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Mating Behavior of the Cabbage White Butterfly, *Pieris rapae crucivora*

VI. Electrophysiological Decision of Muscle Functions in Wing and Abdomen Movements and Muscle Output Patterns during Flight

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Summary. 1. Extracellular recordings of eleven muscles in the pterothorax and the first abdominal segment were carried out to electrophysiologically decide their functions in wing and abdomen movements and to reveal their output patterns during flight.

2. Muscle functions were decided; That is, DLM.2, BM.2, SM.2, DLM.3, BM.3, and SM.3 as wing depressors, DVM.2, DVM.3, and OM.3 as wing elevators, DLM.1a as an abdomen elevator and VLM.1a as an abdomen depressor.

3. During flight, rigid phase relationships were observed among the muscles except for BM's. DLM.2, SM.2, DLM.3, SM.3 and DLM.1a fire synchronously during downstroke, while DVM.2, DVM.3, OM.3, and VLM.1a fire synchronously during upstroke.

4. BM's were characterized by the particularly expedient firing patterns during flight. They showed a tendency to synchronize usually with DLM's but may be active at any phase of the wing beat cycle.

5. These muscles were classified into depressor and elevator groups in terms of their function in flight and phase relationship (Fig. 9).

Introduction

An already mated female cabbage white butterfly, *Pieris rapae crucivora* Boisduval, responds to the mating behavior of a conspecific male by assuming the mate refusal posture (hereinafter abbreviated as MRP) (Obara, 1964). The posture is characterized by the maintained depression of the fore- and hind-wings and elevation of the abdomen. The previous anatomical investigations designed to identify the muscles responsible for MRP, that is, for the wing depression and abdomen elevation revealed main fore-wing depressors and an abdomen elevator (Obara and Nakagoshi, 1974). The elevator of the fore- and hind-wing and the depressor of the abdomen were also determined. The hind-wing depressor, however, remains to be otherwise identified because the mechanics of the hind-wing movement are obscured by the remarkable deformations of the metathoracic cuticular structures and appear not to be successfully explained solely from an anatomical view point.

In order to substantiate these anatomical results, to reveal the depressors of the hind-wing, and to understand the output patterns of these pterothoracic flight muscles, their electrical activities during flight movements were investigated. Motor patterns of the abdomen depressor and elevator during flight were also examined, because the wing beats are always accompanied by slight oscillatory

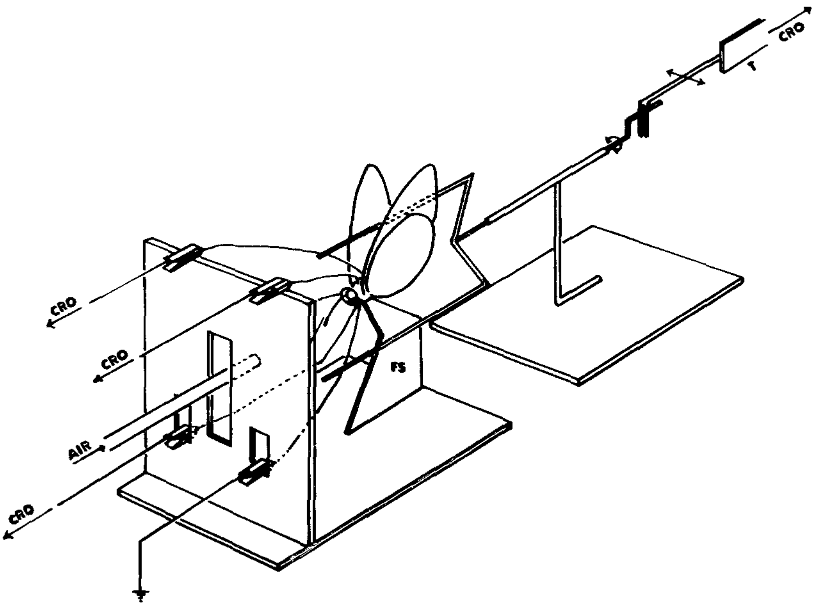


Fig. 1. Recording procedure of muscle action potentials during flight and the apparatus to register wing movements. The operative part of WMMA consists of glass capillaries

abdominal motions in antiphase with the wings, which was observed in highspeed photographs taken for another purpose (Obara, 1970). The output patterns of these pterothoracic and abdominal muscles will demonstrate electrophysiologically their roles in wing and abdominal motions and are expected to provide some information on the relationship between flight and MRP motor patterns in both of which wing and abdomen movements are involved.

