

The Nervous Control of the Indirect Flight Muscles of the Honey Bee*

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Summary. 1. Changes in wingbeat frequency and action potential frequency were highly correlated in response to changes in body temperature. Wingbeat frequencies of 50 to 175 Hz occurred simultaneously with action potential frequencies of 9 to 26 Hz in response to changes in body temperature over a range of 16 to 34° C.

2. Weighting the wings lowered the wingbeat frequency and the action potential frequency simultaneously.

3. The oxygen consumed per action potential was constant before and during flight (1.16 and 1.14 $\mu\text{l/g/min}$ respectively). The average in-flight oxygen consumption was 1171 $\mu\text{l/g/min}$ at standard temperature and pressure.

4. Action potentials in the dorsoventral muscles occurred during a preferred phase of the wingcycle. Action potentials in the dorsolongitudinal muscles usually occurred with no significant phase preference to the wingbeat.

5. The role of the dorsoventral muscles as a timer of the flight system is discussed in light of the results presented.

A. Introduction

Many insects of the orders Diptera, Hymenoptera, Coleoptera and Hemiptera possess flight muscles which seem to have rather unique properties: the wingbeat frequency in many species exceeds 100 Hz. However, the electrical activity in the muscles which drive the wings does not seem to occur in phase with, or at the frequency of, the movements of the wings. The action potential frequency has been described as being from 2 to 6% of the wingbeat frequency (Pringle, 1949; Chadwick, 1953; Wilson and Wyman, 1963).

Since the discovery of these unique neuromuscular systems much attention has been focused on them. The contractile processes have

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been studied by Pringle and co-workers in detail (Pringle, 1967). The asynchrony in the flight system is believed to be due to a sensitivity to stretch that is inherent in the muscles.

Others have concentrated on the patterns of electrical activity present in these muscles. They tried to determine at what level in the nervous system and by what means these patterns are generated (Wilson, 1968). Several types of activity patterns have been found in different insects (Wyman, 1966, 1969) and a general model has been proposed to account for these patterns: All are believed to be produced by a single mechanism which employs shared excitation to motor neurons and a lateral inhibitory network. The different output patterns seen in different animals depend on the degree of shared input and the strength of the inhibition (Wyman, 1969). Unfortunately, little is known of the significance of these patterns in the actual flight behavior (Wilson, 1968).

Although these specialized muscles and their patterns of electrical activity have individually been studied in detail, relatively little attention has been given to the interaction of the two. Gross power output is the only flight parameter that is generally thought to be under at least partial control of the asynchronous flight system (Nachtigall and Wilson, 1967). However, the few studies that have been made on asynchronous muscles as possible flight controllers have revealed interesting results. Wingbeat frequency changes have been shown to be well correlated with changes in the action potential frequency, but a causal relationship has not been established between the two (Wilson and Wyman, 1963; Nachtigall and Wilson, 1967; Esch and Bastian, 1968). Changes in the patterns of motor neuron activity have been shown to occur in response to visual stimuli which simulate varying ground speed (Smyth and Yurkiewicz, 1966). Unfortunately, no records were made of the mechanical activity during these experiments. Complex patterns of innervation to the asynchronous flight muscles of a bee were found by Ikeda and Boettiger (1965) and led them to the conclusion that these muscles could have a more complex role in the regulation of the flight mechanism.

These few studies lead one to believe that the asynchronous flight muscles might function as more than a simple power source for flight. However, a strong objection to any proposed regulatory capabilities of these muscles is that no temporal relationship, other than random, is known to occur between any phase of the wingbeat and the time of action potential occurrence (Pringle, 1949; Wilson and Wyman, 1963). It is this lack of synchrony between action potentials and wingbeats that contrasts so sharply with the properties of synchronous flight systems (Wilson, 1964).

The flight system of the honey bee was studied in an attempt to clarify the relationships between the electrical and mechanical activities during flight.

B. Materials and Methods

1. Electrical Recordings. The action potentials were recorded extracellularly in all experiments. Platinum, silver or copper wire electrodes with diameters from 25 to 200 μ , insulated except at the tip, were used whenever the experiment did not require precise placement of the electrodes. Precise electrode placement could be achieved with a micromanipulator and glass micropipettes filled with soft solder. These had a tip diameter of 10 to 25 μ .

Two recording configurations were used. In most cases one or two wire electrodes were placed in the thoracic musculature while a third indifferent electrode was placed in the head. This configuration gave a clear record of the muscle potentials occurring in the immediate vicinity of the recording electrode. In the experiments where micropipettes were used, the thoracic vibrations associated with the wingbeats caused a recording artifact. This undesirable effect was eliminated by recording in the absence of an indifferent electrode (push-pull recording).

