

NEURO-MUSCULAR CONTROL OF DIPTERAN FLIGHT

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INTRODUCTION

Since the discovery of the myogenic nature of the main flight-muscle rhythm in flies and some other insects (Pringle, 1949; Roeder, 1951) there has been wide agreement that the nervous command to individual motor units must be one in which the significant aspect of the temporal code is the average frequency of nerve impulses and not detail of sequential pattern or the phase of each impulse (Wilson & Wyman, 1963; Burton, 1964; Barber & Pringle, 1966). There are interesting impulse-sequence patterns among the motoneurons in the flight control systems of flies (Wyman, 1966); but as far as we can tell these have no significance for the muscle performance although they must indicate something of the co-ordinating mechanisms within the thoracic ganglion. Although some isolated studies support the argument that the code must be a pulse-frequency modulation, one in which pulse phase is irrelevant (Wilson & Wyman, 1963), there has yet been no careful study of the correlation between nervous events and aerodynamic output in myogenic insects.

It has also been assumed that there is a spatial coding, or a differently varying command to different muscles, for the control of such behaviour as turning. This seems necessarily true, but there has been little study of this aspect of control. Such a study is presently needed, for example, as background for attempts at correlating optomotor reaction behaviour with underlying nervous phenomena.

We have tried to obtain a broad picture of muscle function and nervous command of the whole flight musculature in normal flight and during strong turning in flies. The results have been correlated as far as possible with the known features of wing kinematics (Nachtigall, 1966).

MATERIALS AND METHODS

Several species of muscoid flies, mostly calliphorids, were used. The genera included *Muscina*, *Sarcophaga* and *Calliphora*. We are not aware of any significant differences between the species used relative to these experiments.

We have used the anatomical guide of Ritter (1911). Fig. 1 shows the muscles and their names. Table 1 provides some information on muscle function.

An open-jet wind-tunnel was constructed. Its features are illustrated and described in Fig. 2. The working section was at least 3.5 cm. in cross-sectional diameter and

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3 cm. long. Smoke lines and cotton threads were used to demonstrate a completely satisfactory degree of laminarity in the working section. Wind-velocity differences within the working section were not measurable with a hot-wire instrument, except for a 1.3 % decrease in velocity along its length. There was no swirl (rotary turbulence) in the working section; stream lines were parallel to the tunnel axis.

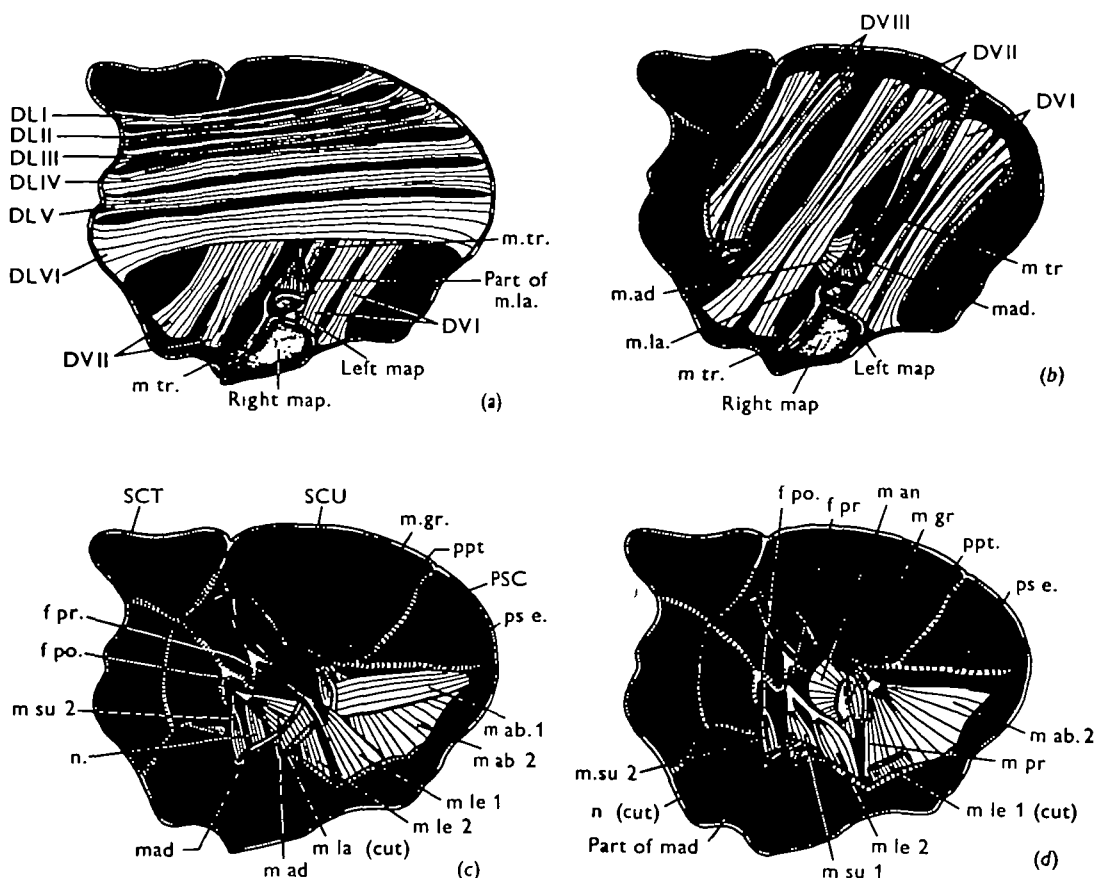


Fig. 1. Sketch of the flight structures in the left half of the thorax of a calliphorid fly, seen from the inner side. Anterior is to the right. The muscles were drawn as in a Bouin's-fixed specimen, hence they have a shrunken appearance which gives a clearer separation between the major bundles. (a) Dorsal longitudinal indirect wing depressors (fibrillar, myogenic muscles). (b) Dorsoventral indirect wing elevators (fibrillar, myogenic muscles). (c) The more medial direct wingbase muscles (tubular, neurogenic muscles). (d) The more lateral direct wing base muscles (tubular, neurogenic muscles). For muscle names see Table 1. Skeletal names are as follows: PSC, praescutum; SCU, scutum; SCT, scutellum; map., mesapophysis; mad., mesapodema; ppt., parapterum; ps.e., praescutellar edge; f.pr., foramen praealare; f.po., foramen postalare (after Rutter, 1911).

For observations on the control of power a flight balance was used (Fig. 3). With the balance it was possible to keep the fly within the working section of the wind-tunnel and to measure its lift and air speed under conditions which do not interfere with the normal production of lift and thrust. The balance was damped so that rapid transients were integrated and could not be measured directly.

Table 1. *The flight-muscles of flies*

(The abbreviations are as in Fig. 1.)

