Timing of forewing elevator activity during flight in the locust

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Summary. 1. The time interval between the onset of depressor activity and the onset of the subsequent elevator activity (the D–E interval) was measured in the forewing muscles of tethered flying locusts. The D–E interval was also measured following the removal of all sensory input from the wings.

2. During long flight episodes (up to 4 min) in intact animals the D–E interval varied slowly with time but this variation was not related in any consistent manner with slow changes in wingbeat frequency. Consequently the phase of the onset of elevator activity in the depressor cycle usually changed slowly during a long flight episode (Fig. 1). These changes in phase were not large. Typically phase values for a single animal remained within the range of 0.35 to 0.4.

3. For short periods of flight activity (ca. 15 s) the cycle-by-cycle values of the D–E interval were linearly related to the corresponding values of cycle period (Fig. 2). However, the slope of the linear regression lines varied for different short periods of flight selected from a prolonged flight episode (Fig. 3). Moreover, the slopes depended on whether the animals were flown in a wind stream or in the absence of a wind stimulus (Fig. 7). For animals flown in a wind stream the slopes were distributed around a value close to that expected for a phase-constant motor pattern. The absence of wind reduced the slopes of the regression lines relating the D–E interval to cycle period (Figs. 7, 8). In this condition the motor pattern was often close to latency-constant.

4. The characteristics of the timing of elevator activity were similar in animals flying in upright

and inverted positions regardless of the wind conditions (Figs. 5, 6, 8). Intracellular recordings from elevator motoneurons also failed to reveal any changes in the pattern of synaptic input when the animal was inverted (Fig. 9).

5. Our previous finding that deafferentation results in a major change in the characteristics of the timing of forewing elevator activity (Pearson and Wolf 1987) was confirmed. Following deafferentation there is an increase in the D–E interval (from about 20 ms to 50 ms) and a corresponding increase in the phase of the onset of elevator activity (from about 0.4 to 0.6) (Figs. 10, 11, 12). From these and other data we conclude that phasic afferent signals are necessary for determining the timing of the onset of elevator activity in intact flying animals.

Introduction

Considerable information has now been obtained regarding the influence of sensory input on the motor pattern for flight in the locust (Altman 1982; Wendler 1974, 1982; Möhl 1985; Neumann 1985; Horsmann and Wendler 1985; Reye and Pearson 1987). Based on the fact that phasic afferent input can entrain and reset the flight rhythm (Wendler 1974; Horsmann and Wendler 1985; Pearson et al. 1983; Reye and Pearson 1987) it is now generally agreed that sensory input is involved in rhythm generation (Altman 1982; Wendler 1982; Pearson 1987). What is less clear, however, is the extent to which phasic afferent feedback is involved in establishing specific features of the motor pattern. In fact, there is presently a lack of agreement about whether afferent feedback establishes any specific feature of the motor pattern. Stevenson and

Abbreviation: *EMG* electromyogram

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Kutsch (1987) have reported in a study of the motor activity of hindwing muscles that deafferentation results in only a slowing of the flight rhythm and does not produce a qualitative change in the flight motor pattern. Specifically they reported that the motor pattern was phase constant in both intact and deafferented animals with a phase value for the onset of elevator activity in the depressor cycle of about 0.6. This led them to conclude that 'peripheral feedback is not essentially required for structuring the basic flight motor pattern'. That is, the generation of the flight motor pattern depends on the functioning of a central oscillator that is sped up by afferent input, as was originally proposed by Wilson (1961). On the other hand we have reported that deafferentation produces a qualitative change in the timing of elevator activity with respect to the depressor activity in forewing muscles (Pearson and Wolf 1987). In particular, our recordings showed that the phase of the onset of elevator activity in the depressor cycle increased from about 0.4 in intact animals to about 0.6 following deafferentation. Our data further indicated that deafferentation caused a change in the motor pattern from one in which the onset of elevator activity occurred at an almost constant latency following depressor activity to one in which the phase of the elevator activity in the depressor cycle remained constant. In a footnote to their article Stevenson and Kutsch reported that inverting intact animals changed the pattern from phase-constant to latency-constant in both forewing and hindwing muscles. This finding suggested that the reason why we had observed a change in the motor pattern following deafferentation was because the data were obtained from animals tethered in an inverted position. However, in our original study we did examine the timing of elevator activity in upright animals as well as in inverted animals and reported that there was no difference.