The electrical activity was amplified with a Tektronix type 122 differential preamplifier (coupling time constant 1 sec) and recorded with a Precision Instrument PI 6104 tape recorder (FM mode, at 3.75 ips). Subsequent analysis of the data recorded on magnetic tape was performed with a Nuclear Chicago model 7100 Data Retrieval Computer and a general purpose digital computer.

2. Mechanical Recordings. In order to record the vibrations of the thorax associated with the wingbeat, the animal was fixed to a phonograph cartridge with a stiff rod that was attached to the animal's notum. This technique gives a roughly sinusoidal recording with a fundamental frequency equal to the wingbeat frequency of the animal.

3. Temperature Control and Measurement. In order to manipulate the environmental temperature, the apparatus was housed in a plastic box and placed on a thermoelectric chiller. The box was equipped with a 6 volt 15 candle power lamp bulb which served as a heat source and a fan for slowly circulating the air. By adjusting the cooling rate of the chiller the air inside the box could be maintained at any temperature between 15° and 40° C. The relative humidity in the box was 55% at 15° C and 30% at 34° C.

The temperature of the air inside the box was monitored with a Yellow Springs Instrument (Sargent No. 8175040) thermistor probe which was used in conjunction with a Beckman Type R Dynograph. The animal's body temperature was monitored with a Fenwal microthermistor (GB32J2) which was waxed to the dorsal surface of the animal's thorax. This method has been shown to be equivalent to temperature measurement with a thermocouple inserted into the muscle (Esch, 1960). The output of the thermistor was recorded in a second channel of the dynograph. With this setup a running account of the animal's body temperature and the environmental temperature was available.

4. Measurement of Oxygen Consumption. A modified version of a coulometric respirometer described by Heusner, Stussi and Dreyfus (1965) was used to measure the uptake of oxygen before and during flight. During these experiments the electrical activity, mechanical activity and the animal's body temperature was measured along with the animal's oxygen consumption.

5. Correlation of Wing Position and Muscle Potential Occurrence. The waveform of the output of the phonograph cartridge varies from experiment to experiment, making it impossible to consistently identify various positions of the wings from

this output. In order to relate the time of occurrence of an action potential to a specific phase of the wingbeat, a stroboscopic flash was used. The flash was triggered by the phonograph output. By adjusting a delay circuit the flash could be synchronized with any desired part of the wingcycle. Frequency fluctuations in the flight pattern had no effect on the phase of the wingbeat to which the flash was locked because the phonograph cartridge accurately followed the frequency changes. This apparatus permitted visual identification of a particular phase of the wingbeat.

The pulses which drove the flash, the muscle potentials and the cartridge output were recorded on a magnetic tape. The analysis of the temporal relationships of these data were performed with the data retrieval computer in time histogram mode. The digitized output of this computer analysis was punched onto standard paper tape and further processed in a general purpose digital computer.

C. Results

Observations on the electrical and mechanical activities of the indirect flight muscles have previously been presented along with a preliminary description of the action potential, wingbeat frequency relationship (Esch and Bastian, 1968). This study is a continuation of our earlier work.

1. The Effect of Temperature and Loading on Action Potential and Wingbeat Frequencies

The strong correlation that is observed between the wingbeat and action potential frequency in flies and bees (Wilson and Wyman, 1963; Esch and Bastian, 1968) can be explained in several ways. Such a relationship would be observed if the action potentials were the stimulus for contraction of the asynchronous flight muscles; but, this causal relationship is generally considered unlikely since stretch has been shown to be an adequate stimulus for contraction (Machin and Pringle, 1959). Secondly, this relationship could arise if information about the wingbeat were fed back to the central nervous system where it could modulate the motor neuron output. This second possibility implies that the patterns of motor neuron discharge are very important to the flight behavior of the animal. The observed correlation could also be due to some influence of the mechanical activity of the muscles on their electrical activity. However, changes in muscle length do not alter the time of occurrence of an action potential and contribute less than 1 mV to its amplitude (Machin and Pringle, 1960). Another possible source of the observed correlation could be some other variable which affects the action potential and the wingbeat frequency simultaneously. This possibility had not been considered previously. Temperature is the best candidate for such an interfering variable since it is known that both the wingbeat frequency and the electrical activity are temperature