Abbreviations in Fig. 1		Nomenclature		Functions		Author
Fibrillar (myogenic muscles)	Ritter (1911) (<i>Calliphora vicina</i>)	Williams & Williams (1943) (<i>Drosophila repleta</i>)		Method of functional analysis	General agreement	
DL I-VI*	Musculus dorsalis	Median dorsal muscle	Wing depression	Inference from mechanics	General agreement	
DV I-III*	Musculus dorso-ventralis	Anterior tergosternal muscle, tegal tendon of coxa, lateral oblique muscle	Wing elevation	Inference from mechanics	General agreement	
Tubular muscles						
m.tr. (DV IV)*	Musculus trochanterus (musculus dorso-ventralis IV)	Tergal depressor of the mesothorax†	The jump muscle Main starter muscle and take-off-jump muscle	Leg cutting Electrophysiology and force transduction	Behrendt (1940) Nachugall & Wilson	
Medial wingbase muscles (Fig. 1c) *						
m.ab 1	Musculus latius†	Dorsal furco-entopleural muscle†	Controls thoracic 'click' mechanism	Inference from mechanics	See Pringle (1957) for general discussion	
m.le 1	Musculus abductor alae primus	Second basalar muscle	Main controller of wingbeat frequency	Electrophysiology	Nachugall & Wilson	
m.le 2	Musculus levator alae primus	Anterior muscle of the first axillary	Draws wing forward	Pulling with forceps	Ritter (1911)	
m.ad	Musculus levator alae secundus	Two internal muscles of the fourth axillary	Extension of flexed wing	—	Williams & Williams (1943)	
m.au. 2	Musculus adductor alae	(One of the) two external muscles of the fourth axillary†	Elevates and draws wing somewhat back	Pulling with forceps	Ritter (1911)	
m.au. 1	Musculus supinator alae secundus	Posterior muscle of first axillary†	Draws wing back after flight (?) Draws wing back during flight	Pulling with forceps	Ritter (1911)	
m.n.	(Not recognized by Ritter)		Depresses anal wing portion	Pulling with forceps	Nachugall & Wilson Ritter (1911)	
Lateral wingbase muscles (Fig. 1d)						
m.ab. 2	Musculus abductor alae secundus	Sternobasalar muscle	Draws wing horizontally forward	{ Pulling with forceps Electrophysiology	Ritter (1911) Nachugall & Wilson	
m.pr.	Musculus pronator alae (we could not distinguish from m.ab. 2)		Depresses anterior wing border (?)	{ Pulling with forceps	Ritter (1911)	
m.au. 1	Musculus supinator alae primus	Posterior muscle of the third axillary	Depresses anal wing portion	Pulling with forceps	Ritter (1911)	
m.gr.	Musculus gracilis	First basalar muscle	Not known	—	—	
m.an	Musculus anonymus	Third and fourth basalar muscles	Not known	—	—	

* Indirect muscles

† This muscle is often called the pleurosternal muscle (Pringle, 1957). It seems arbitrary to call it a direct wing muscle.

‡ Homology with *Calliphora* is not clear.

(?) We agree on all functions not indicated by question mark.

A very small 5-way plug and socket was made and was attached to the pendulum of the flight balance. The removable plug carried two leads for a tiny phone crystal carrying a stiff $50\ \mu$ wire which was waxed to the rear tip of the scutellum. No wax was allowed in the scutellum fissure or on the scutellar levers. Wingbeat frequency, and to some extent wingbeat amplitude, could be recorded by means of this transducer. Another lead was inserted into the fly's abdomen as a reference or ground electrode. Two further wires provided active leads which were implanted in the flight muscles. For muscle recordings $15\text{--}25\ \mu$ copper or stainless steel wires, insulated except at the tip, were used.

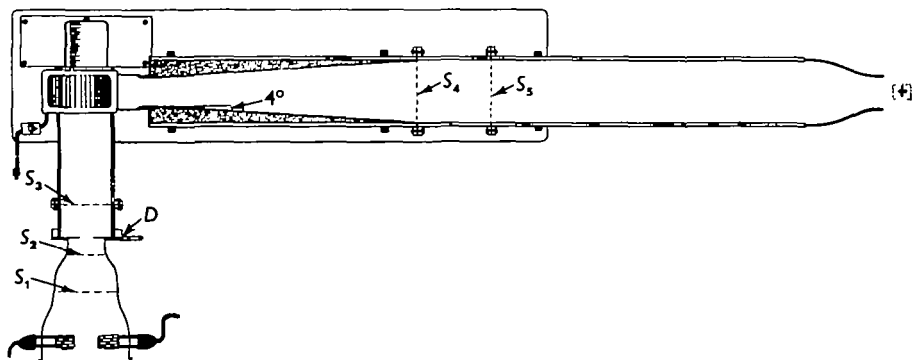


Fig. 2. Wind tunnel (drawn to scale). Wind velocity was regulated by means of the diaphragm *D* in the overflow tube. The motor velocity was constant. A four-coil heater, regulated by a variac, was sometimes used to hold the temperature at $25\text{--}29^\circ\text{C}$. Effects of room turbulence and the diaphragm edge were diminished by three medium-mesh wire screens, S_1 , S_2 and S_3 , in the inflow system. Turbulence generated by the tunnel itself was kept low by a very small wall angle (4°) in the diffuser. Turbulence was damped further by two fine screens S_4 and S_5 (60 and 120 meshes/in., respectively), a very long settling distance, and a well-shaped nozzle with a contraction ratio of 4:1. The outflow diameter was 5 cm.

Experiments ran as follows. A fly was mounted with wax by its abdomen tip to the special plug. (We felt thoracic mountings should be avoided as they may interfere with the natural vibrations.) Under a dissecting microscope the leads were prepared. The plug was then connected to the socket on the pendulum and the whole balance was quickly adjusted to zero conditions. Flight was initiated by starting the wind tunnel and/or eliciting the tarsal inhibition-reflex rebound. The wind speed was manually adjusted to keep the pendulum vertical. Lift was recorded by frequent readings verbally recorded on one channel of a four-track tape recording. Wingbeat vibrations and the two muscle recordings were continuously recorded in the other channels. At the end of a recording session the fly was killed with a drop of ether, weighed, preserved, and dissected in order to ascertain electrode positions. The lift scale on the balance was calibrated in terms of percentage of body weight for each fly.

Turning behaviour and the function of the smaller thoracic muscles was not studied in the flight balance. Instead, special techniques were used in several types of experiment. The techniques will be described in the relevant discussions of the results.

Out of more than 200 experiments about eighty flies gave useful performances, but relatively few of these flew for long periods. The damage associated with electrode implantation and the restraint of the force transduction seem sharply to reduce the willingness of the animals to fly.

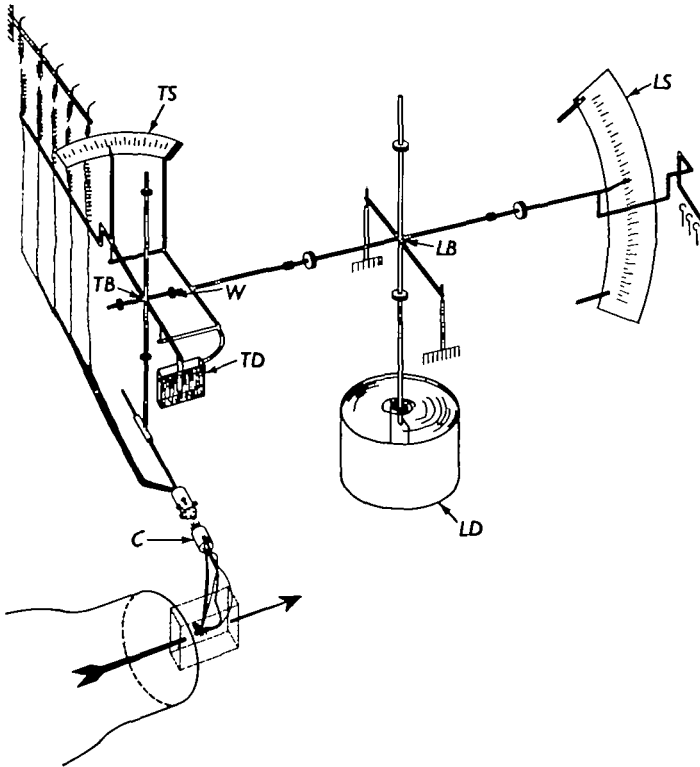


Fig. 3. Aerodynamic balance (not to scale). Constructed of fine brass tubes, it is primarily a lift-sensitive balance (pendulum and beam *LB*), bearing a thrust-drag sensitive balance (pendulum *TB*) on one end. Each pendulum has an oil and vane damper (*LD*, *TD*). Each balance also bears four sets of weights, the ones on the horizontal beams being for precalibration, the ones on the vertical pendula being for sensitivity control. Lift was read on the lift scale *LS*. Wind velocity was always adjusted by observing the thrust scale *TS* and manipulating the diaphragm of the wind-tunnel until the thrust-drag pendulum was vertical. Since this measure was exactly correct only when the lift balance was horizontal, the wind velocity was preadjusted to the preferred mean flight speed with the horizontal beam locked. Animals which flew well then tended to keep the same speed over long periods. The drag of electrode and suspension wires was prebalanced by shifting weight *W*. A weight platform (near the lift scale), at the same distance from the centre of rotation of the lift balance as the fly, was used to calibrate the lift scale after each flight by adding wire weights. A five-way plug carried the mechanical mounting for the fly (see text), electrical leads, and the transducing crystal *C*. The plug was inserted into a socket on the thrust-drag pendulum. The five fine insulated wires lay in the axis of rotation of the balance. Between this axis and a fixed external suspension the leads consisted of 20 cm. long coils of $5\ \mu$ copper wire. The restoring forces of these coils were negligible compared with the fly's weight. The whole balance was shielded from the wind by a Plexiglass cover, except for the lower portion of the thrust-drag pendulum which projected through a hole in the cover. The fly's movements did not exceed the limits of the working section (dotted solid). Since the balance was damped, short duration (1.5 sec.) lift variations were not indicated but were integrated into the average lift.