The present investigation was made as the third section of the studies on the MRP of the mated female.

Material and Methods

The butterflies used were raised through the larval stages on cabbage leaves. Butterflies of both sexes were used for the investigation.

All experiments employed tethered animals as shown in Fig. 1. For the investigation of muscle activities during flight, an animal was prepared for tethering by severing all of the legs at the trochanter and by removing the scales from the ventral portion of the mesothorax. The mesothoracic episternum was then cemented to the tip of a vertical fixing stage (FS) with beeswax.

Recording electrodes 2 to 3 mm long with 50 μ silver lead bound were inserted into the pterothoracic flight muscles and those in the first abdominal segment through a small hole punched in the exoskeleton with a fine insect pin and fixed at the base with beeswax. These electrodes consisted of approximately 25 μ stainless steel insect pins which were tapered by electrolyzing in 1 N HCl solution and insulated with enamel except at the tip. A common indifferent electrode consisting of 50 μ silver wire was inserted into the body cavity below the intersegmental membrane between the prothorax and the mesothorax and connected to ground.

All of these operations were performed while animals were anesthetized with CO_2. Animals showed normal behavior patterns including flight movements less than 20 min after ceasing CO_2 anesthesia. They were thus kept still for at least 30 min before the experiments.

Table 1. List of muscles examined in this experiment

Common name (at present paper)	Abbrevation	Nomenclature (Obara and Nakagoshi, 1974)	Function (Obara and Nakagoshi, 1974)
Mesothoracic dorsal longitudinal muscle	DLM.2	t. 1–5	depression of fore-wing
Mesothoracic dorso-ventral muscle	DVM.2	t-p.1–4, t-cx.1, 2, 4, 5, t-tr. 1, 2	elevation of fore-wing
Mesothoracic basalar muscle	BM.2	p-cx. 1	depression and pronation of fore-wing
Mesothoracic subalar muscle	SM.2	t-cx. 6	depression and supination of fore-wing
Metathoracic dorsal longitudinal muscle	DLM.3	t. 5	?
Metathoracic dorso-ventral muscle	DVM.3	t-p. 1, 3, t-cx. 1-3, 5, t-tr. 1, 2	elevation of hind-wing
Metathoracic oblique muscle	OM.3	t. 7	elevation of hind-wing
Metathoracic basalar muscle	BM.3	p-cx. 1	?
Metathoracic subalar muscle	SM.3	t-cx. 6	?
Dorsal longitudinal muscle in the first abdominal segment	DLM.1a	t. 4	elevation of abdomen
Ventral longitudinal muscle in the first abdominal segment	VLM.1a	s. 2	depression of abdomen

Animals were stimulated to start flight movement usually by touching the abdominal tips lightly with a soft brush or by blowing air on the head when required. Flight lasted for a few seconds without any more stimuli.

The eleven muscles examined are listed in Table 1. Electrical activities of these muscles were recorded bilaterally to investigate the phase relationships between the same muscles on both sides. In order to evaluate the effect of presumed sensory feedback from the wing region on the flight motor pattern, the bilateral recordings were made before and after the entire removal of the right wings. On the other hand, simultaneous recording of different muscles was carried out unilaterally on the left side. A total of 210 animals were used for the investigation.

Following the experiments, animals were fixed with 70% alcohol for the location of an electrode tip which was visually confirmed in the musculature of carefully dissected specimens under the binocular microscopic observation.

Left wing movements were monitored concurrently with a wing movement monitor apparatus (hereinafter abbreviated as WMMA) shown in Fig. 1. By depressing the lower lever of WMMA, the wing downstroke produces a clockwise rotation of the horizontal axis, which is displayed as a downward deflection of a beam on the CRO by way of a transducer (T). An upstroke operates the WMMA in reverse by pressing the upper lever counterclockwise, causing the beam to deflect upwards.