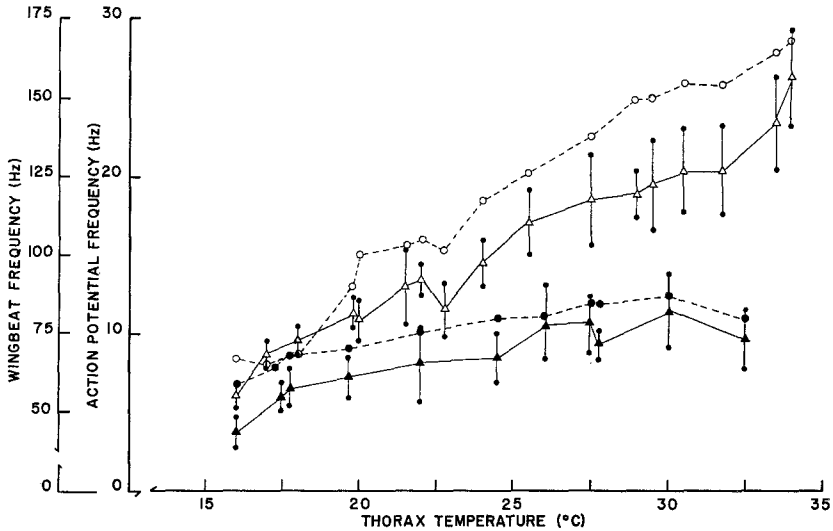


Fig. 1. The relationship between action potential frequency (triangles) and wingbeat frequency (circles) for animals with weighted and unweighted wings at various body temperatures. The vertical bars on the triangles represent + and — one standard deviation for the mean action potential frequency. The maximum range of variation of wingbeat frequencies during an individual measurement was 7 Hz; therefore, no indication of the standard deviations were included

dependent (Del Castillo *et al.*, 1953; Chadwick, 1953; Esch and Bastian, 1968).

We found that variations in action potential frequency and wingbeat frequency in response to temperature changes are highly correlated (Fig. 1, open symbols). A modification of the experiment enabled us to determine if both variables were simultaneously influenced by temperature.

The addition of weight to the wings of insects slows the wingbeat frequency (Sotavalta, 1947, 1952). If the muscle potential frequency and the wingbeat frequency are truly related then both should decrease in response to the extra weight on the wings. However, if the relationship is a temperature artifact then the wingbeat frequency should drop with the extra weight, but the action potential frequency should not. Fig. 1 (closed symbols) shows that the relationship is not a temperature artifact.

2. The Oxygen Consumption Before and During Flight

Previous observations showed that the asynchronous flight muscles of the honey bee contract with individual twitches in response to normal

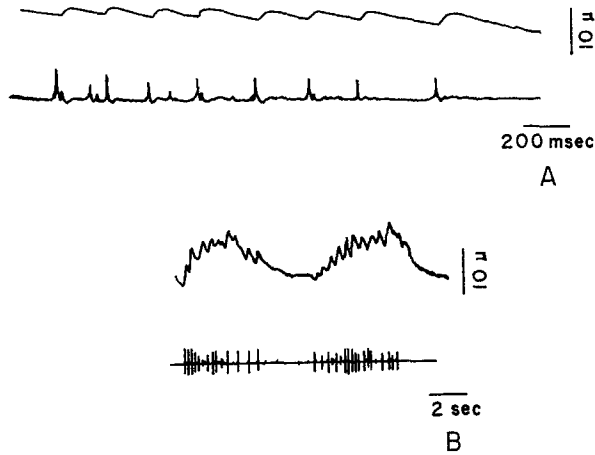


Fig. 2. Mechanical and electrical activity of the asynchronous flight muscles of the honey bee during pre-flight behavior: A Low frequency electrical activity; B Summation in response to higher frequency activity

nervous excitation prior to flight (Esch and Bastian, 1968), Fig. 2. Others have demonstrated that preparations of bumblebee flight muscles contract in response to artificial electrical stimulation (Ikeda and Boettiger, 1965). It seems that these muscles function in a manner similar to most other striated muscles before flight, by contracting in response to an action potential. However, at the onset of flight changes occur: the muscles contract in response to a stretch and the ratio between mechanical and electrical events changes.

It has not yet been demonstrated that the fast stretches and releases of the muscles during flight do not cause some local unmeasurable potential changes to occur which supplement the activation caused by the observed action potentials. Machin and Pringle (1960) suggested that these unmeasurable potentials could occur in deep clefts in the muscle membranes. They would then increase the activation by the usual coupling process which must be present in all striated muscles. If such a mechanism was operating during flight, then the activation associated with a pre-flight action potential would be less than the total activation caused by an in-flight action potential plus the local unmeasurable potentials.

We used pre-flight and in-flight oxygen consumption as a measure of the activation process in these muscles. The amount of work that the muscles can do is determined by the degree of activation; and, the oxygen consumed is a direct measure of the total amount of work done.