The primary aim of the present study was to reexamine the issue of whether deafferentation alters the motor pattern in forewing muscles, and to assess whether tethering intact animals in upright and inverted positions leads to different conclusions regarding the effect of deafferentation on the motor pattern. To do this we first examined the pattern in intact animals flying in an upright position to characterize the normal motor pattern. We then compared the motor patterns in intact animals flying in upright and inverted positions and confirmed our earlier finding that inverting the animal does not alter the timing of elevator activity. Finally, we compared in more detail than previously the motor patterns recorded in intact

and deafferented animals. The main result of this study was that deafferentation qualitatively changes the timing of forewing elevator activity. The results are consistent with results obtained using intracellular recording techniques which have demonstrated that phasic input from the tegulae determines the timing of onset of elevator activity (Pearson and Wolf 1988; Wolf and Pearson 1988).

Materials and methods

Animals. Experiments were performed on adult male and female *Locusta migratoria* obtained from a colony at the University of Alberta. All animals were aged from 2 to 4 weeks after the final molt.

Electromyographic recordings. Electromyographic (EMG) recording electrodes were implanted into the forewing tergosternal (M83, elevator) and first basalar (M97, depressor) muscles (see Pearson and Wolf 1987 for details). The electrodes (100 μ m copper wires insulated except for the tips) were inserted through holes in the ventral cuticle at the sites where the muscles attach to the sternum. A single electrode was placed in each muscle and the indifferent electrode inserted into the abdomen. The electrodes were waxed to the ventral cuticle and connected to recording amplifiers via a flexible lead approximately 50 cm in length.

A rigid rod was waxed to the pronotum and used to tether the animal in a wind stream (velocity about 2 m/s). Animals were flown in the dark to avoid any influence visual input might have when the animals were inverted. The flight sequences were also longer and more stable in the dark. The experimental protocol and the number of animals examined varied from experiment to experiment. Details are given in Results.

When examining the motor pattern in deafferented preparations the nerve roots 1 of the meso- and metathoracic ganglia were cut before implanting the EMG electrodes. The nerve roots were exposed by removing small pieces of cuticle above each ganglion and the underlying fatty tissue. The wounds were covered with petroleum jelly following nerve transection. All deafferented animals were flown in a windstream in the dark.

Intracellular recordings. In a previous publication we have reported that elevator activity in intact tethered flying animals is initiated by a discrete rapid depolarization which does not occur in deafferented preparations (Wolf and Pearson 1987a). Since these intracellular recordings were performed in inverted flying animals we wished to determine whether this characteristic early depolarization in elevator motoneurons also occurs when intact animals are turned into an upright position. In order to record intracellularly in an intact, upright tethered animal the locust was first mounted in the usual, inverted position (Wolf and Pearson 1987a). The metathoracic ganglion was exposed and a small stainless steel plate was placed under the ganglion as a support. The handle of the steel spoon was short and could be waxed to the ventral cuticle. The soma of an elevator motoneuron was then impaled with a microelectrode. After a stable recording had been achieved the electrode was glued to a metal clasp that had previously been attached to the thoracic sternum. After the electrode had been fixed to this support it was detached from the manipulator (by melting the wax used for mounting) and connected to the recording amplifier via a flexible lead. This then allowed the animal to be turned

Fig. 1 A, B. Slow changes in the wingbeat frequency (top), the D-E interval (middle), and the phase of onset of elevator activity in the depressor cycle (bottom) during prolonged flight episodes in two animals. The EMGs were recorded from forewing muscles (M83, tergosternal and M97, first basalar) with the animals flying upright in a wind stream. Each data point represents the average of 10 consecutive cycles. Flight episodes started at time = 0

activity in the depressor cycle etc. Cycle period was taken as the time interval between the first spikes in successive depressor bursts, and the D-E interval was defined as the time interval between the first spike in a depressor burst and the first spike in the subsequent elevator burst. The phase of the onset of elevator activity in the depressor cycle was calculated as the ratio of D-E interval to cycle period.

