

## MUSCLE ACTIVITY DURING FLIGHT IN SOME LARGE LEPIDOPTERA

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### INTRODUCTION

In order to investigate the physiological mechanisms which generate behaviour, it is reasonable to examine first the movements which constitute the behaviour and next the patterns of activity in the muscles which cause the movements. It is then possible to consider the central nervous processes which produce the excitatory output to the muscles. This type of analysis has been extensively applied in studies on the flight of locusts (Wilson, 1964*a*) and flies (Wilson & Wyman, 1963; Wyman, 1965, 1966). In the present paper this approach is applied to the flight of Lepidoptera.

Flying insects are favourable objects for the investigation of central nervous interactions by which patterned motor outputs are produced because of the relative simplicity and rhythmic nature of the flight movements and because of the relatively small number of motor units involved. The muscles and skeletons of insects from different orders have many similar features, and it seems probable that these similarities represent homologies (Snodgrass, 1927, 1935; Pringle, 1965). Morphological evidence suggests that there is also a high degree of homology among the components of the nervous system (Schmitt, 1962). Therefore, if insect flight has evolved only once, it is reasonable to expect similarities among the flight-control systems of various insects, and any differences present should be explicable as evolved modifications of a primitive mechanism. Proceeding from this point of view the author began a comparative study on the Lepidoptera. Such a study should help to define those properties which are common to the flight-control systems of all insects and could also uncover differences from which new ideas about the central nervous mechanisms can be derived. Lepidoptera were chosen because the group includes animals which differ widely in size and in wingbeat frequency and because preliminary work by M. Konishi (unpublished) indicated that there are interesting variations in the patterns of muscle activity in different species of this group.

The first study of muscle activity during flight in the Lepidoptera was made by Roeder (1951). He showed that in the moth *Agrotis* there is synchrony between muscle potentials and wing movements. Contractions of the muscles are initiated by the motor impulses, and the alternating activity of antagonistic muscles is timed by the nervous system. This type of flight system is called 'neurogenic' or 'synchronous', in contrast to the 'myogenic' or 'asynchronous' flight system of flies and wasps. In the latter case, the wing movements occur at a greater frequency than the muscle potentials and phase

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relationships between the two are random (Pringle, 1949; Roeder, 1951; Wilson & Wyman, 1963).

In those insects with a synchronous flight system regular phase relationships between muscles are necessary. In the best-studied example, the locust *Schistocerca gregaria*, the wingbeat frequency varies only slightly, and the motor units are tightly coupled during flight (Wilson & Weis-Fogh, 1962). The motor patterns are not due to morphological connexions which permit only one rigid sequence of interactions between the neurons of the flight-control system. Instead, different phase relationships are possible during different types of behaviour. For example, the same muscle pair may be synergists in flight and antagonists in walking (Wilson, 1962). Nor are the phase relationships set by timing cues from peripheral receptors, because the pattern persists when phasic sensory information is eliminated (Wilson, 1961, 1964c; Wilson & Wyman, 1965). The patterns of motor unit activity must therefore result from labile, functional interactions among neurons in the central nervous system.

In contrast to the uniform wingbeats of locust flight many large-winged Lepidoptera fly with wingbeats which vary widely in amplitude and frequency. This paper describes patterns of activity in muscles which move the wings and discusses correlations between muscle activity and the amplitude of the wingbeats. A brief account of this work has been published (Kammer, 1964). Wingstroke amplitude was measured in order to provide an indication of the mechanical output of the flight muscles. Because of the variability of the amplitude it was necessary to make continuous observations of the wing position during each stroke. In most previous studies on Lepidoptera, either an average amplitude was taken, usually by observing the blurred movement of the wings, or each stroke was sampled stroboscopically a small number of times (Marey, 1874; Voss, 1914; von Uexküll, 1924; Magnan, 1934). For this order of insects there is still no comprehensive study of wing movements under proper aerodynamical conditions, comparable to the studies on locusts (Weis-Fogh, 1956; Jensen, 1956) or on flies (Nachtigall, 1966). In the present investigation motor unit activity was characterized in terms of burst length and wingbeat period. The *burst length* is the number of times a motor unit is activated in each wing stroke. The *wingbeat period* is the interval from the beginning of one burst to the beginning of the next burst in the same unit. It includes one cycle of up and down movement of the wings. In order to elucidate properties of the flight-control system the observed patterns were compared with patterns previously described for locusts (summarized by Wilson (1964a), and by Weis-Fogh (1964)).

#### MATERIALS AND METHODS

Most of the experiments were performed on moths of the family Saturniidae, including *Antheraea polyphemus* (Cramer), *Hyalophora cecropia* (L.), *H. (Callosamia) promethea* (Drury), and *Samia cynthia* (Drury). These animals were obtained as pupae, primarily from dealers in the midwestern United States. Adult monarch butterflies, *Danaus plexippus* (L.), collected locally, were also used.

All the experiments employed tethered animals, and 'flight' as used in this paper refers to rhythmic wing movements of normal amplitudes made by an animal under the experimental conditions. An animal was prepared for tethering by severing the

legs at the trochanter or proximal end of the femur and by removing the scales from the ventral portion of the mesothorax. Then the base of the mesothoracic coxae and stumps of the legs were cemented to the tip of a vertical supporting rod with a mixture of beeswax and rosin (Colophonium). With this arrangement, wing movements were unimpaired except in a small space below the animal, and in most cases the wingstroke amplitude was not so large that the wings touched the supporting rod. The animals usually flew readily after being handled or after stimulation with a puff of air or a light touch. It was not necessary to use a wind tunnel. Additional stimulation was sometimes provided by holding tissue paper soaked with acetic acid near the antennae of the animal.

Muscle potentials were recorded by means of electrodes inserted into the mesothoracic flight muscles through small holes punched in the exoskeleton with a fine insect pin. The electrodes consisted of  $50\ \mu$  or  $100\ \mu$  copper wire, insulated except at the tip. A ground lead, a larger copper wire, was inserted into the abdomen. Muscle potentials of 1 to 10 mV. were readily obtained.

In one set of experiments the nerves from receptors on the wings were cut. There is one large sensory nerve running from each wing to the central nervous system. An opening near the anterior edge of the base of the wing was made by removing the tegula or by cutting the membranous cuticle. The nerve, visible between air sacs of the thorax, was then severed with fine scissors. Success of the operation was verified by the absence of responses to tactile stimuli to the wing and by post-mortem dissection.

Wing movement in a vertical plane was monitored continuously by photographing the wing position on horizontally moving film by means of a Grass C-4 camera. The animal was positioned with its head toward the camera and with the leading edge of its left wing perpendicular to the axis of the camera lens. A small ball of aluminum foil was waxed to the leading edge of the left forewing as a marker and was illuminated from above by a narrow beam of light. A simultaneous record of muscle potentials was obtained by reflecting an image of the oscilloscope screen into the lower half of the lens aperture by means of a front surface mirror (Pl. 1, fig. 1). Because the wing marker was displaced horizontally as well as vertically when the wing moved, precise temporal relationships between wing position and muscle activity cannot be deduced from direct observation of the film, although they can be calculated.

The filmed records of muscle potentials and wing movement were measured and the data were transferred to punched cards by means of a Gerber Digital Data Reduction System and subsequently processed by a computer. Programs written by R. J. Wyman were employed in the analysis.