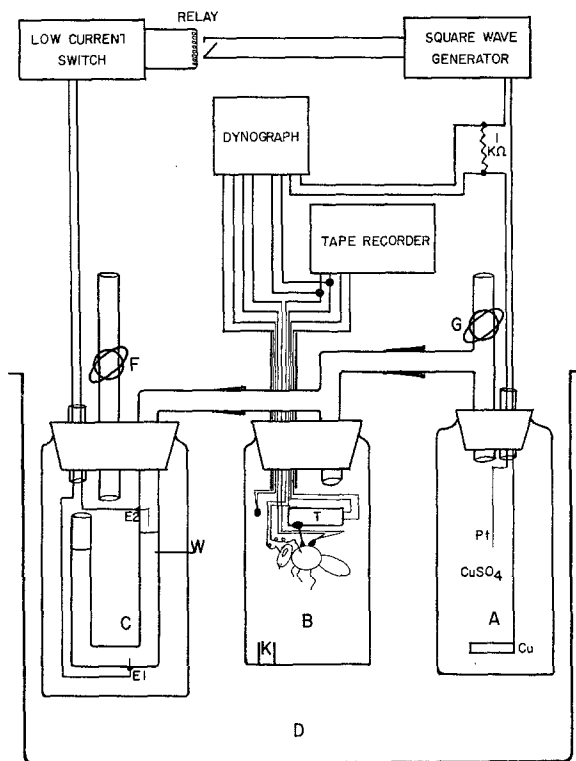


Fig. 3. The coulometric respirometer: *A* Electrolysis vessel; *B* Experimental chamber; *C* Manometer vessel; *D* Water bath; *E*₁ and *E*₂ Sensing electrodes; *F* and *G* Stopcocks; *K* KOH vessel; *T* Phonocartridge; *W* Water column

The R. Q. of the honey bee is 1.0 (Jongbloed and Wiersma, 1934) and the contribution of tissues other than flight muscle to the in-flight oxygen consumption is 1 to 2% (Harvey and Haskel, 1966). Oxygen consumption, action potential frequency, wingbeat frequency, and body temperature were simultaneously measured with the apparatus diagrammed in Fig. 3. The amount of work done per action potential is relatively constant. Referring to Fig. 4, there is no difference in the amount of oxygen consumed per action potential *before* or *during* flight. The average amount of oxygen consumed per action potential in the 12 measurements made prior to flight was $1.16 \mu\text{l}$ (variance = 0.0705) and the average for the 21 measurements made during flight was $1.14 \mu\text{l}$ (variance = 0.0878). Since a *t*-test between these means is not significant, a supplementary mechanism can not be operating during flight to increase the activation of the muscles.

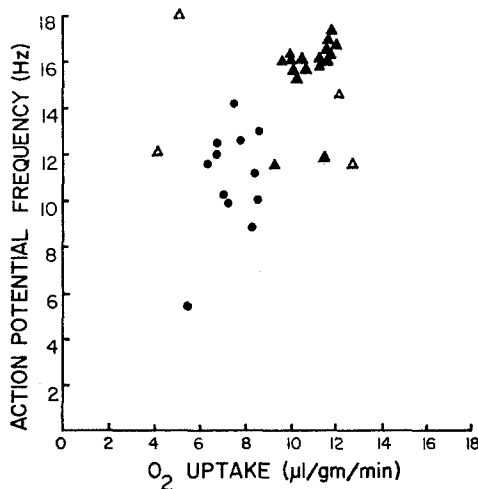


Fig. 4. Relationship between oxygen consumption and action potential frequency. Circles represent measurements made during pre-flight activity. Triangles, measurements during flight. Open triangles, erratic measurements associated with the beginnings and the ends of flights. These results are from one animal and are typical of ten experiments judged as acceptable. O₂ values are at standard temperature and pressure. The absolute values of oxygen consumed per action potential varied from animal to animal but were constant for an individual

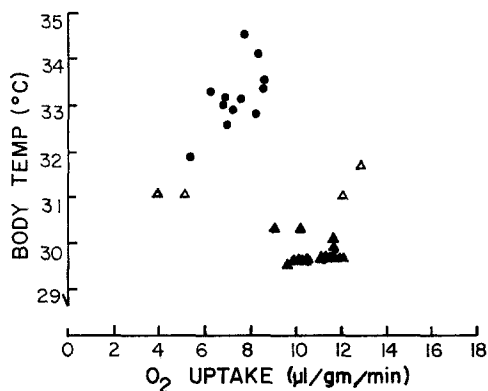


Fig. 5. The relationship between body temperature and oxygen consumption. The specifications of Fig. 4 also pertain to Fig. 5