Results

Timing of elevator activity in intact animals

Animals flying in a wind stream. In the initial part of this study we examined the timing of elevator activity in forewing muscles (first basalar and tergosternal) during the first 4 min of flight episodes performed in a wind stream in the dark. The animals (9 males and 3 females) were tethered in an upright position. Figure 1 plots, for 2 animals, the wingbeat frequency, the D-E interval and the phase of the onset of elevator activity in the depressor cycle against time (each point is the average of 10 consecutive cycles). The most obvious feature of these plots was that none of the measured parameters remained constant throughout the flight episode. The wingbeat frequency, for example, always declined progressively following an initial period of about 1 min during which it was fairly con-

into an upright position. The animal was suspended from the pronotum by the recording leads and flight activity was initiated by a brief wind puff. The recording assembly was small enough to be entirely accommodated on the thoracic sterna without impairing normal wing movements. The legs of the locust had to be cut at the femur-tibia joint, however, because they would have interfered with the electrode mounting.

Data analysis. The analysis of all EMG data was performed using a DEC LSI-11 computing facility. Input to the computer consisted of TTL pulses corresponding to the onset of elevator and depressor bursts in each flight cycle. To obtain these TTL signals the EMGs were first fed into a window discriminator. The resulting pulses were passed through monostable circuits in order to reject multiple spikes in the elevator and depressor bursts and to obtain a signal corresponding to the onset of the elevator and depressor activity in each cycle. Data from flight sequences lasting up to 5 min (approximately 6000 cycles) could be stored on disc and later analyzed by a variety of programs designed to calculate the relationships between D-E interval and cycle period, or the phase of the onset of elevator

Fig. 2. Linear relationship between the D-E interval and the corresponding cycle period. Each scatter plot represents a short segment (15 s) of flight activity sampled near the beginning of a prolonged flight episode in 3 different animals. Linear regression lines fitted by least squares analysis

stant. The D-E interval usually increased as wingbeat frequency declined (Fig. 1 A) but changes in the D-E interval were not related in any consistent manner with changes in wingbeat frequency. This was indicated by the variation in the phase. For example, at the beginning of the episode shown in Fig. 1 A the mean D-E interval increased

while the wingbeat frequency remained constant thus yielding a progressive increase in the phase. By contrast, in the middle of the episode shown in Fig. 1 B the D-E interval remained fairly constant while the wingbeat frequency declined thus yielding a progressive decrease in phase. Slow changes in the D-E interval and the phase were observed at some time during the flight episodes in all 12 animals. Thus it is clear that the flight motor pattern can not be characterized as either strictly latency-constant (i.e. a constant D-E interval) or as strictly phase-constant.

Although slow changes in frequency, phase and D-E interval occurred during long flight episodes, the D-E interval was linearly related to the corresponding cycle period during short periods of flight activity. Examples of scatter plots of the D-E interval versus cycle period are shown in Fig. 2. Data for each plot were collected during a short segment (15 s) sampled from a long flight episode in 3 different animals. The analysis interval of 15 s was chosen to minimize the effect of the pattern being non-stationary (Fig. 1). The plots could be reasonably well fitted by linear regression lines (correlation coefficients were usually in the range of 0.45 to 0.6, $n > 280$). The slopes of the regression lines varied for different segments within a long flight episode. A convenient way to illustrate this variability was to plot the slope of the regression line against the average phase of the elevator activity in the depressor cycle (calculated by averaging the phase values of all cycles) for the different segments sampled from a long episode. Two examples are shown in Fig. 3. Data points for these diagrams were derived from consecutive 15 s segments sampled throughout the two flight episodes shown in