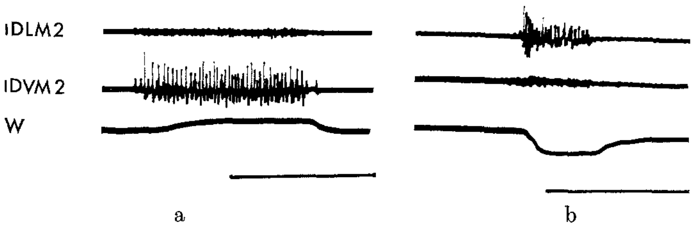


Fig. 2a and b. Muscle potentials recorded from the mesothoracic antagonistic indirect flight muscles during sustained wing elevation (a) and depression (b) response. The upward and downward deflection of the trace, *W*, indicate elevation and depression of wings, respectively. The prepositive *l* means left. The bar represents 0.5 sec

Results

1. General Notes

Animals tethered on the fixing stage stayed alive for a few days as long as they were fed a dilute sugar solution. They showed apparently normal flight movements in terms of wing-stroke amplitude and wing beat frequency. No apparent differences between male and female flight movements and electrical events of the muscles were observed.

Muscle potentials of 2 to 3 mV usually occurred steadily in synchrony with the wing beat. They appeared in bursts which usually consisted of 3 to 6 spikes. In exceptional cases in which somewhat abnormal flight with powerful wing strokes but lower wing beat frequencies was observed, longer bursts contained about a dozen spikes.

The WMMA operated efficiently and yielded data on which it was made possible to determine whether relevant muscles were depressors or elevators. As shown in Fig. 2a, remarkably longer bursts accompanied by sustained wing elevation response were steadily obtained when the wings were forced to depress beyond and below the lower lever of the WMMA. This wing elevation could be safely attributed to the concurrently bursting muscles, which were thus judged to be elevators. Depressors were likewise determined by operating the wings in the opposite direction (Fig. 2b). The functions of all of the nine pterothoracic muscles in flight, that is, depression or elevation of the wing were decided first in this way and then reconfirmed by simultaneous registration of both muscle potentials and wing movements during flight.

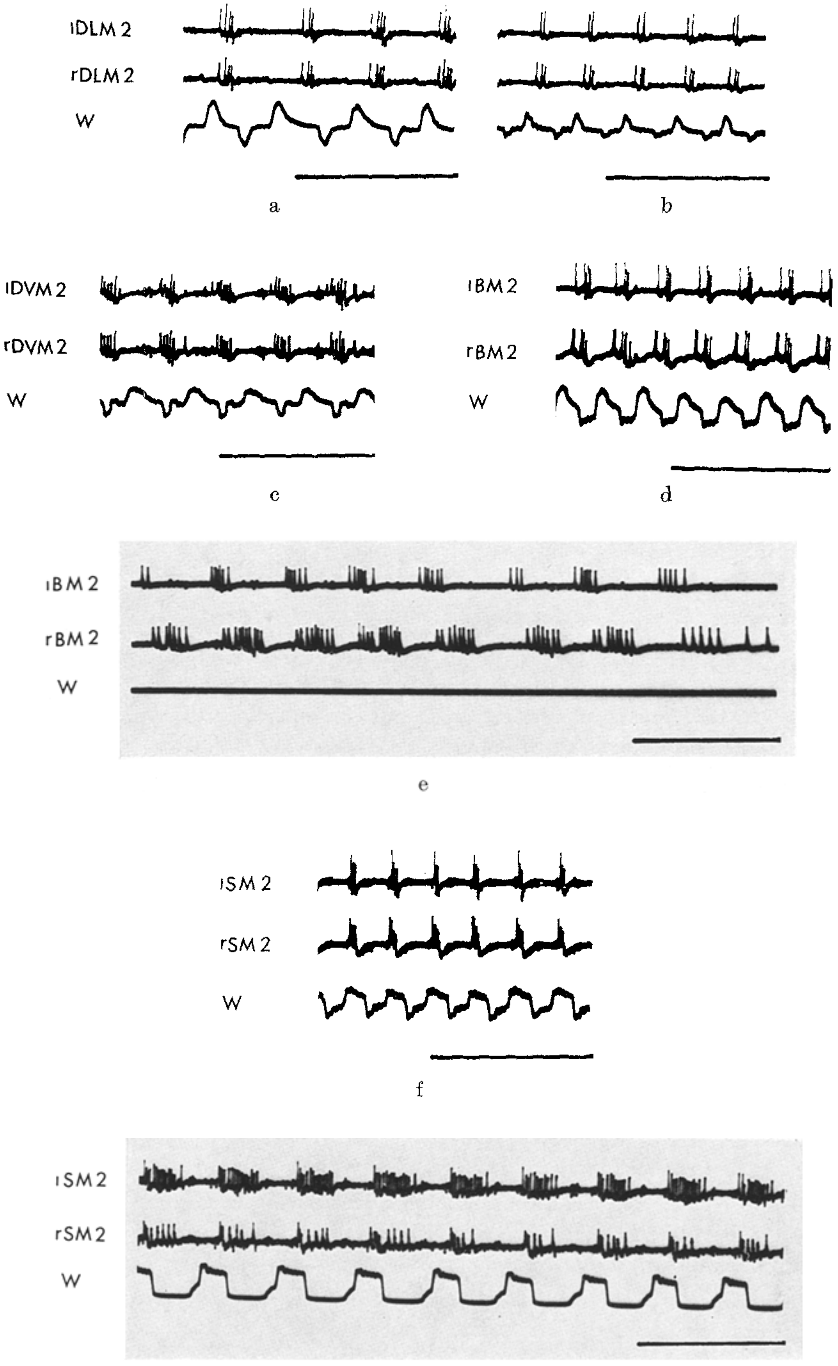
Because of the elasticity of the transducer, however, the WMMA failed to precisely follow the intermediate course of the wing strokes, while the uppermost and lowermost positions of the wing were reliably displayed as upward and downward deflection, respectively.