Body temperature does not give an accurate indication of the animal's state of activity throughout the entire range of behaviors (Fig. 5). The drop in body temperature relative to oxygen consumption

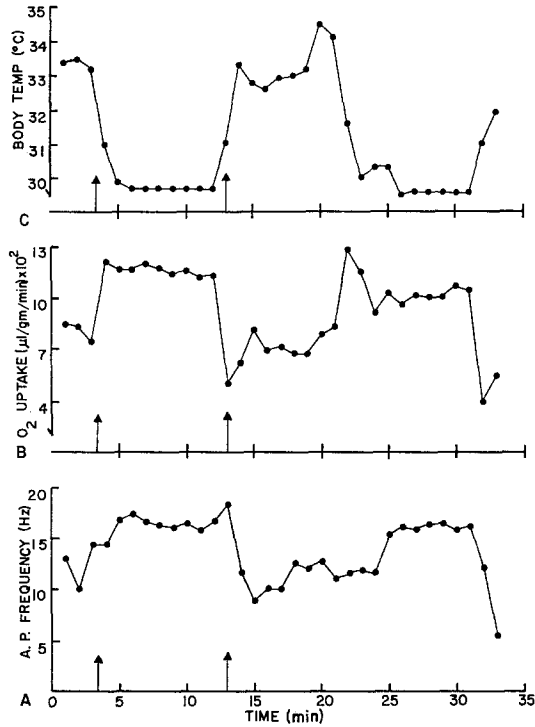


Fig. 6. The course of events during a typical oxygen consumption experiment. Arrows show the start and the stop of a flight

at the onset of flight is primarily due to increased cooling during flight (Sotavalta, 1954). The results of other studies of the oxygen consumed by flying insects compare favorably with the values that we obtained (Davis and Fraenkel, 1940). Therefore, we consider our less conventional method to be accurate.

All oxygen consumption experiments had to meet certain requirements in order to be valid. The most important criterion was that the animals fly for at least five minutes. This minimum flying time insured the recording of enough readings to get an accurate picture of the animal's respiratory rate during flight and that the animal was not damaged.

The course of a typical experiment can be followed with the aid of Fig. 6. Fig. 6A shows the action potential frequency taken over consecutive one minute intervals. Fig. 6B illustrates the oxygen consumed during each interval, and Fig. 6C shows fluctuations in the animal's body temperature. Flight begins 20 seconds into the fourth interval

(first arrow) and ends 15 seconds into the thirteenth interval (second arrow). At the end of flight the body temperature immediately rises and the oxygen consumption drops to a very low level. This is due to the increase in the body temperature associated with the end of flight and the poor heat conduction in the still air. A second flight occurred later in this same experiment. Fluctuations in the body temperature of animals in respiration chambers has been mentioned by other investigators as a source of error (Chadwick and Gilmore, 1940; Davis and Fraenkel, 1940). However, these rapid temperature changes only effect the measurement associated with the interval they occur in, and do not contribute significantly to the overall picture.

3. Phase Relations between the Electrical Activity and the Wingbeats

It has been stated that the action potentials in asynchronous flight muscles of flies occur in all possible phase relations to the wingbeat and that no phase preferences occur (Pringle, 1949; Wilson and Wyman, 1963). However, when large samples of the electrical and mechanical activities of the asynchronous flight muscles of the honey bee were studied, definite phase preferences were found.

Frequency histograms of the time of occurrence of action potentials during a wing cycle were made. Fig. 7 is an example of such a histogram. The distance between the origin and the first peak of the upper plot gives the average time of one wing cycle. The portion of the lower plot corresponding to this time interval contains the frequency distribution of the action potentials occurring in different parts of the wing cycle. It is clear that action potentials occur in all possible phase relations to the wing cycle, but there is a striking phase preference. Preliminary experiments, in which the animals were mounted on a phonograph cartridge and the output of this cartridge was taken as the fundamental frequency of the wingbeat, showed that the phase preference did exist. Then the experiment was modified so that the time of occurrence of the action potentials could be related to a specific position of the wings. The stroboscopic flash system, described under materials and methods, was used to visually identify this relationship. The highest position of the wings was taken as a reference point in these experiments. We used magnetic tape to record the action potentials and the pulses driving the flash. The latter coincided in time to the highest point of the wingbeat. The tapes were then played into two accurately synchronized channels of a data retrieval computer. The instrument measured the times of occurrence of the action potentials relative to the time of the highest position of the wings. These results were further analyzed to see if action potentials really did occur randomly