Fig. 3 A, B. Variation in the slope of the linear regression lines relating D-E interval to cycle period. The slope and average phase were calculated for consecutive 15 s periods of flight activity of the two episodes shown in Fig. 1. Slopes were calculated by least squares analysis (see Fig. 2), and the average phase was determined by averaging the phase values for all cycles. Dotted lines indicate the expected relationship between slope and average phase if the timing of elevator activity in the depressor cycle was phase-constant (see text for explanation)

Fig. 1. A useful feature of this type of plot is that the general characteristics of the motor pattern can be appreciated. A phase-constant pattern for each short segment would be indicated if the points were scattered about a line with a slope of one. This can be understood by first noting that for a strictly phase-constant pattern the slope of the D-E interval versus cycle period relationship is equal to the phase value. In this case a plot of average phase (equal to the phase value) versus slope for a number of segments would result in all the points being superimposed at one position. However, in reality the pattern is not strictly phase-constant because there are uncorrelated variations in the D-E interval and cycle period (indicated by the scatter in Fig. 2) and because the overall pattern is non-stationary (i.e. the average phase value changes slowly during a long episode as shown in Fig. 1). Both of these sources of variability result in scatter of the data points. Scatter of these points around a line of slope equal to one thus indicates that the pattern for each segment is close, but not exactly, phase-constant. On the other hand if the data points in a plot of slope versus average phase for a number of short segments were scattered along the abscissa the pattern would be close to latency-constant. The plots in Fig. 3 show that the slope could vary over a considerable range (0.2 to 0.5) whereas the average phase remained relatively constant (0.35 to 0.45).

Despite the variation in the slope for different segments of a long flight episode, the overall pattern for a single animal was close to phase-constant. This was indicated by the fact that when the slopes for all segments were averaged to give a mean slope for the entire episode, the value of the mean slope was close to the value of the mean of the average phases of all the segments. Averaged data for all 12 animals are shown in Fig. 4. Statistical analysis (t -test) of the data from all 12 animals showed that in half of them there was no significant difference between the mean slope and the mean of the average phases. For 5 animals the mean slope was significantly less than the mean of the average phases ($P < 0.05$) and for 1 animal it was significantly higher. It should be noted that in no animal was the mean slope close to zero, i.e. a latency-constant pattern was not observed in any animal.

In the second part of this study we investigated more fully whether inverting an animal changed the characteristics of the timing of elevator activity. Fourteen locusts were flown in a wind stream for up to 1 min first tethered in an upright position then in an inverted position. This sequence was

Fig. 4. Plot of the mean slope of the linear regression line relating D-E interval to cycle period against the mean of the average phase for 12 animals. Each point displays values for one animal. For each animal slope and average phase were calculated for at least 10 consecutive segments (15 s each) of a prolonged flight episode. These values were then averaged to give the mean slope and mean of the average phase for each animal. Dotted line: expected relationship if timing of elevator activity in the depressor cycle was phase-constant

repeated at least twice for each animal. All trials were made with the animal in darkness in order to reduce any possible influence of differences in visual input in the two tethering positions. Short segments (15 s) of each flight episode were analyzed to determine the slope of the linear regression line relating D-E interval to cycle period. The average phase was also calculated for each segment. From 2 to 4 consecutive segments were analyzed for each flight episode. Figure 5 shows histograms of the slopes and the average phase values for all the segments of flight activity that were analyzed in the 14 animals. Two features can be seen in these histograms. First, there was considerable variation in the slopes whereas the average phase remained relatively constant. These data are similar to those obtained previously by analyzing short segments of long-lasting flight episodes (see Fig. 3). The second feature is that there was no noticeable difference in the data obtained for upright and inverted animals. Statistical analysis failed to reveal any significant effect of inverting the animal on either average phase or slope ($P > 0.1$, t -test). That the position of the animal was irrelevant with regard to the timing of elevator activity was also apparent when the mean slope in upright animals was plotted against the mean slope in the same animals when inverted (Fig. 6). In this plot it can be seen that the points cluster around a line of slope equal to one. This means that if the mean slope was