RESULTS

I. *The myogenic indirect muscles*

These relatively large muscles each contain several motor units. The neuromuscular control seems to be of the 'fast' type; that is, nerve impulses trigger one-to-one large non-facilitating muscle action potentials (Boettiger, 1957). These are about 5–7 msec. in duration. Even with large, apparently extracellular, wire electrodes the muscle

potentials can be more than 10 mV. in amplitude and positive in sign. For illustration see Fig. 4. In these muscles the train of arriving nerve impulses excites, but the contractions occur at a high frequency and independent of the phase of the nerve impulses. The strength of the contractions is, however, regulated by the frequency of arriving impulses. See Pringle (1965) for a discussion of this type of muscle control.

We have not carefully studied many of our records for evidence of sequential patterning of muscle impulses. Qualitatively the records appear similar to ones studied previously (Wilson & Wyman, 1963; Wyman, 1966; Wilson, 1967). We have found again that the several motor units of one muscle fire at almost exactly the same frequency even though each unit shows a wide frequency range. Therefore, for bilateral inter-muscle comparisons it is not necessary to record from all the units of each muscle or even to place the two electrodes exactly in homologous parts, although we did attempt to do the latter.



Fig. 4. (a) Burst discharges in an elevator muscle of an etherized housefly. (b) A similar burst occurs in an elevator muscle at the beginning of flight in another housefly. Subsequently, the wingbeat vibrations (middle line) develop and the muscle potentials acquire the usual pattern. Time mark (lower line), 10 cyc./sec.

One new pattern of nervous activity is worth describing here. This pattern, shown in Fig. 4, may also be characteristic of the beginnings of flights when the spike frequencies are very high. It could be elicited by a small, sublethal drop of ether applied to the fly's head. This pattern of repeated high-frequency bursts of impulses is reminiscent of the behaviour of motor units in the neurogenic flight systems of grasshoppers or butterflies.

Sometimes before flight had started action potentials occurred in the indirect flight muscles in a pattern which did not appear to be distinguishable from that during flight, but these were not accompanied by the slightest movement of the scutellum. This activity could last seconds or minutes and might cease altogether or there could be a transition to flight. The observation of this kind of activity in the indirect muscles tends to confirm the notion that a separate starting mechanism is needed to initiate the mechanical oscillations. Similar neuromuscular activity has been observed in the apparently mechanically quiet flight muscles of honey bees (Esch, 1964) and of the bug *Oncopeltus* (Wilson, unpublished). Esch suggests this activity is associated with 'preflight warm-up' in bees.

A. Lift control

We were interested in measuring a parameter related to the aerodynamic power output of the whole animal. In order to reduce the complexity of the total number of recorded variables we chose to measure only the lift, but to do this in flights during which air speed, and therefore thrust, remained virtually constant. In long smooth flights lift might change by 300 % or more while mean wind velocity was steady. (During rapid transients lift and thrust tended to vary together but we were not equipped to make quantitative analyses under these conditions.)

The discharge frequencies in the indirect muscles are strongly correlated with lift. Fig. 5*a* illustrates this relationship for units in the left and right dorsoventral muscles during 2 min. of flight in which lift varied from 300 % to 0 % of body weight. The left and right muscles vary together almost exactly. Fig. 5*b* presents in more detail a part of the record in Fig. 5*a*. One can see here that there is a smaller scatter of interval variation at higher frequency and also that there is a compensatory reaction to very short or very long intervals in the immediately following interval.

Contralateral dorsal longitudinal muscles also undergo similar frequency variations which are correlated with lift. That all the indirect muscles tend to vary together as lift changes is indicated by Fig. 6, which shows the behaviour of a left dorsoventral muscle unit and a right dorsolongitudinal muscle unit. This similarity of behaviour between elevator and depressor muscles is not *a priori* predictable, but neither is it surprising.

A species of fly showing tendency to hover, *Stomoxys calcitrans*, weighing only 5 mg., was similarly observed. Since the balance had not been constructed for work on such small animals only a crude index of lift was possible. Nevertheless a clear similarity to the larger flies was apparent.

The correlation of impulse frequency in each of two muscles with lift was qualitatively analysed in thirteen further experiments. There was no deviation from the above results.

B. Turning

Strong turning reactions can be recognized by asymmetry of the wing movements. The extreme condition is that in which one wing makes normal flight movements while the other is folded over the back in the rest posture. That this extreme reaction is natural was shown by Magnan (1934) who made filmed records of this behaviour in freely flying flies.

In front of our wind-tunnel strong turning reactions were rare. The good aerodynamic conditions tended to sustain straight flight. In the absence of a wind short flights with spontaneous turning reactions were common. We chose to study the behaviour of the muscles during these easily observed turns. Since it was not technically feasible to present a correctly varying wind speed and angle during the rapid shifts in behaviour, we used the next best condition of no wind at all. While this is not perfectly natural it at least allows the fly to experience the wind forces he generates himself rather than ones externally and arbitrarily applied.

In this extreme turning reaction which results in a virtual turn at a point in a freely flying animal, maximal differences in muscle activity could be expected. It

would seem possible that the muscles of the still wing might not be activated at all except for the adductor. This is not the case for the indirect muscles. Fig. 7 shows the results for homologous units in the right and left dorsal longitudinal muscles during 108 short flights during which only one wing was open and beating. Of these,