Fig. 2 and other results described below showed that cross-talk between recording electrodes was quite negligible, though the action potentials of large antagonists were occasionally registered as small deflections.

2. *Electrical Activity of Individual Flight Muscles*

Mesothoracic Flight Muscles

Electrical activities of the four main mesothoracic flight muscles are shown in Fig. 3. The largest flight muscles, DLM.2's, on each side of the body, always



a b c d e f g

Fig. 3. Muscle potentials recorded from four mesothoracic flight muscles during flight. The prepositive *l* and *r* indicate left and right, respectively and the trace, *W*, shows wing movements. These abbreviations are commonly employed through Fig. 9. Each bar in Fig. 3 through Fig. 8 represents 0.5 sec

discharge synchronously and depress the fore-wing (Fig. 3a). Usually left and right DLM.2's fire simultaneously even after differently loaded by removing the right wings, though a slight difference in burst length between them appeared occasionally (Fig. 3b). This was the case in all the other muscles examined except for BM.2 and BM.3. Fig. 3c shows the rhythmical and synchronous discharges of left and right indirect elevators (DVM.2).

BM.2 shows particular motor patterns and although depressing the fore-wing, it may be active at the upstroke phase of the wing beat cycle (Fig. 3d, Fig. 6a). Left and right BM.2's may fire quite independently of each other even when animals with all four wings intact were allowed to fly unrestricted by freeing them from the WMMA (Fig. 3e).

SM.2's as well as BM.2's are direct depressors, but the motor outputs of the left and right SM.2's are always synchronous (Fig. 3f). The difference in burst length between the two SM.2's is slightly larger as compared with indirect muscles (Fig. 3a, c). This is more conspicuous when the left SM.2 was relatively overloaded by removing the right wings, in which not only the burst length but the spiking rate within bursts are different between the two SM's (Fig. 3g). Synchronization of the burst, however, still persisted.

Metathoracic Flight Muscles

Functional and electrophysiological homology was found between anatomically homologous mesothoracic and metathoracic flight muscles. Indirect DLM.3 and DVM.3 produced depression and elevation of the hind-wing, respectively, and synchronous discharges between these muscles on the left and right side were obtained as shown in Fig. 4a and b. BM.3 depressed the hind-wing and usually showed longer bursts than BM.2 (Fig. 4c). But the motor patterns of BM.3 were essentially homologous with those of BM.2; Left and right BM.3's may fire independently of each other and at various phases of the wing beat cycle. SM.3 is another important direct depressor, which showed synchronous discharge patterns (Fig. 4d). The slight difference in the burst length between left and right SM.3's were noticed as in SM.2's.