Fig. 5. Histograms of the average phase (top) and slope of the regression lines relating the D-E interval to cycle period (bottom) for animals flying in an upright position (left) and inverted position (right). Data for these histograms from 14 animals. At least 3 short periods (15 s) of flight activity were analyzed for both tethering positions in each animal. Arrows: mean values of distributions

Fig. 6. Mean slope of the regression lines relating the D-E interval to cycle period for an inverted animal (ordinate) plotted against the value observed in the same animal when flying in an upright position (abscissa). Each point represents data for a single animal ($n=14$). Note that the data points cluster around a line with a slope of one thus indicating that inverting an animal caused no change in the D-E interval versus cycle period relationship

small in an animal flying in an upright position it was also small when the same animal was inverted. Thus we failed to observe any change in the motor pattern when the animal was inverted. In particular, our data clearly failed to demonstrate that inverting the animal changed the motor pattern from phase-constant to latency-constant. Had this been the case data points in Fig. 6 would have been clustered around the abscissa with values in the range of 0.1 to 0.6.

Animals flying in the absence of a wind stimulus. Our finding that the motor pattern in all animals was close to being phase-constant was not in agreement with the results of our earlier study (Pearson and Wolf 1987) in which the pattern varied from almost phase-constant in some animals to almost latency-constant in other animals. Re-examination of our previous data revealed that the latency-constant pattern occurred most often in those animals which flew with wingbeat frequencies below 17 c/s (Fig. 3 in Pearson and Wolf 1987 shows that most of the lines that extend below 17 c/s have slopes close to zero). Unfortunately, in the present study we were unable to confirm that low wingbeat frequencies tended to result in a latency-constant pattern because in none of the animals that flew in a windstream did the wingbeat frequency fall below 19 c/s (range 19.2–23.2).

However one condition that reliably resulted in the animals flying with low wingbeat frequencies (about 16 c/s, range 13.2 to 18.8) was the absence of a wind stimulus. A brief wind puff often initiated a flight episode in a tethered animal that lasted for more than a minute (in some cases up to 20 min). Analysis of the timing of elevator activity during these episodes showed that the D-E interval was similar to that observed in animals flying in a windstream. Average values for the D-E interval in 12 animals flying in a windstream and 9 animals flying in the absence of wind were 17.4 and 18.5 ms, respectively. As a consequence of the D-E interval remaining almost constant at the lower wingbeat frequencies the average phase of the onset of elevator activity in the depressor cycle

Fig. 7. **A** Time course of wingbeat frequency (top) and D-E interval (bottom) in an animal flying initially in the absence of a wind stimulus and then in a wind stream. Time of onset of the wind stimulus indicated by the large arrowhead. Each data point represents average of 20 consecutive cycles. Note that the wind stimulus increased the wingbeat frequency from about 15 to 20 c/s but only slightly influenced the D-E interval. **B** Scatter plots of the D-E interval versus cycle period for the two sections marked *a* and *b* in **A**. Note that the slope of the linear regression line was increased when the animal flew in the wind (from 0.01, $r=0.03$ to 0.22, $r=0.29$)

decreased from about 0.4 to about 0.3 (compare Figs. 5 and 8). Another effect of flying the animals in the absence of a wind stimulus was to reduce the slopes of the regression lines relating the cycle-by-cycle values of the D-E interval to the cycle period (Figs. 7, 8). For short segments (15 s) of flight activity in the absence of wind the slopes of the regression lines were in the range of 0 to 0.25 with the majority below 0.2 (Fig. 8 left). The fact that the slopes of many segments were close to zero and significantly less than the average phase ($P < 0.001$, t -test) meant that for these segments the pattern was close to latency-constant. An example of a segment with an approximately latency-constant characteristic is shown in Fig. 7. This figure also shows that presentation of a wind stream increased the wingbeat frequency and increased the slope of the regression line relating the D-E interval to cycle period.