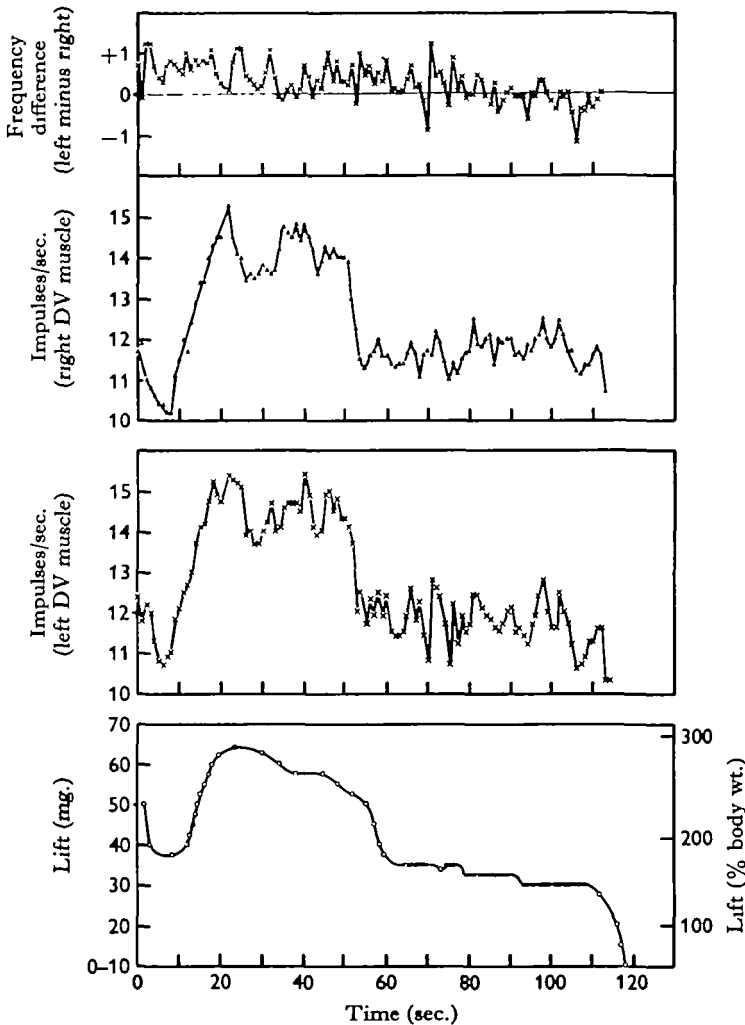


Fig. 5. (a) A graph of muscle action potential frequency and lift. The four records display the lift, the discharge frequency in left and right dorsoventral muscles, and the ratio of the discharge frequencies on the two sides. The last 2 min. of a $2\frac{1}{2}$ min. flight are presented. Small lift variations would be smoothed by the balance damping. Lift was constant during the long bars on the lift record. Although both muscles undergo wide frequency change, there is little relative change between them. Both are strongly correlated with lift. (b) A portion of the record of (a). The lift record (●) was adjusted to compensate for balance delay. The irregular variation of spike intervals in the left dorsoventral muscle is less at higher lift or higher frequency. Single very long or very short intervals are compensated in the next interval (see connecting lines). (c) Two examples of the original records of muscle action potentials from the flight shown in (a). The top set of records is from the 15th sec. of flight when lift is high. The bottom set is from 114th sec. of flight at low lift. Upper traces are from left, lower traces from right dorsoventral muscle units number IV.

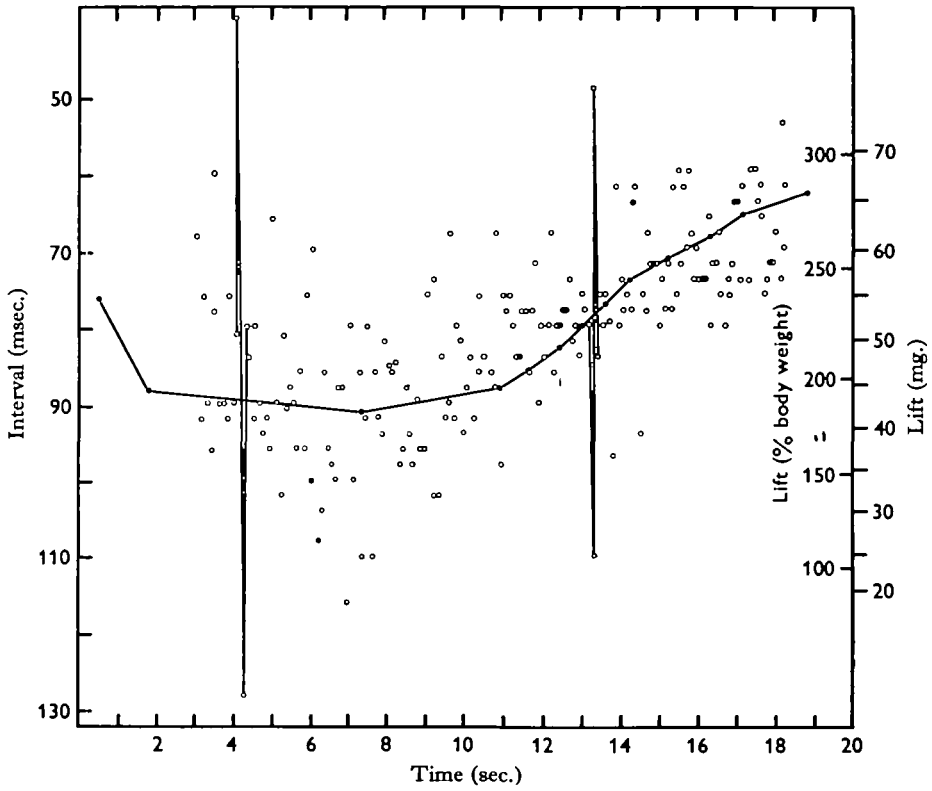


Fig. 5. (b)

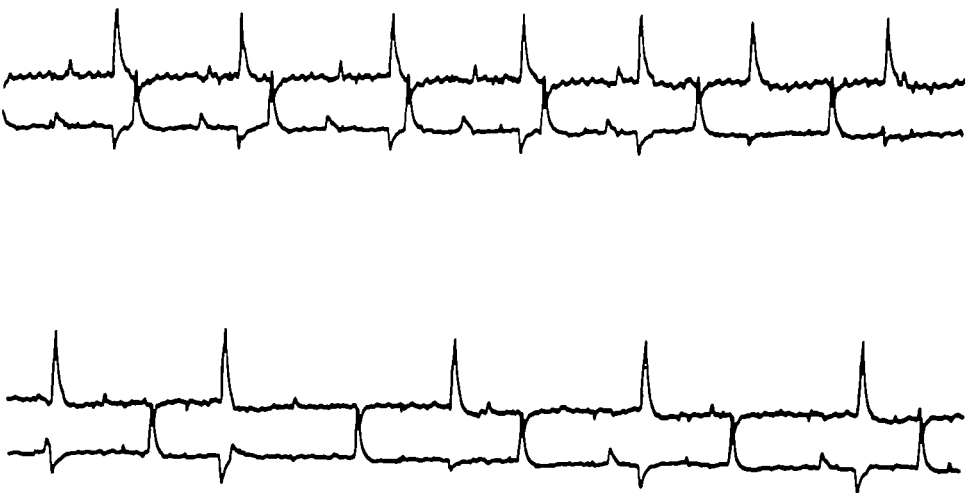


Fig. 5. (c)

in 52 only the left wing beat, in 56 only the right wing beat. The ratio of right frequency divided by left frequency varied from 0.75 to 1.25, with a dominant value of about 1.0, but the ratio was independent of which wing was beating.

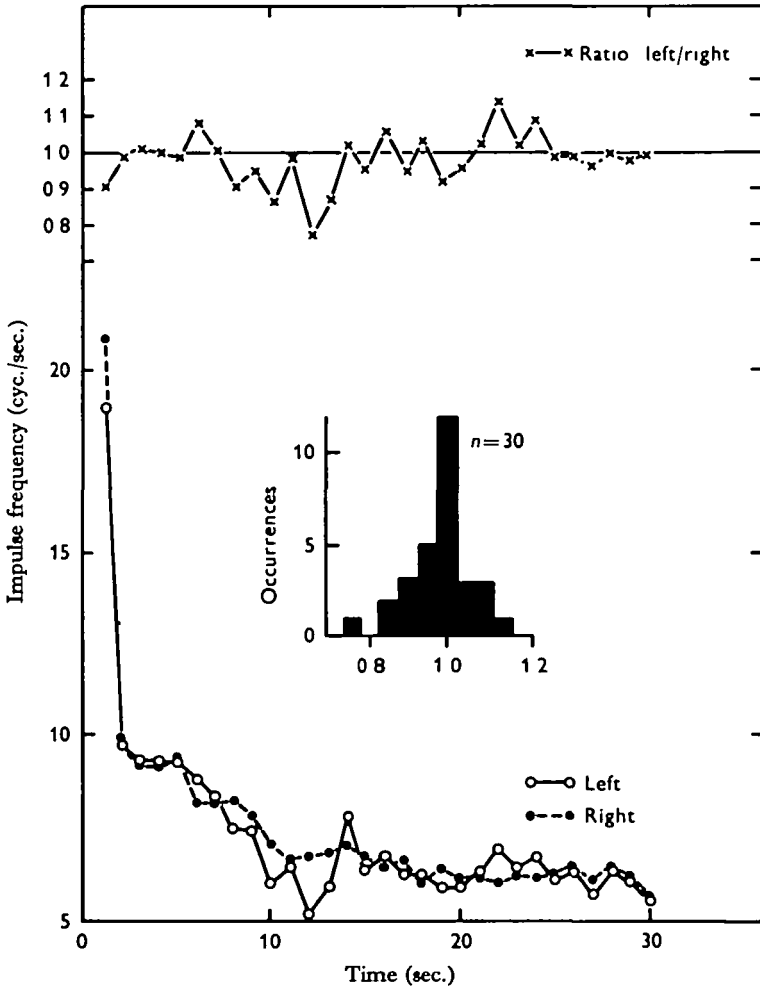


Fig. 6. Impulse frequency of a left dorsoventral and a right dorsal longitudinal muscle in *Muscina* as a function of time after start of flight. The impulse frequencies remained approximately steady, at 6½/sec., for 1 min. after the portion of flight plotted. Above: ratio of left to right muscle impulse frequencies during each succeeding second of flight. Insert: histogram of left over right frequency ratios for the 30 sec. Below: left and right muscle impulse frequencies in 1 sec. intervals. Note frequency is maximal at start and declines to a relatively steady value in a few seconds. The left/right ratio is independent of frequency.