OM.3 runs roughly parallel with DVM.3, therefore it was decided to elevate the hind-wing. This was demonstrated electrophysiologically as shown in Fig. 4e. Like other indirect flight muscles, OM.3's on both sides discharged simultaneously and usually their burst lengths were negligibly different.

Muscles in the First Abdominal Segment

DLM.1a which elevates the abdomen fired in synchrony with the downstroke of the wing, whereas VLM.1a as an abdomen depressor fired with the upstroke (Fig. 5a, b). Left and right muscles of the former discharged simultaneously and their burst lengths were almost equal. This was also the case in the latter.

3. Phase Relationship among Different Muscles

Phase Relationship among Mesothoracic Flight Muscles

Fig. 6a shows a simultaneous recording of the three mesothoracic depressor activities during flight. It indicates that DLM.2 and SM.2 obviously fire concurrently and their burst lengths are also nearly identical. On the other hand,

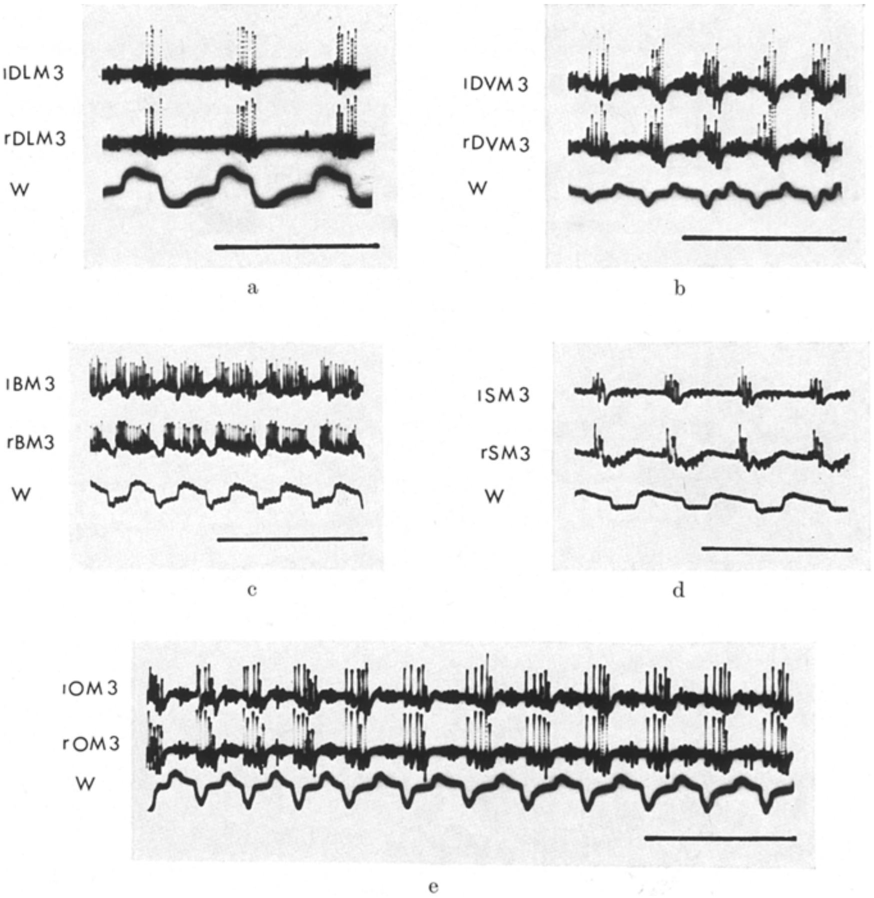


Fig. 4. Muscle potentials recorded from five metathoracic flight muscles during flight

BM.2 fired twice per wing beat in the initial part of the recording and was slightly preceeded by DLM.2 and presumably DVM.2 as a small potential between DLM.2 activities. In the latter part of the recording BM.2 showed a tendency to synchronize more closely with DLM.2. BM.2's characteristic of firing freely at various phases of the wing beat cycle was here again demonstrated.