The procedure for calculating the angle over which the wing moved is illustrated in Pl. 1, fig. 1. For the highest and lowest excursion of the wing in each stroke the position of the aluminum marker was recorded with respect to some convenient reference line, such as the edge of the film. The position of the base of the wing with respect to the same reference line was determined in pictures taken on stationary film. The stills were also used to establish the distance from the wing base to the marker. From these values the angle through which the wing moved in either the upstroke or downstroke was calculated. This angle will be called the *amplitude* of the stroke.

The method employed to calculate the amplitude results in some errors. Because

a sine function was used, a small vertical displacement of the marker is equivalent to an increasingly larger angle as the wing nears a vertical position at the extremes of the stroke. The calculated values are thus least accurate at the points of greatest interest. The inaccuracies at the extremes of the stroke were evaluated by comparing the results obtained by computing the amplitude for each wingbeat, as described above, with results obtained by measuring still photographs. In the latter, the image of the moving wing is blurred, but the maximum amplitude can be measured directly and with more accuracy. The comparison indicated that the actual movement during a stroke covered a larger angle than that given in the results. Therefore, in the data presented only changes in amplitude within an experiment are meaningful. The observed changes in relative amplitude were of such a magnitude that they were not obscured by the error inherent in the method. Because the wing is a three-dimensional object moving in three dimensions, measurements of amplitude from projections of the wing marker onto the plane of the film are subject to some additional errors. The method does not account for certain alterations in wing position, such as changes in the extent to which the wings are promoted or changes in the angle of the stroke plane. Such alterations in wing position would affect the apparent distance between wing base and marker. In order to estimate the magnitude of the source of error, this distance was measured on brief time-exposures taken with stationary film at intervals during the experiment. It did not change appreciably in any one experiment. Therefore any errors from unrecorded changes in wing position are small. The method also does not account for changes in the angle of attack, which might alter the wing displacement resulting from a given amount of force applied to the wing by the muscles. It has been assumed that this factor remains relatively constant within an experiment, and the assumption is supported by the consistency of the general trends within experiments. From experiment to experiment, however, there may be differences in angle of attack, position of the stroke plane, or other stroke parameters which influence the relationship between wingstroke amplitude and work performed by the muscles. Such a difference can explain the results of two experiments in which the same animal and the same muscle were observed on successive days. In the first experiment a given burst length produced only a small wingstroke amplitude, but in the second experiment the same burst length, at about the same wingbeat period, produced a much larger amplitude. Because within an experiment burst length and amplitude were observed to be positively correlated, the difference between the results of these two experiments can be explained most simply in terms of a change in some unrecorded variable, such as angle of attack. Because of the possibility that at different times or in different specimens there may be differences in unrecorded stroke parameters affecting amplitude, and because the calculated values are smaller than the actual values, comparison of the calculated amplitudes from different experiments is not meaningful.

In addition to errors resulting from the method used to determine amplitude, variations in amplitude and also in frequency could result from tethering the animal (Chadwick, 1953). Certainly the aerodynamic conditions in my experiments were not normal. However, in the course of collecting monarch butterflies it became clear that large changes in air speed do occur during free flight. In addition, Urquhart (1960) estimated from his field observations that the stroke amplitude is about  $30^\circ$  in a cruising

monarch butterfly and about  $120^\circ$  in a speeding one. Therefore changes in amplitude and probably frequency are part of normal flight behaviour, at least in monarch butterflies. Even if the variation seen in my experiments were outside the normal range, the correlations between the variables measured still reflect important properties of the flight-control system.

#### OBSERVATIONS ON THE MUSCLES OF THE PTEROTHORAX

In Lepidoptera the mesothorax is larger than the metathorax. Especially striking is the difference between the dorsal longitudinal muscles of the two segments. The mesothoracic dorsal longitudinal muscles run almost the whole length of the pterothorax, while the corresponding metathoracic muscles are reduced to small bundles of short fibres. Considering their size it is probable that the mesothoracic dorsal longitudinal muscles provide most of the power for the downstroke. The other flight muscles in the mesothorax, the direct depressors (basalar and subalar) and the indirect elevators, are at least twice the size of their metathoracic homologs. In the present study only these large mesothoracic muscles were investigated.

Table 1 summarizes the terminology applied to the main mesothoracic flight muscles and gives the function of these muscles in the lepidopteran imago. Descriptions of the anatomy of the thorax and its muscles are provided by Weber (1928) for *Papilio machaon*, by Nüesch (1953, 1957) for *Antheraea* (= *Telea*) *polyphemus*, and by Ehrlich (1958) and Ehrlich & Davidson (1961) for *Danaus plexippus*. Nüesch (1953) also summarizes the descriptions given in some earlier studies on other species. The anatomical terms used in the present paper are based on the position and skeletal attachments of the muscles and are similar to those used for *Schistocerca gregaria* (Wilson & Weis-Fogh, 1962; Wilson, 1962). Additional anatomical features and observations important in understanding how the muscles function are discussed below.

The relative paucity of motor units in an insect, compared with a vertebrate, is apparent in the number of motor units in the mesothoracic flight muscles. According to published accounts (cited above) and my own observations on anatomy, the dorsal longitudinal muscle consists of five units. This number has been confirmed by electrical stimulation in *Antheraea polyphemus* (Nüesch, 1953) and in *Danaus plexippus* (present study). My electrical recordings from the mesothoracic subalar muscle in *Hyalophora cecropia* suggest that this muscle consists of more than two units, but the exact number has not been determined. No physiological studies have been made on the basalar muscles, but the anatomical evidence (cf. Table 1) suggests that there are at least four units present. The number of components of the elevator muscles is also uncertain, but on the basis of anatomy and incomplete electrical records, I estimate that there are approximately eleven motor units on each side. Thus the total number of motor units in the power-supplying muscles of the mesothorax is probably between forty and fifty. A comparable estimate of about 35 units in the mesothorax has been made for the locust (Wilson & Weis-Fogh, 1962; Wilson, 1964a).

There has been some question about the function of the dorsal oblique muscle. In the Lepidoptera this muscle and the dorsal longitudinal muscle are innervated by branches of the same nerve (IIN1b of Nüesch, 1957), and probably all but one of

Table 1. *Names and functions of the mesothoracic muscles important in flight*

	Generalized insect (Snodgrass, 1935)	<i>Disostetra</i> (Snodgrass, 1929)	<i>A. polyphemus</i> (Nüesch, 1953, 1957)	<i>D. plexippus</i> (Ehrlich & Davidson, 1961)	<i>P. machaon</i> (Weber, 1928)	Function in flight
er						
al	mA	81	dl <sub>1a-s</sub>	50-54	(dlm <sub>1</sub> )	Depressor
	1A	? 82	dl <sub>5</sub>	49	—	?
	—	? 82	dl <sub>8</sub>	61	dlm <sub>8</sub>	Elevator
	C	83	dv <sub>1a</sub>	56, 62	dvm <sub>1,s</sub>	Elevator
	I	84	dv <sub>1b</sub>	57	dvm <sub>8</sub>	Elevator
al	J	89	dv <sub>8</sub>	58, 63	dvm <sub>2,s</sub>	Elevator
axal		90	dv <sub>4</sub>	65	dvm <sub>6</sub>	Elevator
		91	dv <sub>6</sub>	60		
l	P	103b, c	dv <sub>2a</sub> & b	59, 64	dvm <sub>4,7</sub>	Elevator
	1E', 2E'	97	pv <sub>1</sub>	69	pm <sub>1</sub>	} Pronator, depressor
	3E'	98	pv <sub>8</sub>	70	pm <sub>8</sub>	
	P	—	pv <sub>9</sub>	71	—	Supinator, depressor
ar	3E''	99	pv <sub>4,s</sub>	79, 80	pm <sub>7,s</sub>	?
	1E''	—	p <sub>1</sub>	81	pm <sub>9</sub>	? Adjust lateral thoracic capsule
	G	86	pv <sub>7</sub>	84	—	