The same result was observed in several animals and for other muscle pairs. Not always were the mean ratios identical during right and left turning, but the differences were always small, mostly under 10 %, the maximum observed difference being about 20 %. Absolute spike frequency varies widely, as has been shown already in the section on lift, so these small maximum side-to-side differences seem likely to be of small significance. However, during turning absolute frequency changes are conspicuous.

Even while the two sides show little or no difference between them, both sides increase in frequency during the turn. For example in one fly, while both wings were beating the average impulse frequency was 6/sec., but when either right or left wing was still the impulse frequency was about 19/sec. This was the maximum difference

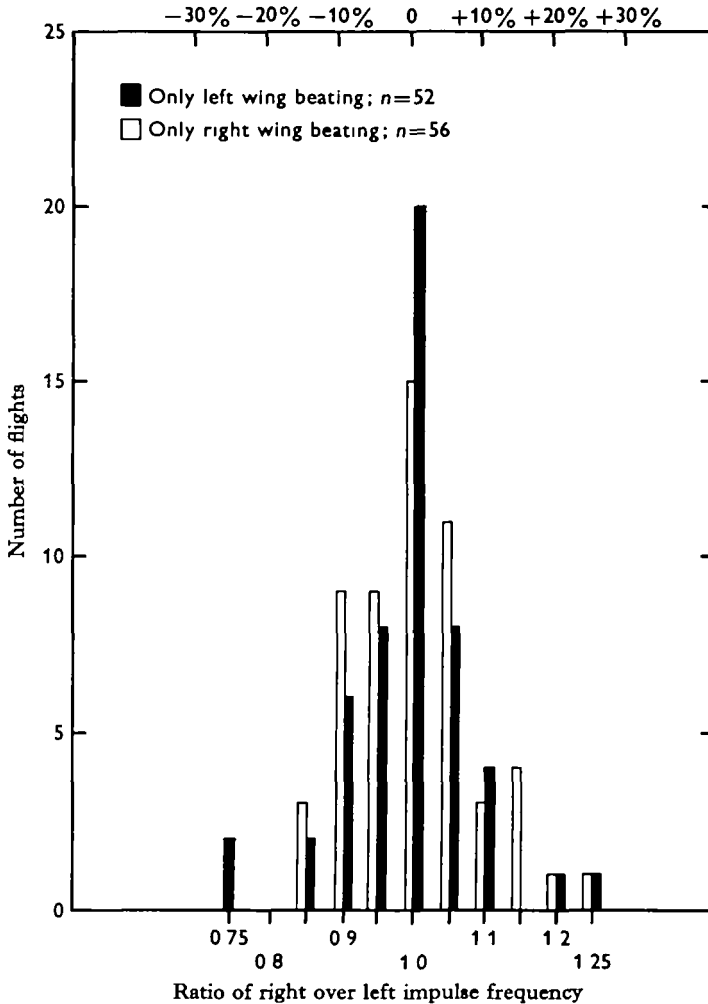


Fig. 7. Histogram of relative impulse frequencies in left and right dorsal longitudinal III muscles of a calliphorid fly. There were 1683 impulse intervals in 108 short flights. In every flight one wing was beating, but the other was completely folded over the abdomen in the rest posture. Each flight in still air lasted 0.5–6.0 sec., the whole series taking about 20 min. Note that the distribution of left/right ratios does not depend upon which wing is beating. The distribution is also similar to that of Fig. 6, taken from one flight of a fly which was not apparently turning.

observed (see Fig. 8). The higher frequency of both sides during turning may be explained by consideration of the energy needed to keep the thoracic oscillation going. With one wing folded it is quite possible that the resonant properties of the thorax are relatively out of order and that a higher state of excitation is required to keep up a useful amplitude of the one beating wing.

C. Conclusions

The myogenic indirect muscles in calliphorids receive approximately the same nervous commands. The impulse frequency varies strictly with lift, but there is little or no difference between frequencies for different muscles either as lift changes or

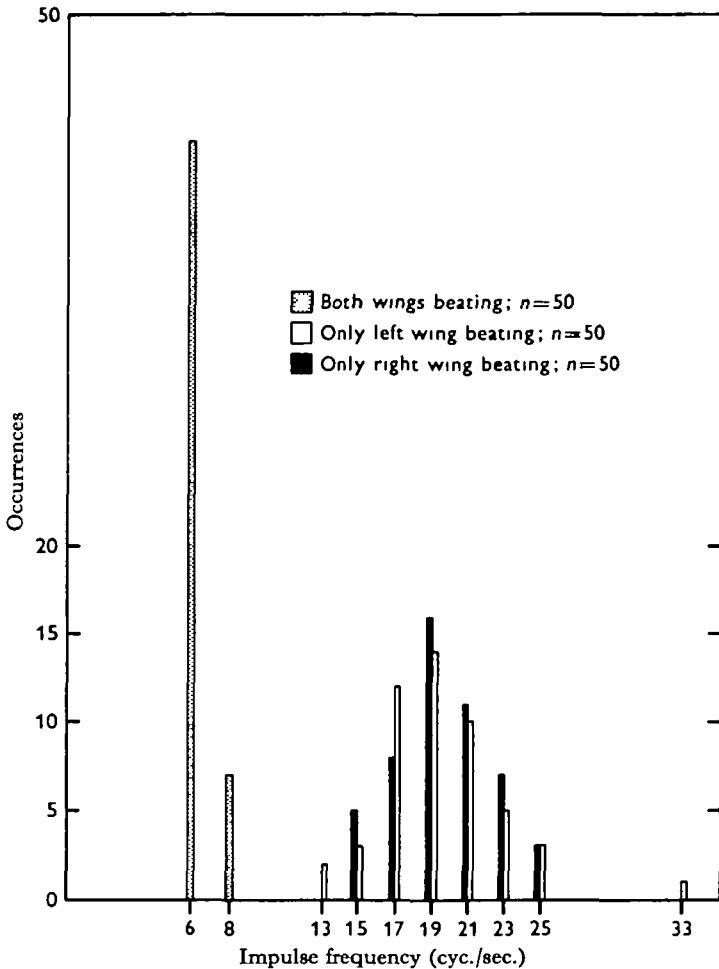


Fig. 8. Histogram of absolute impulse frequencies of a left dorsal longitudinal muscle unit under three conditions of near symmetry or maximal right and left turns. There were 1638 impulse intervals in 150 short flights. The frequencies were always lower when both wings were beating at the same amplitude. The frequencies were equally higher for either direction of turn.

during turning. At least, to a first approximation the indirect muscles comprise a flight power motor which has only a single variable parameter, namely, potential power output regulated by the mean frequency of excitation of all its motor units.

How then is steering accomplished?

II. The direct muscles

Except for the implications that could be drawn from anatomical studies little is known about the functions of these small muscles. They are mostly white in colour,

are not fibrillar in gross structure, have a relatively large sarcomere length compared to the indirect muscles, and have muscle action potentials of relatively short duration (2–4 msec.). The innervation frequency may be as high as about 200/sec. for short periods. Our recorded potentials were mostly positive in sign but only about 2 mV. in amplitude. These muscles presumably do not oscillate actively at the wingbeat frequency, but maintain relatively slow contractions, and can be considered tonic muscles in the flight system. The anatomical arrangement suggests a diversity of functions (see Table 1). We have been able to record from most of the direct muscles and can make some definite statements as to the role of several of them in flight.