The alternate firing shown in Fig. 6b reveals the antagonistic relationship between DLM.2 and DVM.2. During normal flight, the last action potential of the DLM.2 bursts precedes the first one of the DVM.2 bursts by about 23 msec (23.1 ± 4.5 msec, N = 27).

Phase Relationship among Metathoracic Flight Muscles

The three metathoracic depressors showed essentially the same motor patterns as those in the mesothorax (Fig. 7a). DLM.3 fired in synchrony with SM.3, with the burst length of the latter being slightly longer. In this specimen the continuous discharge of BM.3 is particularly notable, though it usually fired more phasically.

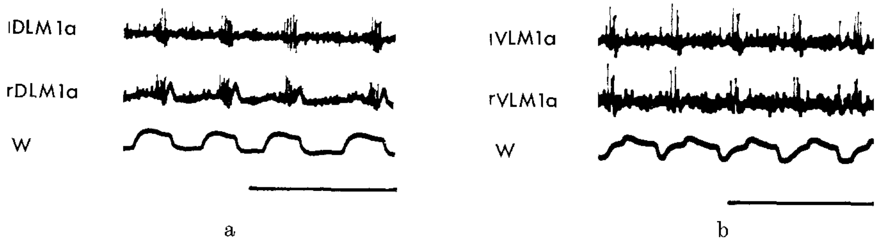


Fig. 5. Muscle potentials recorded from two longitudinal muscles in the first abdominal segment during flight. The slow upward deflections of the trace immediately after rDLM.1a are artifacts

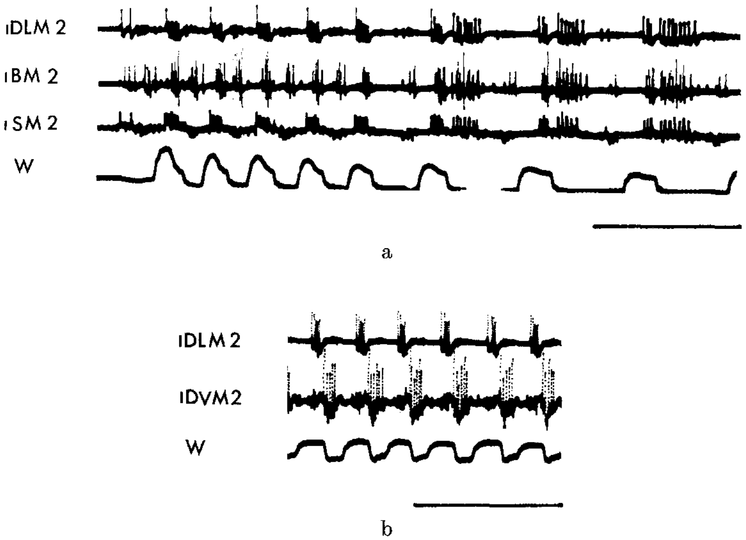


Fig. 6. Muscle potentials simultaneously recorded from different mesothoracic flight muscles during flight

The relatively large OM.3 discharged simultaneously with DVM.3 and the burst length of the two was almost identical (Fig. 7b).

Phase Relationship among Different Muscles in Different Segments

Simultaneous recording of indirect pterothoracic muscles and those in the first abdominal segment clearly revealed the phase relationship among these muscle activities. As shown in Fig. 8a and b, DLM.2, DLM.3 and DLM.1a always fired concurrently and the synchronous firings were also steadily obtained among DVM.2, DVM.3 and VLM.1a. These results substantiate electrophysiologically the observation of the abdomen movement in antiphase with the wing.

On the contrary, the bursts of the homologous BM.2 and BM.3 showed only rough synchronization with the different number and timing of spikes (Fig. 8c). This was the case when animals were freed from the WMMA (Fig. 8d).