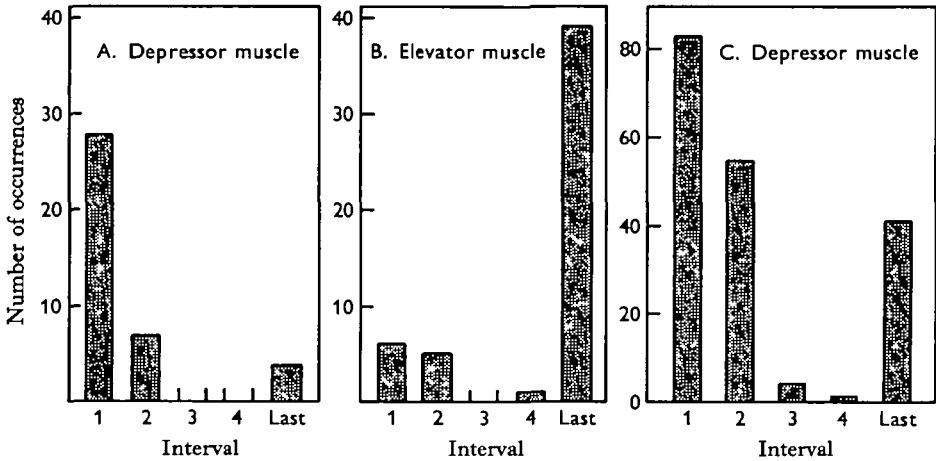
their motor axons originate in the prothoracic ganglion (Nüesch, 1953, 1957). The dorsal oblique muscle runs dorsoventrally between the scutum and an apodeme variously describes as projecting from the postalar portion of the epimeron (in *Danaus*, Ehrlich & Davidson, 1961), from the postnotum (in *Sphinx*, Berlese, 1909; in five species, Maki, 1938), or combinations of these (in *Eacles*, Michener, 1952). The fact that the dorsal oblique muscle runs parallel to the tergo-coxal muscle and other indirect elevators suggests that it is an elevator muscle. This supposition has been verified by electrical records obtained during flight. In the hawk moth *Mimas tiliae* the dorsal oblique muscle is an elevator, activated alternately with the dorsal longitudinal muscle. In the locust *Schistocerca gregaria* the 'oblique lateral dorsal muscles' (Snodgrass, 1929, 1935) are represented by two small muscles which, because of their arrangement and extremely small size, cannot supply significant power for wing movement (Wilson & Weis-Fogh, 1962). In *Periplaneta americana* the dorsal oblique is a large muscle running between the tergum and the second phragma (Carbonell, 1948). On the basis of anatomical studies on *Blatella germanica*, Tiegs (1955) postulated that the dorsal oblique muscle is a depressor. However, similarities in innervation and attachments suggest that the dorsal oblique muscle in cockroaches is homologous with the muscle of the same name in Lepidoptera. In order to determine if it has the same function in both groups, a few experiments were performed on *Periplaneta americana*. Muscle potentials were recorded during tethered flight, and wing position was observed with a stroboscope, as described previously (Wilson & Weis-Fogh, 1962). The results showed that the muscle is activated when the wings are at the bottom of the downstroke. Therefore the dorsal oblique muscle of *Periplaneta* is an elevator, as in the Lepidoptera. In insects with an asynchronous type of flight system physiological evidence for the function of a muscle is difficult to obtain. In flies the dorsal oblique muscle is a large muscle running parallel to the tergo-coxal muscle. Because of its position it is thought to be one of the main elevators of the wing (Snodgrass, 1935; Tiegs, 1955; Smart, 1959). In belostomatid bugs the mesothoracic dorsal oblique muscle runs obliquely between an anterior, dorsal portion of the tergum and a ventrolateral extension of the post-phragma. Barber & Pringle (1966) concluded that this muscle causes supination and possibly depression of the wing, but evidence for the latter role is slight. It may be that the dorsal oblique muscle is an elevator in this group also.

## EXPERIMENTAL RESULTS AND DISCUSSION

### A. Muscle activity during a wingstroke

The indirect flight muscles of Lepidoptera, like those of the locust (Wilson & Weis-Fogh, 1962), are of the fast type. A single stimulus elicits a large, brief depolarization of the cell membrane and a strong, rapid twitch; with repeated stimulation the muscle potentials show no facilitation. In the monarch butterfly and saturniid moths the wingbeat frequencies are relatively low, 5–12 per second. During a wingstroke each motor unit is briefly tetanized with a burst of 3–7 impulses (Pl. 1, fig. 2; Pl. 2). This multiple firing contrasts with the single or double excitation of a motor unit in locusts and in those Lepidoptera with a higher wingbeat frequency, such as skippers and hawk moths (Roeder, 1951; Moran & Ewer, 1966; Kammer, 1967). In the locust

*Schistocerca gregaria* the duration of a twitch in the dorsal longitudinal muscle in response to a single stimulus is approximately equal to the duration of the downstroke (Neville & Weis-Fogh, 1963). A single muscle twitch at 35° C. lasts 33 msec. A second stimulus after a brief interval, 5–8 msec., increases the tension significantly but has little effect on the duration of the contraction. A similar comparison between duration of a muscle twitch and duration of a wingstroke may be made for the Lepidoptera. In butterflies and moths with low wingbeat frequencies, a single up or down stroke may last 40–100 msec., while a single muscle twitch lasts approximately 20 msec.



Text-fig. 1. Position of the longest interspike interval within the burst. The data were sorted by counting the number of bursts in which the longest interval was the first, or the second, . . . , or the last interval. For short bursts, the longest interval could be both the second and last interval (burst length = 3), or both third and last (burst length = 4), etc.; in these cases the interval was tallied in the 'last' column. A. *Samia cynthia*, depressor muscle. Half of the bursts consisted of three muscle potentials, and the other half of four and five impulses. B. *Samia cynthia*, elevator muscle. The data were taken from the same flight as in A. Burst lengths ranged from three to seven, and one-third of the bursts consisted of three impulses. C. *Danaus plexippus*, depressor muscle. Bursts were longer than in A and B, averaging about five potentials. (In addition to the data presented, the record contained 80 other bursts in which there was no recognizable maximum interval.) For all cases the longest interval was almost never in the middle of the burst. For depressor muscles the longest interval was most frequently the first, and for elevators the longest was most frequently the last.

The latter value was obtained for the dorsal longitudinal muscle of both the monarch butterfly *Danaus plexippus* and the saturniid moth *Samia cynthia* at 35° C. In these animals maintenance of tension for the entire stroke, i.e. for 40–100 msec., requires stimulation of the muscle three to several times per stroke. The repetitive excitation which occurs during flight functions in prolonging the duration of contraction as well as in increasing the tension generated.