In order to study these muscles we usually flew animals in still air. They were waxed by the scutum directly to a mechano-electric transducer. A set of micro-manipulators was used to position fly and electrodes. A recording probe of $10\ \mu$ fine stainless steel wire, insulated except at the tip, was inserted into one of the muscles. After the recording the fly was killed with ether and the electrode wire was rigidly waxed to the thorax wall. The tissues were fixed with formalin and subsequently a post-mortem examination was made to discover the exact location of the electrode tip. It was not easy to obtain reliable records from the small muscles. Data were accepted only if later examination showed the electrode tip to be completely surrounded by fibres of the muscle concerned. For certain muscles the problem was made somewhat easier by the fact that the muscle itself was nearly surrounded by air sacs, thus providing insulation from other muscle action currents. Other direct muscles lie very near the large myogenic muscles and current spread is a serious problem.

A. Control of turns

The extreme turns discussed in section I B involve major changes in average wing posture affecting the stroke plane setting relative to the body and also changes in the amplitude of wingbeat in the stroke plane. The main muscles which can be suspected of regulating this posture are Ritter's musculus abductor alae II and musculus adductor alae. Ritter showed that the abductor can draw the wing open and forward and the adductor can draw it backwards toward rest posture. These muscles are among the relatively easy direct muscles for electrical recording. Out of several dozen experiments three very good ones gave extensive results with near-perfect electrode positioning. Fig. 9 gives a sample record.

In one experiment seventy-three transitions from *only right wing beating* to *only left* to *only right* were filmed. In every set the result was that the left adductor muscle fired only when just the right wing was beating and was silent when only the left wing was beating.

In another experiment both wings beat most of the time but there were brief (*c.* 1 sec.) interludes of *only left wing beating*. The left abductor II fired only when only the left wing was beating. It was not active in normal flight or in the few cases when only the right wing was beating.

The abductor was once observed to be active in a non-flying animal. This fly held his wing nearly straight out, but did not vibrate it (quite abnormal in *Calliphora*, but reminiscent of sexual displays in other flies). The wings were repeatedly drawn forward quickly about 25° and went back slowly to the perpendicular position. The forward motion was accompanied by potentials in the musculus abductor.

So during the extreme turns the abductor muscle fires only when only the ipsilateral wing is beating; the adductor fires only when only the contralateral wing is beating. (Consequently there must be a reciprocal relationship during a sharp turn. The inside wing is drawn back to the rest posture; the outside wing is drawn more forward than usual. High-speed films (Nachtigall, unpublished) indeed show that the beating wing is drawn somewhat more forward than in normal straight flight.) The adductor-abductor pairs may also work in less extreme fashion. They have been observed to fire at a lower rate when there is not a visually obvious turn in progress.

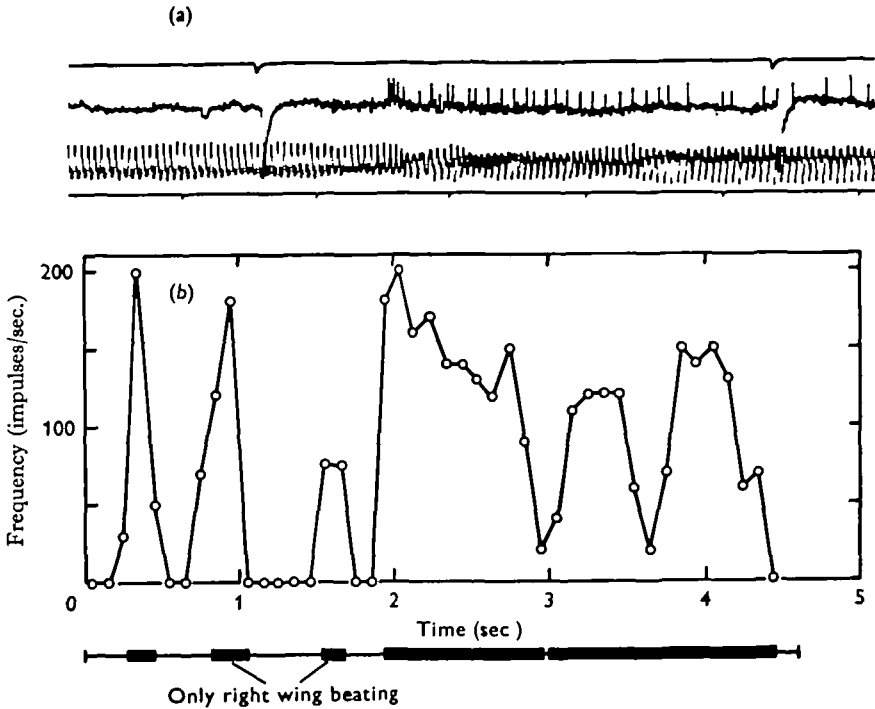


Fig. 9. Electrical discharge in the direct muscles of two calliphorid flies. (a) Line (i): an indirect left dorsoventral muscle recorded at low gain. Line (ii): the left abductor secundus begins to fire during a previously normal flight when the right wing ceases to beat. Line (iii) below: the mechanogram of scutellar movements undergoes a change correlated with the turning attempt. Line (iv): time mark, 10 cyc./sec. (b) Impulse frequency in the left adductor as a function of time. One example from seventy-three similar records. When the adductor is highly active it draws the left wing back into rest posture and only the right wing beats.

From the above we conclude that the adductor and abductor muscles of each wing comprise an antagonistic pair with negatively correlated activity which regulates the stroke angle. The homologues on the two sides seem also to be negatively correlated. Neither muscle set is necessary to maintain an average stroke-plane posture and therefore these muscles may be active only during turns or in other transient conditions. The normal stroke plane could be a passively stable one once the main flight motor is oscillating. When one wing is partially or fully folded over the back the stroke amplitude is decreased, probably due to poorer resonance conditions and different relationships of parts in the hinge. Decreased amplitude of one wing results in less aerodynamic power on that side and therefore a rolling and yawing torque toward that side.

Other muscles (pronators, supinators, levators) might be used principally or importantly in turning also. We have been able to record potentials from all these muscles, but not under circumstances which allowed observations of correlated kinematic wing parameters.

B. Wingbeat-frequency control

Wingbeat frequency and amplitude, aerodynamic power, and impulse frequency in the indirect muscles are all positively correlated, so it is not necessarily easy to identify the causal relationships. On the basis of knowledge of the basic properties of myogenic flight muscles it seems improbable that the innervation frequency in the indirect muscles has much to do with their oscillation frequency (Machin & Pringle, 1959). The flight power system is considered to be a highly resonant one and wide changes in frequency are probably possible only if the resonant properties of the thorax are adjusted. The pleuro-sternal muscle or Ritter's *musculus latus* has been thought to play this role. Contraction of the *musculus latus* should draw the basal pleural wall inward, presumably stiffening the whole thoracic mechanism. Two kinds of experiments were possible which tend to confirm this hypothesis that the *musculus latus* is the main frequency-determining muscle.

First we wished to know if impulse frequency in this muscle is a function of wingbeat frequency. This was a very difficult task and we were fully successful only once in twenty-five experiments. The muscle is a thin sheet of fibres; it is very deep in the thorax so that stereotaxis was difficult and movement of the electrode relative to the muscle was likely to occur because of its relatively distant attachment to the skeleton. In addition the muscle is in between the large flight muscles. In the best experiment sixteen short flights were recorded. In each flight wingbeat frequency began high and declined and so did the frequency of discharge in this muscle. There was a nearly linear relationship, with wingbeat frequency varying from 260 to 170 cyc./sec. and spike frequency from 190 to 50 cyc./sec. (see Fig. 10).

Next, two mechanical events were correlated: the wingbeat frequency and the degree of inward movement of the pleural base (episternal region). These were recorded with a special streak camera. For a description of technique and a sample record see Fig. 11. In most cases measured there was a high correlation of high wingbeat frequency and greater inward movement of the pleuron base. The displacement at the high-frequency beginning of flight was about 40 μ . This decreased as flight weakened, but return to the rest posture occurred only when flight stopped. Additional observations of some flies under the dissecting microscope with simultaneous amplification of the flight tone gave the same qualitative result.

