherent rhythm of contraction in the indirect flight muscles. The effects obtained which suggested such a possibility have been shown to be due to a prolonged subnormal excitability of the nerve fibres after the passage of an impulse; this is possibly comparable with that found by Grundfest (1939) in mammalian *B* fibres. The exact character of this effect can, however, only be decided by further study.

#### SUMMARY

1. Kymographic recordings have been made of the movements of the tergo-sternal muscles of *Locusta migratoria* when the motor nerve is stimulated at different frequencies and intensities.
2. At low intensities of stimulation the muscles respond with discrete twitches regardless of the frequency of stimulation. At high intensities and frequencies the



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muscles contract with a smooth tetanus. These findings confirm the earlier observations of Voskresenskaya (1947).

3. Electrical recordings from the tergo-sternal (muscle 113), dorsal longitudinal (muscle 112) and depressor extensor (muscle 129) of the locust wing, as well as from the muscle corresponding to the latter in the cockroach, show that the discrete twitches obtained at high-frequency and low-intensity stimulation arise from a long period of subnormal excitability of the nerve and are not to be attributed to any inherent rhythm of contraction in the muscles.

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One of us (D.W.E.) wishes further to record his thanks to Prof. James Gray for his kindness in according him the hospitality of the Zoological Department in Cambridge and to J. W. S. Pringle for his advice and assistance.

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## EXPLANATION OF PLATE 9

Electrical responses from the indirect flight muscles of *Locusta migratoria*. The stimulus artifact can be seen as a fine line. The muscle response is a well-defined diphasic break. *a*, *b* and *c* are records from the dorsal longitudinal muscles at three intensities. *d* is a record from the tergo-sternal muscle.