Analysis of patterns within bursts of muscle potentials should provide clues about the mechanisms of the flight-control system. The interspike intervals within a burst are somewhat variable. Mean values ranged from 6 to 17 msec. in different animals (Table 3). In an analysis of patterns within bursts of muscle potentials from a flying locust, Waldron (1967) found that the first interval was usually the longest. A similar pattern occurred in the burst of two electronic neuromimes coupled positively (D. M.

Wilson and I. Waldron, in preparation). Both cases can be explained on the basis of positive feedback: as the coupled units become active, they excite each other, and the firing frequency increases. This hypothesis could explain the occurrence of long intervals at the beginning of a burst. The termination of bursts might also be influenced by any positive feedback between units generating a simultaneous burst. It is possible that cessation of firing may be initially due to fatiguing of the neurons. As neurons fail to fire the excitation each delivers to the others would decrease. This reduction in excitation, coupled with fatigue, may precipitate the end of activity. If this mechanism is correct, then long intervals at the ends of bursts would be expected. With this hypothesis and the results from locusts in mind, the data from saturniids and the

Table 2. *Changes in interval length within bursts*

Burst length	No. of bursts	Mean $\pm$ s.d. for interval number					
		1	2	3	4	5	6
A. Depressor muscle, <i>Danaus plexippus</i>							
2	1	9	—	—	—	—	—
3	3	6.3 $\pm$ 2.1	6.0 $\pm$ 1.7	—	—	—	—
4	55	7.3 $\pm$ 2.0 *	6.5 $\pm$ 1.1	6.1 $\pm$ 1.3	—	—	—
5	59	6.4 $\pm$ 1.5	6.3 $\pm$ 1.0 *	5.2 $\pm$ 0.8 *	6.1 $\pm$ 1.8	—	—
6	29	5.6 $\pm$ 1.4	6.3 $\pm$ 1.2 *	4.8 $\pm$ 0.7	5.1 $\pm$ 0.9 *	6.4 $\pm$ 2.3	—
7†	4	6.2 $\pm$ 1.2	6.0 $\pm$ 1.2	5.0 $\pm$ 1.4	4.0 $\pm$ 0.8	4.5 $\pm$ 1.7	7.0 $\pm$ 3.4
B. Elevator muscle, <i>Samia cynthia</i>							
2	21	13.0 $\pm$ 5.5	—	—	—	—	—
3	27	8.3 $\pm$ 1.7 *	15.1 $\pm$ 6.8	—	—	—	—
4	14	7.1 $\pm$ 2.6	7.4 $\pm$ 1.7 *	16.4 $\pm$ 9.8	—	—	—
5	11	6.7 $\pm$ 2.4	6.2 $\pm$ 2.5	5.5 $\pm$ 1.6 *	19.2 $\pm$ 7.6	—	—
6	8	6.9 $\pm$ 1.5	5.2 $\pm$ 1.8	5.2 $\pm$ 2.0	7.8 $\pm$ 3.7 *	13.6 $\pm$ 5.6	—
7	2	5.5	5.0	6.5	6.5	9.0	15.5

\* Adjacent intervals are significantly different at the 5 % level (Student's *t*-test, double-tailed).

† Intervals 1 and 4 are significantly different.

monarch butterfly were analysed in two ways. First, counts were made of the number of bursts in which the longest interval was first, or last, or, in bursts containing more than three impulses, second, third, or fourth. Text-fig. 1 shows some typical results. The longest interval rarely occurred in the middle of the burst. In depressor muscles the longest interspike interval within the burst was most often the first. In elevator muscles, however, the longest interval was most often the last. Secondly, bursts were sorted according to length, and the means of successive intervals within the bursts were calculated. Table 2 presents some of these results. In many bursts, particularly of depressor muscles, the firing rate within the burst increases first and then decreases. This pattern is clearly compatible with the hypothesis discussed above. In other bursts, particularly of elevator muscles, there are no short intervals in the middle of the burst, and the firing rate decreases monotonically. In locusts Waldron (1967) has found that the firing rate within bursts of three and four impulses increases. In the lobster cardiac ganglion Maynard (1955) describes units in which the firing rate first increases and then decreases and other units in which there are only decreases in firing rate within the burst. Although other explanations are also possible, all these cases can be explained on the basis of positive feedback and fatigue; the differences

might lie in the strengths of the excitatory coupling and the rates at which the neurons fatigue.

### B. *Correlations among wingbeat period, burst length, and amplitude*

A striking feature of the wing movements of the monarch butterfly and the saturniid moths is the variation which occurs in the stroke from cycle to cycle within a particular flight. Three aspects of the flight were examined: burst length (number of muscle potentials per motor unit per wing beat), wingbeat period, and amplitude of stroke. These three variables are interdependent and usually positively correlated. Text-fig. 2A shows an extreme case in which there is no dependence of amplitude on burst length, while burst length and period are positively correlated. The opposite extreme, in which only burst length and amplitude are correlated, is also encountered (Text-fig. 2B). More common are samples in which both amplitude and period show some positive correlation with the burst length (Text-fig. 2C; Pl. 1, fig. 2). The correlations obtained for several flights are given in Table 3.

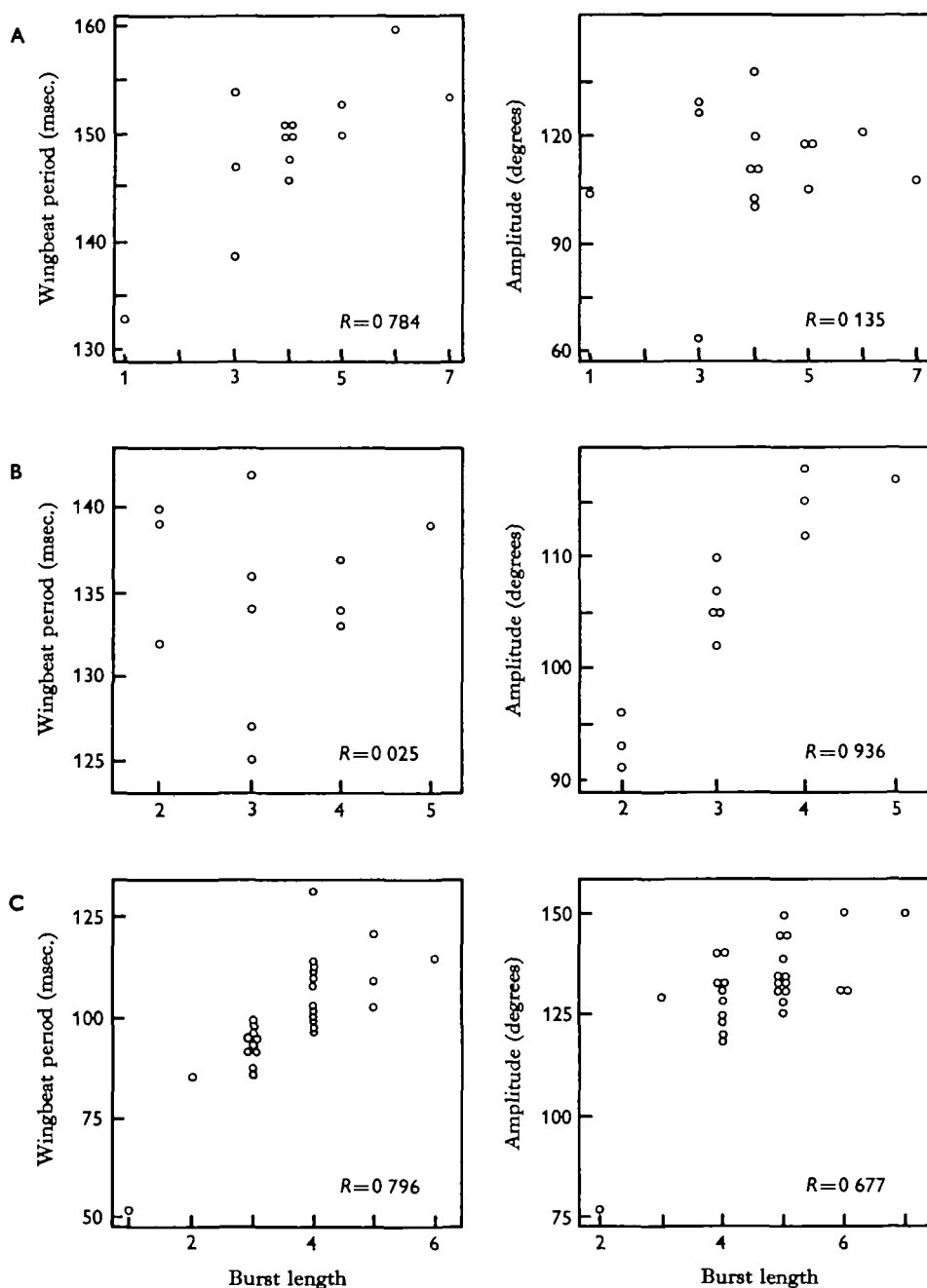
Because the three variables are interdependent it is convenient to discuss correlations between two while the third is considered to be constant. In the following paragraphs some of the implications of the correlations are discussed from this point of view.