Fig. 8. Histograms of the average phase (top) and the slope of the linear regression lines relating the D-E interval to cycle period (bottom) for animals flying in absence of a wind stimulus and tethered in either upright (left) or inverted (right) positions. Data for these histograms obtained by analyzing short segments of flight activity (15 s) in 9 upright and 5 inverted animals. Between 2 and 4 values were obtained from each animal. Arrows: mean values of distributions

We also examined the effect on the D-E interval of tethering animals in an inverted position and allowing them to fly in the absence of a wind stimulus. Figure 8 shows that neither the average phase nor the slope of the regression lines relating D-E interval to cycle period was altered by this procedure ($P > 0.1$, t -test). The histograms in Fig. 8 were derived from 9 animals tethered in an upright position and 5 animals tethered in an inverted position. We were unable to compare satisfactorily the data from individuals flying first in an upright position then in an inverted position because in the absence of a wind stimulus none of the animals produced long flight episodes in both conditions.

The reason why the timing of elevator activity was almost latency constant in animals flying in the absence of a wind stimulus in both upright and inverted positions was revealed by intracellularly recording from elevator motoneurons (Fig. 9). It can be seen in these recordings that the onset of elevator activity was produced by a discrete rapid depolarization lasting about 20 ms (see also Wolf and Pearson 1987a, 1988). The timing of this depolarization relative to the preceding depressor activity was influenced only to a minor extent as wingbeat frequency decreased below about 18 c/s (see superimposed records on right of Fig. 9). The decline of wingbeat frequency was associated with the gradual appearance of a second longer-latency component in the elevator depolarizations. These characteristics of the elevator depo-

Fig. 9. Depolarizations recorded intracellularly from elevator motoneurons in 2 animals flying in upright (top) or inverted (bottom) positions. EMG monitor shown below each record was from the hindwing subalar muscle, 129. In each set of records 3 individual cycles are shown recorded at progressively longer cycle periods. These individual records are superimposed on the right. Note the general similarity of depolarizations recorded in upright and inverted animals, and that in both situations the onset of elevator activity was produced by a rapid depolarization the rise-time of which was only slightly changed as cycle period increased. Furthermore, depolarizations produced at longer cycle periods were qualitatively different from those occurring at shorter cycle periods, i.e. increases in cycle period are not associated with a proportional broadening of the depolarizations produced at short cycle periods

Fig. 10. EMGs recorded from the forewing first basalar (D) and tergosternal (E) muscles of an intact locust (upper traces) and a deafferented animal (lower traces). Values given above each set of records indicate the phase of the onset of elevator activity in the depressor cycle. These records illustrate the general observations that deafferentation led to a slowing of the flight rhythm, an increase in phase, and an increase in the number of spikes per cycle

Fig. 11. The phase of the onset of elevator activity in the depressor cycle is plotted for the first 1000 cycles of flight sequences recorded in 2 intact animals (top) and 2 deafferented animals (bottom). Each data point represents a single wingbeat cycle. Note increase in phase following flight onset and the generally larger phase values in the deafferented animals

larizations remained the same in animals flown in either upright or inverted positions (Fig. 9), and were consistent with the results of our EMG analysis (Fig. 8).