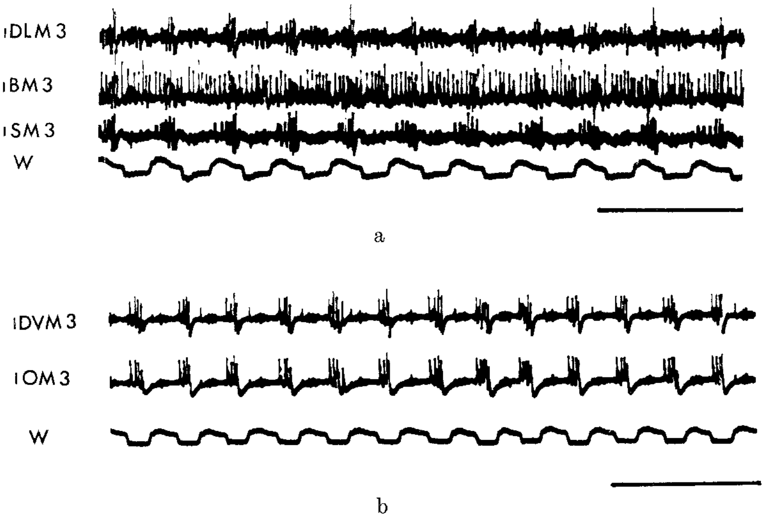


Fig. 7. Muscle potentials simultaneously recorded from different metathoracic flight muscles during flight

Discussion

The results obtained clarified the function of nine pterothoracic flight muscles in flight. They also revealed the qualitative phase relationship among those muscles including two longitudinal muscles in the first abdominal segment during flight movement. The muscles investigated can be classified into depressor and elevator groups according to their function in flight and the phase relationship among them, which was schematically diagrammed in Fig. 9.

It is obvious from Fig. 9 that the motor pattern during stationary flight is characterized by a remarkably rigid activity relationship among the three longitudinal muscles in the depressor group and all four muscles in the elevator group. The burst timing and length of the three in the depressor group are identical and this is the case with the electrical events of each muscles on both sides of the body within the respective segments. The same thing occurs with the four muscles in the elevator group. Apart from the burst length which showed some dependence upon the presumed peripheral feedback, the apparently fixed phase relationship among these muscles including SM's persisted when the two side wings were considerably differently loaded. Although final interpretation requires more precise experiments, it seems likely that the burst timing is coordinated rather centrally as in such insects as locusts (Wilson and Wyman, 1965).

During flight the longitudinal muscles and those of the elevator group fired antagonistically. Judging from the large volume of the pterothoracic DLM's and DVM's, it seems reasonable that the main power for the wing depression and elevation is produced by these muscles. The wing depression may be modified delicately by the activity of SM's which fire synchronously with DLM's but have some dependence upon the presumed sensory inputs from the wing region. BM's seem to exert more important effects on the depression of the wing. Since they