*Burst length and amplitude.* At any given wingbeat period the amplitude of the stroke is positively correlated with the number of impulses per wingbeat. It is reasonable that more muscle activity should produce a bigger movement.

*Wingbeat period and amplitude.* At any given burst length there is a positive correlation between wingbeat period and amplitude (e.g., Pl. 2a, b). If a given burst length produces a certain average velocity of movement, at longer periods the wing will move farther and the amplitude will be greater. If the velocity of movement resulting from a given burst varies with the pattern of the burst, no prediction about the resulting amplitude can be made without further information. In either case, at higher frequencies, the activity of antagonistic muscles may overlap to a greater extent. Energy can be wasted in this way, resulting in a smaller amplitude. This occurs in locusts (Wilson & Weis-Fogh, 1962).

*Burst length and period.* At any given amplitude there is a positive correlation between wingbeat period and burst length (e.g., Text-fig. 2A). This correlation implies that the production of large-amplitude strokes at a shorter wingbeat period is accomplished with fewer activations of the muscles, although the velocity of the wing in each stroke must be greater. This increased velocity could be achieved by differences in the timing between impulses within a burst, by phase changes between muscles, by phase changes between motor unit activity and wing position, or by the recruitment of additional motor units. Changes in the phase relationships of muscles (Text-fig. 3, a, b) or of muscle activity with respect to wing position are known to occur, but they have not been carefully examined.

In locusts the opposite correlation holds: burst length is increased at shorter wingbeat periods (Wilson & Weis-Fogh, 1962; Waldron, 1965). If the flight-control systems of locusts and Lepidoptera are homologous, one model of the basic mechanism of pattern generation should suffice for both groups, and only small changes should be required to convert the postulated mechanism from one which generates more pulses



Text-fig. 2. Correlations between burst length, wingbeat period, and stroke amplitude in three different samples of flight. A. Positive correlation between wingbeat period and burst length but an insignificant one between amplitude and burst length. B. Positive correlation between amplitude and burst length but an insignificant one between wingbeat period and burst length. C. Positive correlations between burst length and wingbeat period and between burst length and amplitude.

Table 3. *Summary of results*

The correlations among wingbeat period, burst length, and amplitude are of particular interest. (Correlation coefficients significant at the 0.01 level are shown in bold-face type.)

Animal and muscle	No. of wing-beats	Period (msec.)		Burst length		Mean inter-spike interval within a burst	Correlation coefficients		
		Mean	S.D.	Mean	S.D.		Burst length and period	Burst length and amplitude	Period and amplitude
<i>Danaus plexippus</i>	28	99.7	14.0	4.6	1.0	6	<b>0.796</b>	<b>0.677</b>	<b>0.659</b>
(dorsal longitudinal muscle)	21	107.1	10.7	5.0	0.8	6	0.481	0.166	-0.147
	39	107.1	8.9	5.2	0.7	6	<b>0.323</b>	<b>0.525</b>	-0.036
	46	96.8	11.0	4.6	1.0	6	<b>0.718</b>	<b>0.694</b>	<b>0.805</b>
	52	102.9	5.8	4.9	0.6	6	<b>0.369</b>	<b>0.724</b>	<b>0.393</b>
	77	91.6	4.5	3.9	0.5	7	0.167	<b>0.513</b>	0.119
	39	107.8	7.5	2.8	0.6	9	<b>0.464</b>	<b>0.403</b>	<b>0.556</b>
	72	118.0	10.7	2.7	0.8	8	<b>0.336</b>	<b>0.656</b>	0.170
<i>Hyalophora cecropia</i>	35	123.4	6.4	3.3	0.5	16	<b>0.497</b>	<b>0.429</b>	<b>0.582</b>
(subalar muscle)	45	122.4	4.1	3.6	0.5	15	0.237	<b>0.548</b>	0.232
<i>Samia cynthia</i>	14	148.1	8.8	3.3	0.6	17	<b>0.692</b>	<b>0.713</b>	<b>0.874</b>
(dorsal longitudinal muscle)	22	141.0	6.4	3.3	0.8	14	<b>0.651</b>	<b>0.840</b>	<b>0.815</b>
(elevator muscle)	14	149.0	6.4	4.1	1.4	10	<b>0.784</b>	0.135	<b>0.505</b>