There is a three-way correlation of impulse frequency in the *musculus latus*, inward movement of the basal part of the pleuron, and wingbeat frequency. It seems that only the *musculus latus* can thus draw in the pleural parts and it is probably the basic frequency-determining muscle. However, we have not found the relationship between wingbeat frequency and inward pleural position to be quantitatively constant. Other factors must affect wingbeat frequency. Mechanical ones, such as wing loading which may vary with stroke plane, come to mind. Further analysis of this problem will probably require a much more detailed understanding of the wing joint mechanism than we have now.

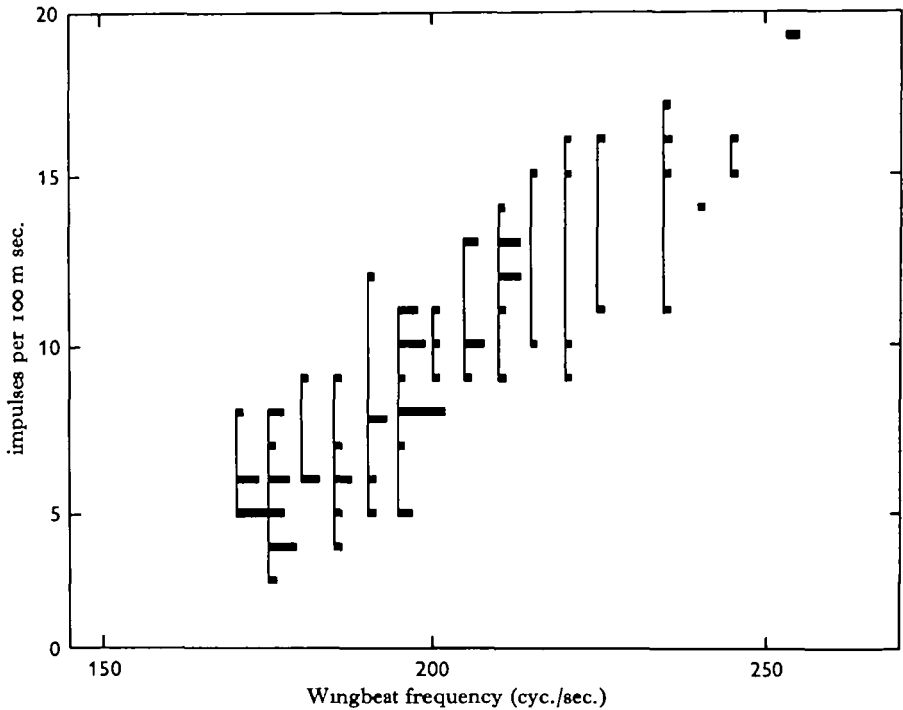


Fig. 10. Impulse frequency in the musculus latus (pleurosternal muscle) as a function of wingbeat frequency in *Muscina*. Wingbeat frequencies are lumped in units of 5 cyc./sec. Each 100 msec. interval is represented by one black square. Horizontal bars indicate several similar observations at the same point along the thin vertical line of constant wingbeat frequency. 553 impulses were divided over 921 sec. intervals in 16 short flights. Sampling many short flights was necessary in order to obtain values for high wingbeat frequencies which occurred only at the beginning of flight. Although there were no impulses in the musculus latus at wingbeat frequencies below 170 cyc./sec., the fly sometimes flew at only 150 cyc./sec. near the end of flight.

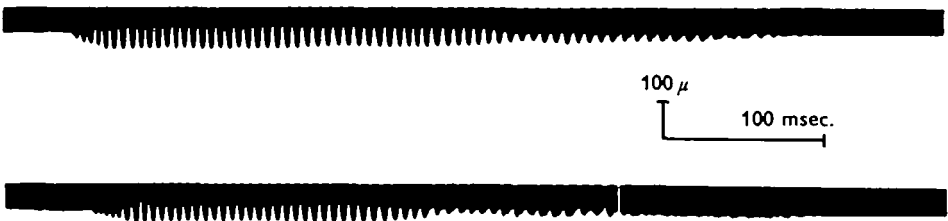


Fig. 11. Two streak camera records of the oscillations of the episternal wall of a 14.5 mm. long sarcophagid fly during short flights. The fly was fixed by its abdomen tip to a rigid wire attached to a microscope slide and flown without wind. Light, interrupted by the edge of the episternal wall, passed through a $\times 10$ microscope objective and was projected on a narrow perpendicularly slotted mask which replaced the lens of a Grass kymograph camera. The film was moved at 25 cm./sec. In these negative prints the black track corresponds to the bright background, the lower edge of the track records the time function of the silhouette of the episternum. Flight start is at the left. The stronger inward movement of the episternum (downward in the figure) at the beginning of flight is correlated with the higher oscillation frequency.

III. The course of flight initiation

A. The starter muscle

The myogenic muscles can be nervously excited without oscillating. One might suppose that this state is unstable and that oscillations would begin spontaneously. However, the state is often rather persistent and normally some special event triggers the first cycle. This oscillation starter is not the same as the wing-opening event (Nachtigall, unpublished high-speed films) since the latter occurs during the first half-dozen wingbeat cycles or even later. The muscle candidate usually proposed for this function is the tergotrochanteral muscle of Ritter's *musculus dorsoventralis IV*. This is a white muscle like the direct wing muscles and is therefore presumed not to be myogenic. It can depress the leg or depress the notum, elevating the wings if they are open. An electrode was placed in the muscle through its insertion on the notum. Good records were obtained from four flies. There was usually one, but sometimes two or three, large spikes a few milliseconds before the first detectable thoracic oscillations. This occurred usually at about the same time as the first discharge in the myogenic muscles. Spikes in the *dorsoventralis IV* then ceased and recurred only rarely later during flight.

The tergotrochanteral muscle can be shown to play a part in the starting procedure by cutting its dorsal insertion or by cutting off the middle legs, which effectively releases it from its origin with respect to the flight parts. The animal cannot now take off normally. However, it still can start, so this muscle is not the only starter mechanism.

Since the *musculus dorsoventralis IV* also depresses the middle leg it cannot just start wing flapping and nothing more. It is also involved in the jump that starts flight. We allowed flies to begin free flights from a platform on a sensitive force transducer. A nearby microphone picked up the flight tone. A latency of about 12 msec. from the beginning of the jump to the first recordable wingbeat was found. Foot impressions in lampblack showed the middle legs exerted more force in the jump than the others.

This role in jumping and flight initiation has been discussed by several previous authors, especially Williams & Williams (1943), Boettiger & Furshpan (1952), and Pringle (1957). Our present results agree with the conclusions of those authors and add the line of electrophysiological evidence and timing measurement. It is interesting that this pair of muscles is probably excited by the largest fibres descending from brain to thorax (the giant fibres of *Drosophila*, Power, 1948).

B. The integrated sequence

From the streak camera records a sequence of events can be identified (Fig. 12) and these can be correlated to particular muscle functions. Usually the same order is followed, but some changes in order are permissible—for example, the rare instances in which the indirect muscles are excited long before flight begins. The usual sequence seems to be as follows.

(1) The *musculus latus* is activated, drawing in the pleural bases and preparing the thorax for the high starting frequency.

(2) 15–30 msec. later there is a starting jump due to a small burst of impulses in the tergotrochanteral muscle. (An outward snap of the pleural base can often be seen at

this time. Following that there may be minute vibrations at least double the usual wingbeat frequency). At the same time all the indirect flight muscles are activated by a synchronous high-frequency burst of motor impulses.

(3) About 3 msec. later the first small-amplitude thoracic vibration is observed.

(4) During the following 10–15 msec. (approx. 6 wingbeats) the wings are gradually drawn forward and the wingbeat amplitude reaches its normal maximum. (Compare with fig. 30 in Nachtigall, 1966.) The opening of the wings could be passive, but it is probably normally aided by the abductor muscles.

(5) By the end of stage (4) the initial high-frequency burst of impulses in the myogenic muscles has terminated and the normal flight pattern is fully developed.