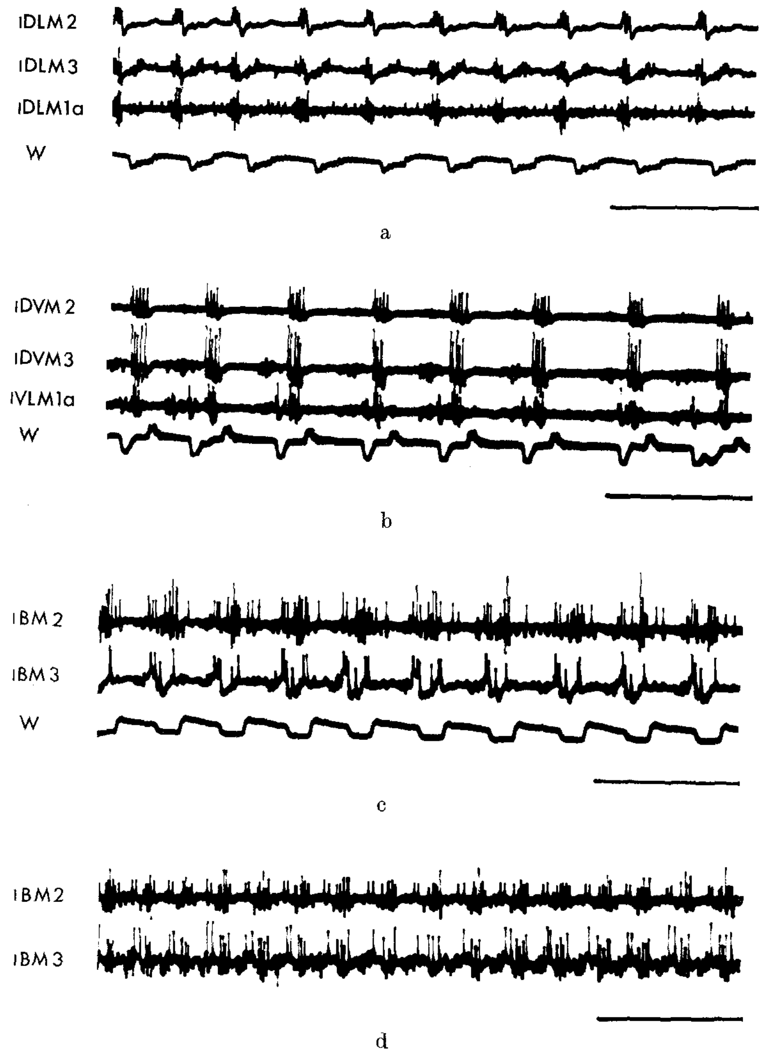


Fig. 8a—d. Muscle potentials simultaneously recorded from dorsal longitudinal muscles (a) and dorsoventral and ventral longitudinal muscles (b) in three different segments. Compared with the synchronous discharges among these muscles, apparently independent firings of BM's in the pterothorax are conspicuous (c, d)

produce the wing pronation as well as depression, it is likely that their expedient activities play an important role in flight control by not only changing the attack angle of the wing but modifying the wing motion during the return stroke as well.

The more precise regulation of the wing motion required by actually flying butterflies would be partially accounted for by other direct muscles such as the third axillary muscles. The changing phase relationship among these direct flight muscles has been reported in some lepidopterous insects. The importance of the

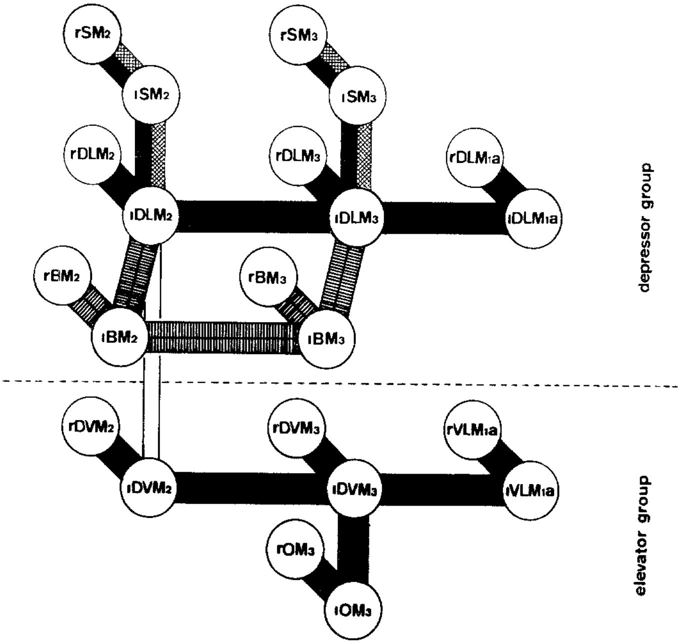


Fig. 9. Classification of, and mutual relationship among, muscles in terms of firing timing and burst length. Black bar: synchronous firing and equal burst length; black and cross-hatched bar: synchronous firing and nearly equal burst length; striped bar: no rigid relationship both in firing timing and burst length; white bar: antagonistic firing

changes in the phase relationship for the control of the flight direction was indicated in a hawkmoth (Kammer, 1971). Right and left mesothoracic third axillary muscles were excited at different phases during a turn performed by a moth (Kammer and Nachtigall, 1973). In several moths it was shown that a different phase relationship among some motor units occurs during different behavior patterns (Hanegan and Heath, 1970; Kammer, 1968, 1970). Functions or roles of such changing phase relationship, however, are not clear with butterflies.

Simultaneous firing of DLM.1a and VLM.1a with pterothoracic DLM's and DVM's, respectively, shows that the abdomen is moved up and down, not passively but actively, in antiphase with the wings. In large saturniid moths with low beat frequences, the possibility was pointed out that the motion of the heavy abdomen could compensate at least in part for the bobbing of the body produced by wing movement (Wilson, 1968). This may also be true for cabbage white butterflies with low wing beat frequencies.

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