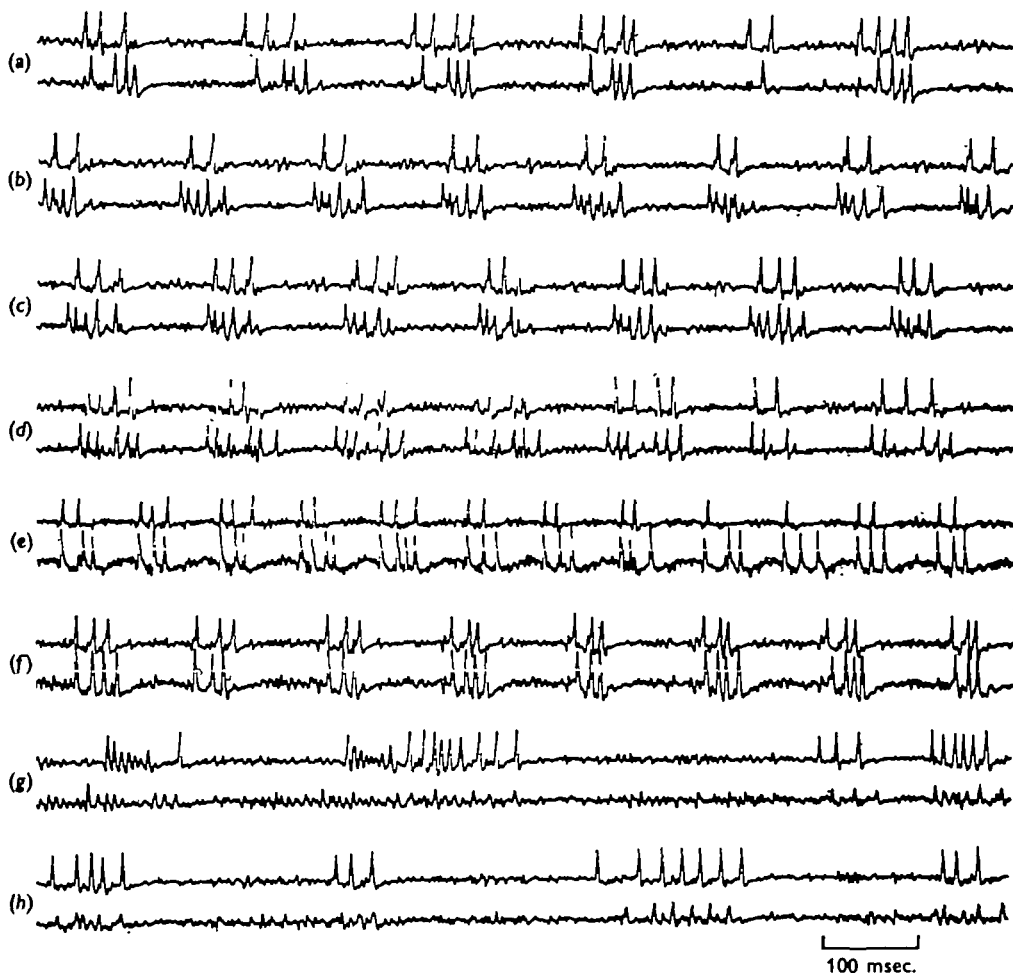
per cycle as wingbeat period decreases to one in which burst length decreases as period decreases. The question of how bursts are generated in each case is discussed later, in section D.

### C. Denervation of the wings

Correlation of stroke amplitude, burst length and wingbeat period, as discussed above, is not surprising, because the contractions of the muscles drive the wings. The observed correlations undoubtedly reflect causal relationships. The possibility of the converse influence, that of wingstroke amplitude on the pattern of motor neuron activity, requires further examination. On the wings there are receptors which could reflexly regulate the motor output to the flight muscles. Chordotonal organs, sensory hairs, and groups of campaniform sensilla are present on or in the wings of a number of Lepidoptera (Vogel, 1911, 1912). Some of these receptors respond when the wing is touched or moved (Sotavalta, 1954*b*), but little is known about their function. In locusts, campaniform sensilla on the hind wings participate in the reflex control of forewing twisting and of lift or body pitch (Gettrup & Wilson, 1964; Gettrup, 1966). Firing frequency in at least some of these sensilla is a function of load and of acceleration of the wing (Gettrup, 1965). Other sensilla, located on the forewings, are involved in the control of flight stability (Gettrup, 1966). By analogy, receptors on the wings of Lepidoptera might detect load or acceleration of the wing, but it is not possible to predict either the stimuli to which they normally respond or their role in reflexly regulating flight.

Sensitivity to some aspect of wing movement was demonstrated in experiments on saturniid moths. The load on the wings was altered by cutting off part of the wings, reducing the length by 50-75 %. The result was an increase in wingbeat frequency of 25-40 %, accompanied by an increase in wingstroke amplitude and only a small

reduction in burst length (Text-fig. 3*e*). The increase in frequency was directly related to the amount of wing removed. When the severed portion was re-attached by wax, the original wingbeat frequency was restored, indicating that the increased frequency is not the result of stimulation arising from the operation nor the result of the deletion of more peripheral receptors. In other experiments the sensory pathway was interrupted by cutting the large nerves which run from the wings to the thoracic



Text-fig. 3. Motor patterns in an intact animal and in the same animal with wings partially cut away and with the sensory nerves from the wings severed. The records show potentials from the subalar muscle (upper traces) and the dorsal longitudinal muscle (lower traces) of *Samia cynthia*.

(a), (b), (c). Animal intact. The three records illustrate phase changes between depressors and some of the normal variation in wingbeat period.

(d). Nerve to left forewing cut. There is little change from normal patterns.

(e). Three-fourths of all wings cut off. The wingbeat period is much shorter, and burst length is decreased slightly.

(f). Nerve to right forewing cut. Compared with (e), the wingbeat period is much longer. Although the load on the wings is reduced, the pattern looks normal (compare with (c)).

(g), (h). Nerves to both hind wings cut. In the absence of sensory input from receptors on the wings, burst length and period are very long

ganglia. When the sensory nerves from either the forewings or the hind wings were cut in an animal with wings partially ablated, the wingbeat frequency dropped from the high value associated with a reduced load to the range characteristic of normal flight (Text-fig. 3f). Cutting the remaining pair of nerves reduced the frequency still further, and burst length increased (Text-fig. 3g, h). When two or four nerves were cut in an animal with wings intact, there were similar but smaller reductions in frequency. In animals with all wing nerves severed, cutting the wings down to small stubs appeared to have no further effect on frequency. However, in most animals with all four nerves cut, the wings were flapped erratically, and the animals did not fly steadily even after strong tactile stimulation. Some of this behaviour could be the result of damage done to the exoskeleton during the cutting of the nerves. In some unpublished experiments, Wilson found that saturniids with wing nerves severed were still able to fly when tossed into the air. Therefore it is reasonable to conclude that the generation of the flight pattern of muscle activity does not depend on timing cues from receptors on the wing. In saturniids excitation from the receptors does influence the wingbeat frequency, as does the excitation from the stretch receptor at the base of the wing in locusts (Wilson & Gettrup, 1963). From wing-cutting experiments it is apparent that in saturniids, which have a low wingbeat frequency, the sensory input from the wings influences wingbeat period and burst length to a greater extent than in a skipper, *Hylephila philaeus*, and a hawk moth, *Mimas tiliae*, both of which have a higher wingbeat frequency. In the latter two species, I found that removal of part or most of the wings has little effect on the wingbeat frequency. Wing-cutting experiments performed by other workers on Lepidoptera have produced differing results. Roeder (1951) reported a decrease in frequency of 5–13 % in *Agrotis* when the wings were amputated close to the thorax. In the saturniid *Nudaurelia cytherea capensis* trimming the wings to an unspecified extent did not alter the wingbeat frequency significantly (Moran & Ewer, 1966). Tiegs (1955), who removed about half of each wing, reported increases of 15 % in two noctuids and in a hawk moth, *Hippotion scrofa*, and an increase of 43 % in another noctuid. A possible explanation for some of the differences is suggested by the results of Sotavalta's (1954a) experiments with noctuids. He found that removal of part of the wings usually caused an increase in wingbeat frequency, up to a maximum of 20–25 % above normal. Further cutting caused either no additional change or a decrease in frequency, sometimes to a value below that of the intact animal. Therefore the results of any one experiment may be determined by the change in load and by the extent to which receptors on the wings are removed by the operation.