Effect of deafferentation on the timing of elevator activity

Sensory input from receptors associated with the wings was removed in 7 animals by cutting nerves 1 of the meso- and metathoracic ganglia distal to the branching of nerve 6 of the anterior ganglia. Only 4 of these animals produced long sequences of flight activity (from 2 to 4 min) that were suitable for analysis. Recordings from the forewing first basalar (depressor) and tergosternal (elevator) muscles demonstrated that in addition to slowing the wingbeat frequency (from an average of about 20 to 12 cycles/s) deafferentation resulted in an increase of the D-E interval from less than 20 ms to between 40 and 60 ms and a corresponding increase in the phase of the elevator activity relative to the depressor cycle (Fig. 10). During the initial portion of a flight episode the phase of elevator activity always increased (Fig. 11). The rate of

Fig. 12. Histograms of the average phase of onset of elevator activity in the depressor cycle in intact animals (top) and in deafferented animals (bottom). Data from intact animals obtained from prolonged flight episodes in 12 animals tethered in an upright position (the same 12 animals represented in Fig. 4). The deafferented animals were also tethered in an upright position. For each intact animal the average phase for a 15 s segment was determined at 0, 1, 2, 3 and 4 min in the prolonged flight episode. Data for deafferented animals obtained from 8 prolonged flight episodes (2 episodes in each of 4 animals). For each episode the average phase was calculated (average of 10 cycles) at every 200th cycle beginning at the onset of flight activity, i.e. approximately every 20 s. Samples were taken more frequently in deafferented preparations because of the progressive change in the average phase with time (see Fig. 11) and because flight episodes in deafferented animals usually did not last for more than 2 min. Arrows: means of distributions

this increase varied from animal to animal (two extremes are shown in the bottom two graphs of Fig. 11), but eventually the phase reached a relatively stable value of between 0.6 and 0.8. By contrast, the phase values in intact animals remained relatively constant between 0.35 and 0.4 at the beginning of the flight episodes (Fig. 11) or they increased only slightly (Fig. 1). A histogram of the average phase obtained by sampling short segments of the flight episodes in deafferented animals is shown in Fig. 12 (bottom). The average phase ranged from 0.45 to 0.8 with a mean of 0.62. This histogram can be compared to that obtained from recordings in intact animals (Fig. 12 top). In intact animals the average phase ranged from 0.25 to 0.45 with a mean of 0.36. Statistical analysis demonstrated a significant difference in the average phase values of intact and deafferented animals ($P < 0.001$, t -test).

Discussion

An important question regarding the patterning of motor activity for flight in the locust is whether

deafferentation alters the characteristics of the motor pattern. In an earlier study (Pearson and Wolf 1987) we reported that for forewing muscles deafferentation produced a delay in the onset of elevator activity relative to the preceding depressor burst (increasing the phase from about 0.4 to 0.6), and that the pattern changed from approximately latency-constant to approximately phase-constant. Subsequently Stevenson and Kutsch (1987) reported that deafferentation did not qualitatively change the motor pattern in hindwing muscles. In both intact and deafferented animals the pattern was phase-constant with phase values of about 0.6. In a footnote to their article Stevenson and Kutsch presented data showing that flying a locust in an inverted position changed the motor pattern in both hindwing and forewing muscles (including the muscles we had studied) from phase-constant to latency-constant. Since the data we had presented were obtained in inverted animals, the implication of these findings was that the changes we had observed following deafferentation were due to our animals being tethered in an inverted position. One aim of the present study was to assess this implied assertion. Three questions need to be considered: (1) is the timing of elevator activity in intact animals phase-constant or latency-constant? (2) does inverting an intact animal change the characteristics of the timing of elevator activity? and (3) does deafferentation alter the basic motor pattern? Each of these questions is considered separately in the following discussion.

Phase-constancy versus latency-constancy

During long episodes of flight activity in upright animals flying in a windstream neither phase nor latency remains constant (Fig. 1). In all animals these values changed slowly throughout the flight episode. Thus the motor pattern is neither strictly phase-constant nor strictly latency-constant. However, for short segments (ca. 15 s) sampled from these episodes the magnitudes of these slow changes were small and the D-E interval was linearly related to cycle period (Fig. 2). The slope of this relationship was often close to that expected for phase-constancy (Fig. 3) and the distribution of slopes for consecutive short segments was usually centered around the value corresponding to a phase-constant pattern (Fig. 4). Thus, by considering the characteristics of short segments of flight activity, i.e. when the pattern is relatively stationary, it was clear that the pattern is close to phase-constant. This is in agreement with the observations of Stevenson and Kutsch (1987) and Waldron (1967).