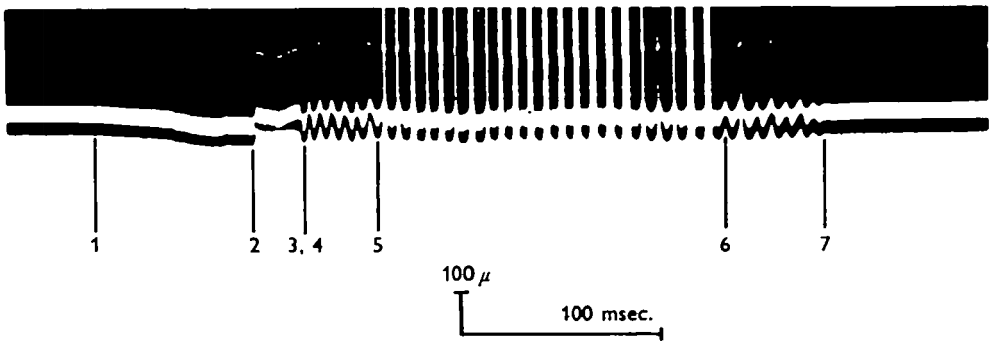


Fig. 12. Streak-camera record of a part of the pleural body wall on the praescutal border, just above the frontal end of the horizontal parapleural slot, during a short flight in *Muscina*. A thick hair (lower track) and a thin hair (upper track), inserting near this point, produce parallel streaks. For description of the recording technique and interpretation of the record see legend to Fig. 11. During each forward excursion the wing shadows the slot, producing a vertical white bar which interrupts the whole record. The numbered arrows indicate significant events in the time course of the flight. (1) Beginning of inward movement of the pleural wall by the musculus latus. (2) Beginning of the starting jump (and possibly the start of oscillations of the indirect flight motor) by the tergotrochanteral muscle. There is a sudden outward movement of the pleural wall followed by very small, very high-frequency oscillations. (3 and 4). First high-amplitude thoracic vibrations are accompanied by small amplitude wingbeats, since the wings are still nearly folded. (5) Full wingbeat amplitude is indicated by the wing interrupting the whole trace. (6) The fly begins to stop: the wings are partly folded, the thoracic vibrations are smaller and of lower frequency. (7) End of flight movements.

DISCUSSION

The most interesting result of this work is the finding of almost total separation of control for the system producing flight power and the ones regulating the use of that power. There is a flight motor—the system of myogenic muscles, which can be fully activated nervously even with the wings still. The aerodynamic power produced by this motor depends upon activity in the system of direct muscles: these control a clutching arrangement, the wing abducting system; and control a frequency-regulating mechanism via the muscles regulating thoracic stiffness. There is also variation in the degree of excitation of the motor itself. Furthermore, the distribution of power from the motor is regulated externally to it by differential effects of the direct muscles on the linkages from motor to wing. The separation of power production and steering mechanisms in flies which has been demonstrated here lends support to Pringle's hypothesis

regarding the control of flight in bees (Pringle, 1965). Pringle concluded from anatomical studies on fresh but not active specimens that separate muscles produced the main power, controlled frequency, controlled wingbeat amplitude, stroke plane, etc.

In many insects such separation of function is not well developed. In locusts or butterflies, for example, certain of the muscles which supply downstroke power (basalar and subalar muscles) must also regulate angle of attack and therefore the lift-thrust relationships within and between wings. In these and other insects with neurogenic flight rhythms the frequency of wingbeat is directly a function of the frequency of motor output from the central nervous system to the main power muscles. The natural frequency of the thorax may also be regulated in order to conserve energy, but changing the thoracic stiffness by itself cannot change wingbeat frequency.

Even in some insects with myogenic flight rhythms the separation is not complete. In the beetle *Oryctes* the basalar muscle is myogenic and aids in the main power oscillation. It has a relatively complex multiple innervation and apparently its power can be differentially regulated in order to vary degree of pronation and wingstroke amplitude on the two sides (Darwin & Pringle, 1959; Ikeda & Boettiger 1965; Burton, 1964).

Great manoeuvrability is not the special asset of myogenic systems with separation of power and control functions. Grasshoppers seem particularly crude, but dragonflies stand at the other extreme, though both have neurogenic flight rhythms. Myogenicity seems necessary only as an adaptation to small size and consequent high wingbeat frequency.

Flies, and probably other very small insects, stand at the apex of evolutionary development of differentiation of control functions for flight. Perhaps the separation of function allows greater efficiency of control by each specialized part. There has been an evolution in flies toward a kind of simplicity of control. To a large extent each function is handled by a spatially distinct anatomical structure. In locusts there needs to be not only a spatial coding of nervous command to the various muscles, with a good deal of complexity of interaction because of shared functions, but also the temporal coding in each line must be carefully regulated and the phasing between the many lines well co-ordinated. In flies no nervous event need be regularly related to the phase of the wingbeat, nor need the nervous impulses be phasically correlated to one another with much precision. Further simplicity is achieved by making some important functions, such as the supination-pronation of the wing during upstroke and downstroke, automatic consequences of the anatomy (Boettiger & Furshpan, 1952) leaving to the nervous system only a margin of additional control for fine differential adjustment. In *Drosophila* the nervous system may even lack the ability to influence angle of attack (Vogel, 1966).

Our results should be of interest to those who are studying optomotor effects on flying insects. If other flies are similar to the ones we studied then motor output correlates of the optomotor turning tendency will have to be sought in the small direct muscles and not in the much more easily monitored power muscles. One visual reflex which does affect the frequency of discharge in the indirect muscles is related to ground speed. Smyth & Yurkiewicz (1966) found that moving the field under a tethered fly forward or backward resulted in changes in frequency in the longitudinal and vertical indirect muscles of both sides. On the other hand air currents aimed from

various directions, while having visible effects on the wing movements, did not affect the discharge rate in the indirect muscles. Nor did Smyth & Yurkiewicz find a differential result with visual stimuli moving to the right or left. Since they did not record from single units they only stress that average frequency for the totality of several units did not change appreciably, but it seems reasonably safe to conclude that the units all behaved alike within each muscle (Wyman, 1966). This seems to be another example showing that control of total power (related to ground speed in this case) involves changes in excitation in the myogenic muscles whereas steering does not.

SUMMARY

1. Electrical activity from the indirect, myogenic muscles of calliphorid flies was recorded during flight. The animals were suspended from an aerodynamic balance in the laminar air-stream from a wind-tunnel. Muscle action potentials, recorded with $25\ \mu$ wire, were 5–7 msec. in duration, up to 10 mV. in amplitude and positive in sign. Frequencies were mostly under 20/sec.

2. Frequencies in all the indirect muscles were similar, but these varied together with changes in aerodynamic power.

3. Frequencies in the indirect muscles of the two sides varied by no more than $\pm 10\%$ during extreme turns to right or left (only left or only right wing beating).

4. Electrical records from the non-myogenic direct muscles were made during tethered flight. The potentials were 2–4 msec. in duration, up to 2 mV. positive and had frequencies up to 180/sec.

5. A nearly linear positive correlation exists between impulse frequency in the musculus latus (pleurosternal muscle), the inward movement of the pleural wall, and the wingbeat frequency, suggesting that this muscle is the basic frequency determiner.

6. Strong turning behaviour is associated with opposed frequency changes in the pairs of antagonistic adductor and abductor muscles of the wings on the two sides of the body.

7. The musculus dorsoventralis IV (tergo-trochanteral) is activated by a short impulse burst at the beginning of flight. It apparently acts as an oscillation starter.

8. Flight initiation normally requires 30–60 msec. Usually activity begins in the musculus latus, which stiffens the thorax. Then simultaneously the myogenic muscles are activated and the 'starter' muscle causes a jump and the beginning of oscillation of the thorax. Then the wings are drawn gradually forward and full wingbeat amplitude develops within the first six wingbeats. Flight begins with maximal lift and wingbeat frequency and a nearly synchronous burst discharge in all the indirect muscles.

9. Power production and the transmission and distribution of power are under separate control. The myogenic indirect motor varies only in total power output, this being influenced by its own state of excitation and by a muscle-controlling wingbeat frequency. Steering is accomplished by non-myogenic direct muscles which are capable of differentially engaging the two wings with the motor.

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