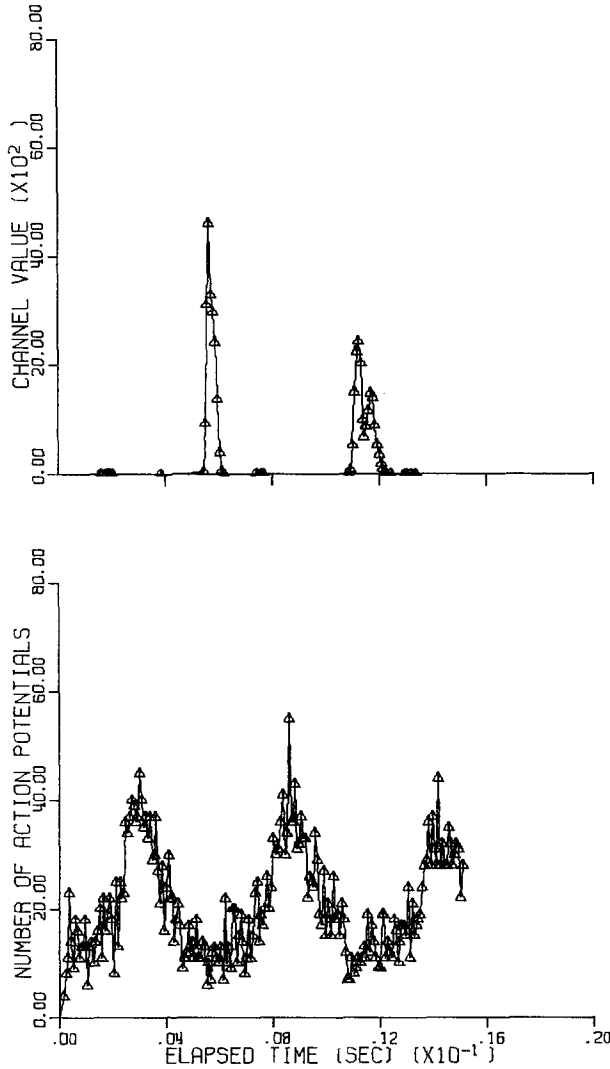


Fig. 7. Distribution of action potentials from a DV muscle within the time of the average wingcycle. Channel value ($\times 10^2$) on the ordinate of the upper plot indicates number of wing cycles. The peaks in the upper plot correspond to the highest point of the wing cycle

during the wing cycle. A chi-square test was used to distinguish significant difference from randomness.

The electrical activity of the dorsoventral (DV) muscles is found to be synchronized with a phase of the wingbeat; and, the muscle potentials

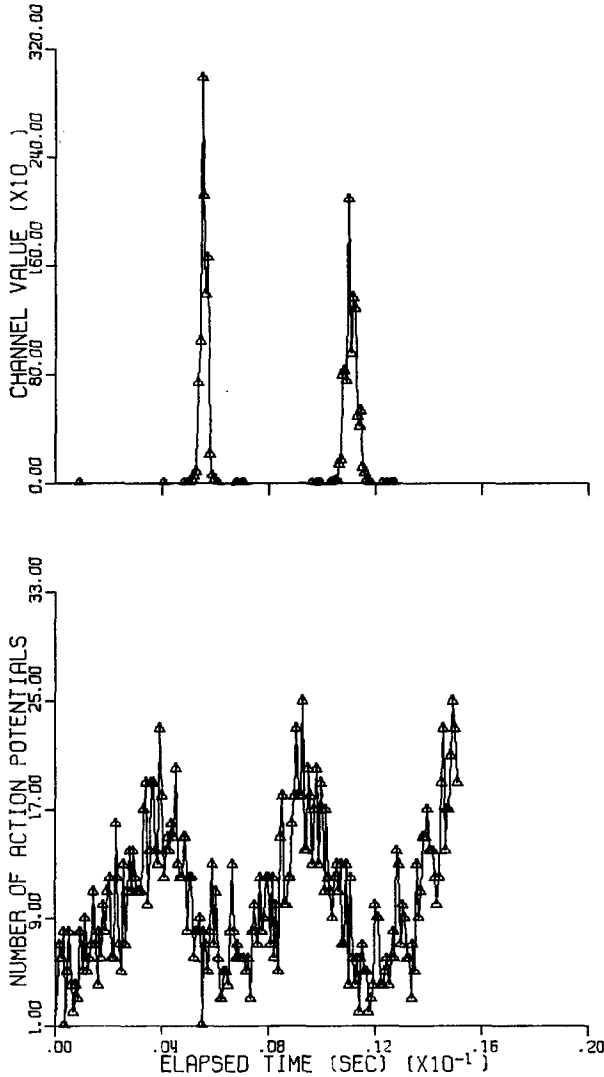


Fig. 8. Distribution of action potentials from a DL muscle showing a significant relationship

do not occur at random during the wingbeat cycle (Fig. 7). The chi-square value for the distribution of muscle potentials in this plot is 384 with 75 degrees of freedom, indicating that the distribution is significantly different from random. Eleven out of twelve experiments showed DV distributions that were significant at the 0.1% level.