The denervation experiments on saturniids demonstrate that sensory input from receptors on the wings increases the wingbeat frequency. It is not yet clear whether the effect is phase-dependent and influential within a single wingbeat cycle, or non-phasic and summed over many cycles. If there is an effect within each wingbeat cycle, the sensory input could be an important mechanism for terminating the burst of motor impulses in each half of a wingbeat cycle. The long bursts which occur when all the nerves from the wings are cut could reflect the absence of this inhibitory control. Alternatively, if the effect is non-phasic, in the absence of a tonic excitatory input the wingbeat period might be long, and the long bursts could be a reflexion of a positive correlation between wingbeat period and burst length. Results with locusts suggest that the latter alternative is more likely. In these animals neither the stretch receptor

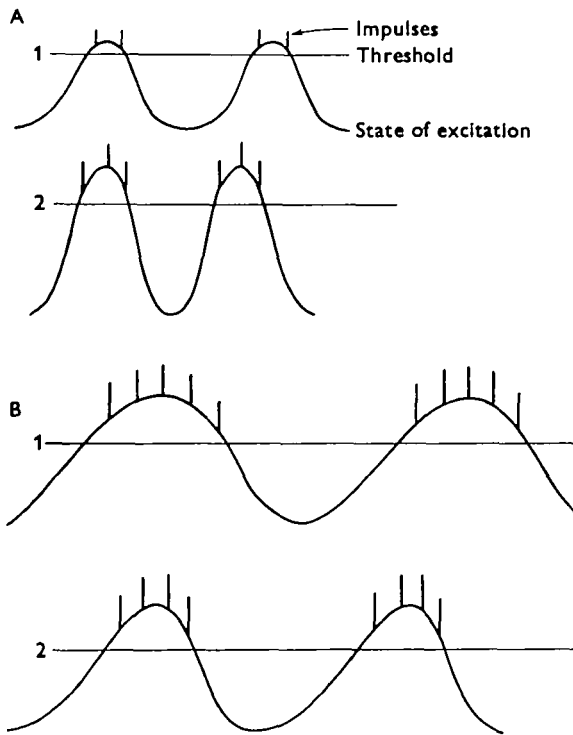
at the base of the wing nor the lift-controlling campaniform sensilla on the hind wings exert much influence phasically within a single wingbeat cycle (Wilson, 1964*c*; Wilson & Wyman, 1965; Gettrup, 1966). Further support for the hypothesis that the sensory input has a tonic effect on the flight control system as a whole is provided by the observation that cutting the nerves to either the forewings or the hind wings has the same effect on the wingbeat period of mesothoracic units. This result means that, at the output level, the effect of input to one pterothoracic segment is indistinguishable from the effect of input to the other segment.

#### *D. The generation of bursts of motor impulses*

The flight-control system, i.e. the assemblage of neurons producing the patterned motor output characteristic of flight, generates burst of impulses in the motor axons. These bursts occur rhythmically and in the proper phase relationships with respect to each other. Proposed models of the system have been discussed by Wilson (1966), and a model based on the data from Lepidoptera will be presented in a subsequent paper. In all these models the overall pattern is a product of interactions within a network of units. The initiation and maintenance of bursts is postulated to depend on fluctuating, excitatory input, including that from positive feedback between synergists. Termination of bursts of activity is accomplished by accumulation of refractoriness, fatigue, or auto-inhibition, in some cases accompanied by a decline of excitation. In most models (Reiss, 1962; Harman, 1964; Wilson, 1964*b*, 1966) silence in the interburst interval is reinforced by inhibition from antagonists. Inhibition between antagonistic units can also keep antagonists firing in antiphase, although this alternating activity can be generated in other ways.

For the purpose of the present analysis of the mechanism of variation in burst length, it is sufficient to consider just one output channel, i.e. one motor neuron, or its antecedent pacemaker, if it has one. The following hypothesis is offered to explain the formation of bursts of different length and the correlations between burst length and period. It is postulated that, because of its interconnexions within the network of the flight control system, the output neuron receives a fluctuating amount of excitation and inhibition. Fluctuations in the input together with properties of the neuron such as refractoriness result in an oscillation of the state of excitation with respect to the average firing threshold (Text-fig. 4). The number of impulses in a burst depends primarily on the length of time that the state of excitation exceeds the threshold. This length of time may be determined by the frequency, the magnitude, and the average level of the oscillation. Magnitude and average level may also effect burst length by influencing the intervals between impulses. When the difference between the state of excitation and threshold is larger, impulses will be produced at shorter interspike intervals. In the locust it has been shown that there is usually a positive correlation between burst length and wingbeat frequency (Wilson & Weis-Fogh, 1962; Waldron, 1965) and that input from certain sense organs increases the average wingbeat frequency (Wilson & Gettrup, 1963; Wilson & Wyman, 1965). To explain the correlation between burst length and period it is suggested that excitatory input to the flight control system increases both the frequency and the magnitude (or average level) of the oscillation of the state of excitation in the pattern-generating

unit (Text-fig. 4A). As a result of the increase in the magnitude of the oscillation the state of excitation is above threshold longer and the burst length is increased. In the saturniids during flight there is a negative correlation between burst length and wingbeat frequency. This correlation can be explained by postulating that increases in the frequency and the magnitude of the oscillation are somewhat separable and that changes in frequency can occur in the absence of a change in the magnitude of the oscillation. Possibly increases in frequency are readily produced in response to small increases in excitation received by the flight-control system. Given a higher frequency, but the same magnitude of oscillation, the state of excitation is above threshold for



Text-fig. 4. Hypothetical mechanism of burst production. Each output neuron is postulated to undergo a cyclic change in state of excitation, at least partly as a function of its overall input and also because of feedback from other members of the system. In this model the burst length depends on the length of time the state of excitation exceeds threshold. In A, constructed to fit the locust data, both the frequency and the magnitude of the oscillation are greater in 2 than in 1. The result of the increase is a longer burst at a higher wingbeat frequency. In B, for the saturniid data, the frequency is greater in 2 than in 1, but the magnitude of the oscillation remains the same. The result is a shorter burst at a higher wingbeat frequency.

a shorter length of time (Text-fig. 4B). The result is a decrease in burst length at the higher wingbeat frequency. In this postulated mechanism frequency and magnitude of the oscillation do not necessarily co-vary. However, there may be circumstances under which both frequency and magnitude change. For example, large increases in excitation received by the flight-control system may increase both frequency and magnitude, and the resulting burst lengths would decline only slightly or show no change at the higher wingbeat frequency. This could explain the observation that in

the wing-cutting experiments (Text-fig. 3e) frequency increased markedly but burst length was only slightly reduced.

The above hypothesis is admittedly speculative and incomplete. At present little is known about the nature and sites of interactions between the units of the flight control system. The explanation has the advantage of resolving in a simple way the differences between locusts and saturniids as observed in the correlations between burst length and wingbeat period. It is compatible with the suggestion, discussed earlier, that the pattern of interspike intervals within a burst is dependent partly on positive feedback between synergistic units. This positive feedback could be an important contribution to the postulated oscillation of the state of excitation of output neurons.

#### SUMMARY

1. The names and functions of the main mesothoracic flight muscles in Lepidoptera are reviewed.

2. The wingbeat period in saturniid moths and monarch butterflies is long and variable. The three parameters, wingbeat period, burst length (number of times a motor unit is activated per wingstroke) and stroke amplitude are interdependent and positively correlated.

3. Partial amputation of the wings in saturniids decreases wingbeat period. Cutting the sensory nerves from the wings increases the period. These results indicate that the influence of wing movement on wingbeat period is mediated by receptors near the base of the wing.

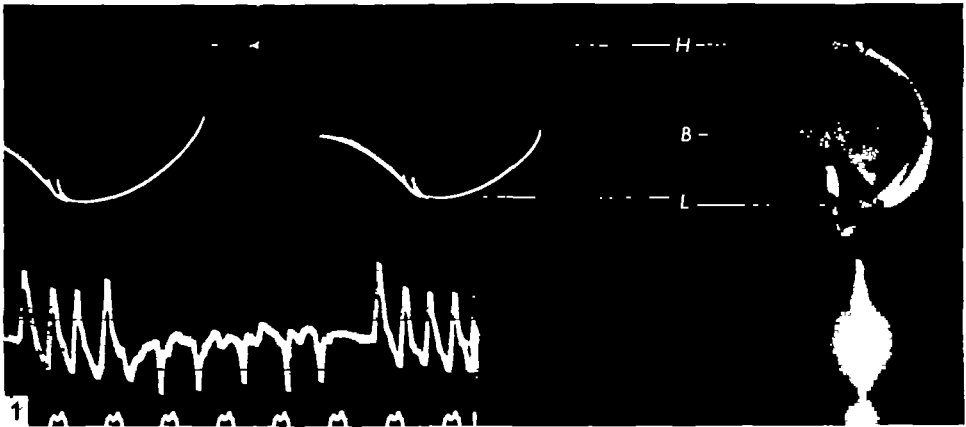
4. The central nervous mechanisms which generate motor patterns during flight in Lepidoptera are discussed and compared with those in locusts. It is proposed that both mechanisms can be described by a single model, with minor differences in the mechanism of burst production.

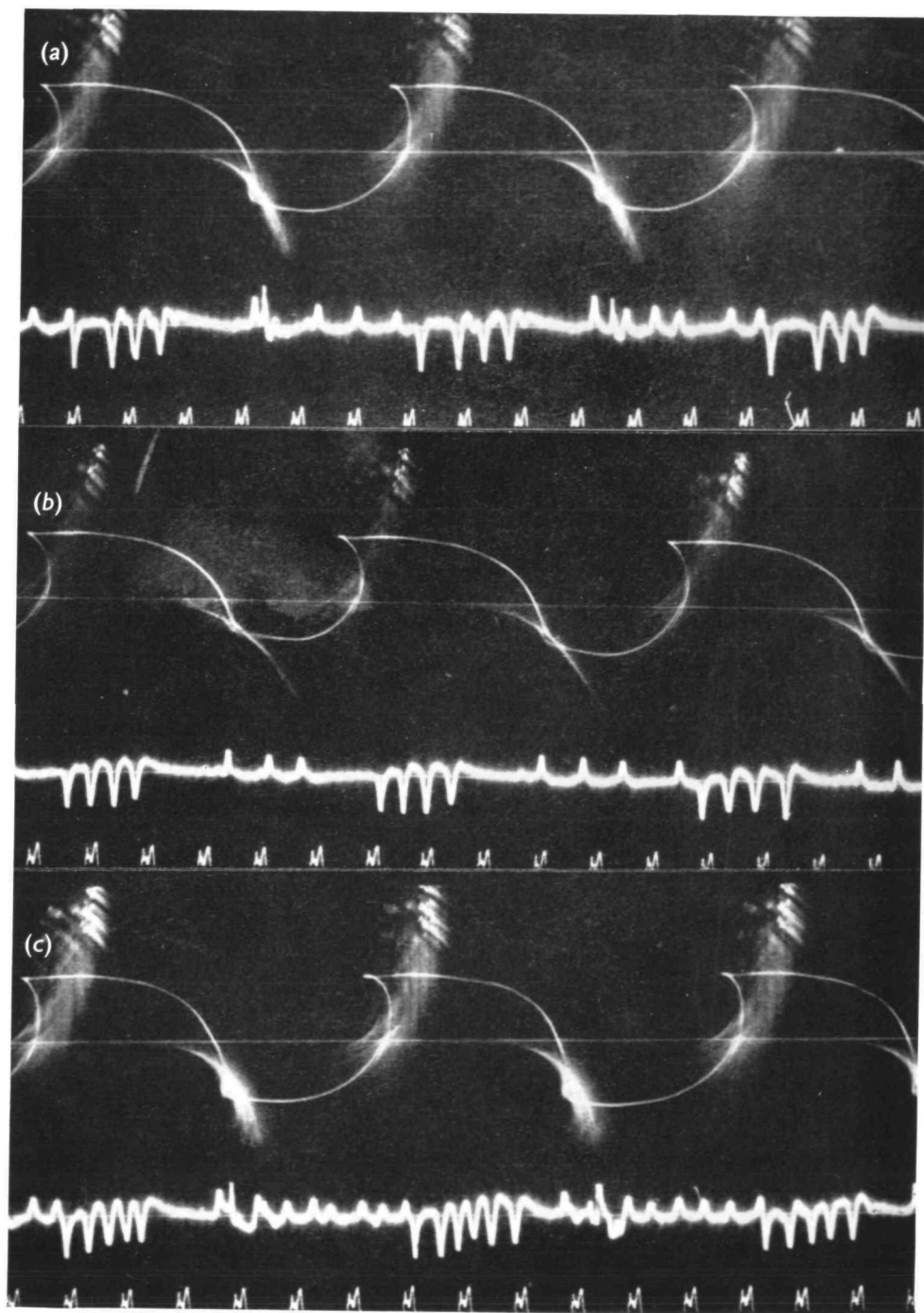
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# EXPLANATION OF PLATES

## PLATE 1

Fig. 1. Method of calculating wingstroke amplitude. An image on stationary film is shown on the right and on moving film on the left. *H*, *B*, and *L* are positions on the film with respect to an arbitrary reference line, the upper edge of the film. *H* refers to the highest and *L* to the lowest position of the wing in one stroke, and *B* is the position of the base of the wing. The radius between the wingbase and the aluminum marker is designated *r*. Using these measurements, the amplitude was calculated as follows:

$$\begin{aligned}\text{angle at top of stroke} &= AT = \arcsin \left( \frac{H-B}{r} \right), \\ \text{angle at bottom of stroke} &= AB = \arcsin \left( \frac{B-L}{r} \right), \\ \text{amplitude of downstroke}_i &= AT_i + AB_i, \\ \text{amplitude of upstroke}_i &= AB_i + AT_{(i+1)},\end{aligned}$$

where *i* designates the *i*th wingbeat cycle, consisting of a downstroke and the following upstroke.

The angle at the top of the stroke was relatively constant. Most of the cycle-to-cycle variation in amplitude was due to differences in *AB*, the angle at the bottom of the stroke.

Fig. 2. Wing position (top line in each record) and potentials from an elevator muscle (middle line, upward deflexions) and a depressor muscle (middle line, downward deflexions) of *Hyalophora promethea*. The bottom line in each record is a 60-cycle time mark. The records show positive correlations between amplitude, wingbeat period, and burst length of the elevator muscle.

## PLATE 2

Wing position and muscle potentials from *Danaus plexippus*. The designations are the same as in Pl. 1, fig. 2. Comparing (b) and (c) there is a positive correlation between any two of the three variables, wingbeat period, burst length, and amplitude. Comparing (a) and (b), in which the burst length of the depressor unit is the same, there is a positive correlation between wingbeat period and amplitude.