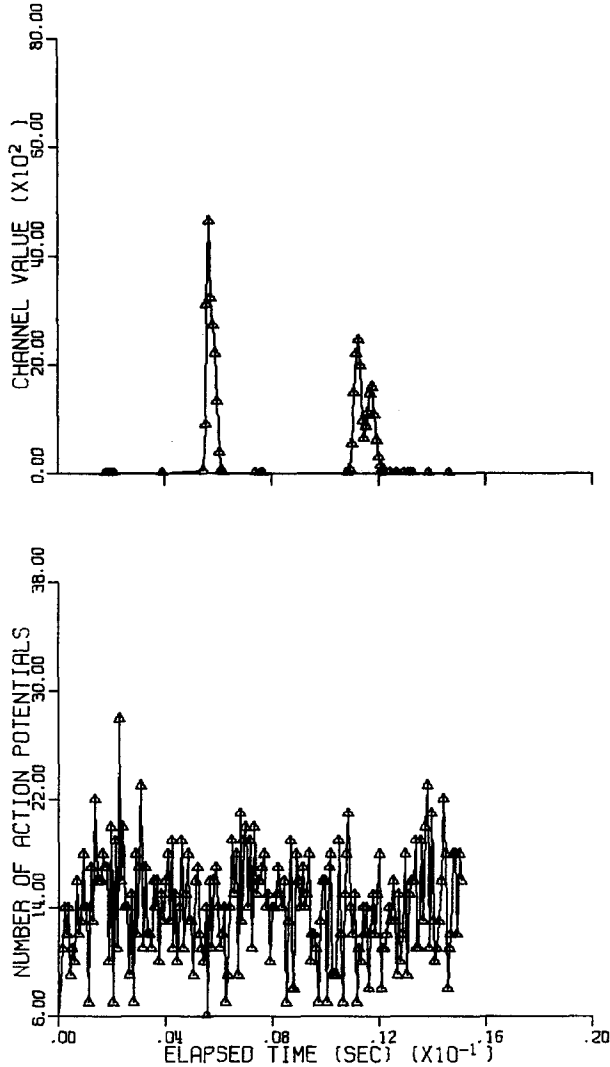


Fig. 9. Distribution of action potentials from a DL muscle showing no relationship

The data for the analysis of the relationship between the occurrence of an action potential and a specific position of the wing in the dorso-longitudinal (DL) muscles was taken simultaneously with the data for the DV analyses. The relationship between the electrical activity of the DL muscles and the wingbeat was not as clear as the relationship observed for the DV muscles. Fig. 8 is a plot of the results of an experiment in which a significant relationship was found. Fig. 9 is a plot

from an experiment in which no relationship was found. The chi-squares for these two plots are 156 with 72 degrees of freedom and 80 with 75 degrees of freedom, the latter is not significant; and therefore, is random. The data for Fig. 9 were taken simultaneously with that for Fig. 7, showing that significant relationships could be present in one muscle group, but not in the other. Only one of the twelve experiments resulted in a distribution of DL action potentials significant at the 0.1% level and six were not at all significant. The difference in the behavior of the two muscle sets is obvious, the synchronization is much stronger in the DV muscles.

It is interesting to know at what time during the wing cycle the peaks of electrical activity occur. The average phase of all distributions of the electrical activity from the DV muscles was calculated using the distribution mean as the measure of central tendency. No phase calculations were made on the data from the DL muscles because the peaks did not occur consistently and their significance was doubtful. Average phase is defined as the time corresponding to the mean of the action potential distribution within the average wing cycle, divided by the time of the average wing cycle.

The mean of the average phase of the twelve DV distributions was 0.55 with a standard deviation of 0.024. This extremely low standard deviation indicates that these peaks of electrical activity occur at a constant phase in different animals.

D. Discussion

The results of our experiments indicate that the current descriptions of asynchronous flight systems have underestimated the importance of the innervation to the asynchronous muscles. The main points of the most recent descriptions of these systems (Pringle, 1965, 1967) are as follows:

1. The high frequency rhythmic contractions of the flight muscles are maintained by a self-oscillatory mechanism that is merely initiated and terminated by motor nerve control.
2. The frequency of the oscillations is determined by the resonance of the muscle-thorax-wing system and it is not under direct central nervous control.
3. Changes in power output are brought about by tonic contraction of accessory muscles which control the amount of stretch applied to the indirect muscles. This third property is believed to be highly developed in bees, resulting in a complete separation of function between power producing and controlling muscles (Pringle, 1965).

4. There is no constant phase relationship between the action potentials in the asynchronous muscles and the cycles of mechanical activity.

Control of Power. The idea, that changes in power output are caused by changes in the amount of stretch applied to the muscles is not consistent with our observations of the oxygen uptake. The constant value of oxygen consumed per action potential before and during flight shows that the increase in tension on the muscles, plus the high frequency stretches of the muscles during flight, does not supplement the activation due to the recorded action potentials; that is, total power output is likely to be under direct neural control. The alternative, that tension changes do not affect total power output, but alter the efficiency of the system, is unlikely because of the work of Nachtigall and Wilson (1967). They measured changes in the aerodynamic lift of flies during flight and found that these were also strongly correlated with changes in action potential frequency. The conclusion is that no changes in power output occur without appropriate changes in the electrical activity of the muscles. However, our results do not eliminate the possibility that power is controlled by simultaneous changes in the excitation of accessory muscles and the power producing muscles. This would enable the animal to simultaneously adjust wing stroke parameters, such as the angle of attack, while varying the power of the oscillations. Sensory feedback from the campaniform sensilla on the wing bases would supply a running account of the stresses on the wings from which the animal could gage the power output. In this way optimal power output could be stabilized. The existence of such a feedback system is supported by the results of the experiments in which the wingbeat frequencies were artificially manipulated. The high correlation observed between wingbeat and action potential frequency, in the absence of a causal relationship, indicates sensory feedback of this sort.

Action Potential Wingcycle Phase Relations. The presence of a significant phase relationship between the time of action potential occurrence and the wingcycle is very surprising, since it has been stated several times that this does not occur (Pringle, 1949, 1965; Wilson and Wyman, 1966). It is possible that the relationship was not detected earlier because the analyses were made on short records of flight. It is also possible that this relationship is peculiar to Hymenoptera. The statements that this does not occur were based on studies of Diptera. This second possibility receives some support from the degree of variability of the bee's wingbeat frequency. The range of natural wingbeat frequencies is from 85 to 240 Hz and rapid changes (10% over a few cycles) have been observed (Herbst and Freund, 1962).

The difference in the behavior of the DV and DL muscles seems to be important, although the significance of these relationships is difficult to assess. Of the two groups, the DV are certainly more complex. This fact was demonstrated by Ikeda and Boettiger (1965). Anatomically, there are branches from three large nerve trunks entering the DV muscles, while branches from only one trunk enter the DL. They also found that different types of action potentials were caused by stimulation of different nerve trunks, indicating different function. They concluded that the DV muscles contribute to the regulation of the wingcycle through the action of this complex innervation.

Since our experiments were performed on intact animals, we have no way of knowing if the action potentials were due to the activity of the primary or secondary innervation; but, since the relationship was present in eleven of twelve experiments it could be that it is a property of both types of innervation. This synchronized electrical activity of the DV muscles could help make rapid frequency changes as well as stabilize any chosen wingbeat frequency. Having the time of peak tension of the DV muscles under neural control enables the animal to vary the time at which the upstroke will begin. Since the DL muscles are deactivated after a downstroke due to their recent contraction, their reactivation will also depend on when the DV muscles contract. If the animal causes the DV muscles to be activated earlier in the cycle, the upstroke will begin sooner and the time of one wing cycle will be shortened. An increase in action potential frequency occurring simultaneously with the timing change of the DV muscles could rapidly change the duration of the wing cycle and adjust the power output proportionally.

This proposed mechanism implies that during parts of the wingcycle an action potential, as well as a stretch, can stimulate a contraction of the DV muscles. This is not unreasonable since it is known that action potentials cause contractions in resting asynchronous muscles and that the morphology of the innervation, as well as the patterns of electrical activity in the DV muscles, are quite different from those of the DL muscles.

This complex behavior of the DV muscles, plus the fact that they consistently make the first contraction at the start of flight (Esch and Bastian, 1968), supports the idea that the DV indirect flight muscles are the most likely candidates for the starter muscles (Bennet-Clark and Ewing, 1968). In the final analysis, changes in power output and wingbeat frequency could be under direct neural control in the honey bee. The results suggest that the DV muscles act as a timer in the flight system.

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