In our previous study (Pearson and Wolf 1987) we concluded that a general characteristic of the flight motor pattern was that the onset of elevator activity occurred at a relatively constant latency with respect to the onset of the preceding depressor activity. In light of the data summarized above we now consider this conclusion to be incorrect. Stevenson and Kutsch (1987) have presented data that suggest that our failure to observe phase-constancy in our previous study was because our data were collected from animals flying in an inverted position. We do not agree with this explanation. This is because we observed no differences in the characteristics of the timing of elevator activity in upright and inverted animals in that study, nor did we observe any difference in the present investigation (Figs. 5, 6, 8). Instead, our incorrect conclusion of latency-constancy can be attributed to two factors. The first is the method we used in that study to pool the data from different animals. The overall mean value of the D-E interval was calculated at each frequency by simply averaging the mean values of the D-E interval for each animal at that frequency (see Fig. 10 in Pearson and Wolf 1987). We now realize that this method of pooling data obscured the dependency of the D-E interval on cycle period that was apparent for about half of the animals. The second factor is that in our initial investigation some animals flew at low wingbeat frequencies (15 to 18 c/s). Examination of the data from these particular animals shows that for all of them (5 out of the total group of 15) there was only a weak dependency of the D-E interval on cycle time. Thus this group of animals biased our pooled data towards an overall latency-constant pattern. We were unable to confirm this finding in the present study because none of our animals flew with wingbeat frequencies less than 19 c/s. Nevertheless we did observe the timing of elevator activity was only weakly dependent on cycle period in animals flown in the absence of a wind stimulus (Figs. 7, 8). In these animals the wingbeat frequencies were low (ca. 16 c/s). If this explanation is correct then one prediction is that in any animal displaying a wide range of wingbeat frequencies (from 15 to 22 c/s) the relationship between D-E interval and cycle period should be non-linear with the dependency of the D-E interval on cycle period diminishing as cycle period increases.

Motor pattern in upright and inverted animals

In a footnote to their article Stevenson and Kutsch (1987) reported that inverting an intact flying animal changed the timing of elevator activity from

a phase-constant pattern to a latency-constant pattern in both hindwing and forewing muscles. We have been unable to confirm this observation for forewing muscles. In both upright and inverted animals flying in a wind stream we have found that the D-E interval is dependent on cycle time, the slope of the relationship is variable, and the mean slope is close to the value expected for phase-constancy (Figs. 5, 6). Furthermore the characteristics of the timing of forewing elevator activity are not altered by inverting animals beating their wings in the absence of a wind stimulus (Figs. 8, 9). Currently we are unable to account for the difference between our results and those of Stevenson and Kutsch. However, it is not due to a difference in the muscles analyzed since Stevenson and Kutsch reported that inverting an animal changed the pattern from phase-constant to latency-constant in the same pair of forewing muscles we examined (tergosternal M83 and first basalar M97).

Effect of deafferentation on the timing of elevator activity

In this study we have confirmed that for forewing muscles deafferentation delays the onset of elevator activity in the depressor cycle such that the average phase values increased from about 0.4 to greater than 0.5 (Figs. 11, 12). In addition, we have now observed that the phase value always increased following the onset of flight activity, beginning at a value close to 0.5 and stabilizing at values between 0.6 and 0.8 (Fig. 11). This marked increase was never seen in intact animals.

The important question is whether these data allow us to draw any conclusions regarding the role of afferent input in generating the flight motor pattern. Here it is first necessary to consider the serious limitations of using EMG data as an approach for establishing the function of afferent input in pattern generation. This is that if the motor pattern is altered and slowed by deafferentation then the change may be due either to the absence of essential phasic sensory signals or to an alteration in the characteristics of the central oscillator as it slows down (i.e. a tonic influence of afferent input on the pattern generator). On the other hand the absence of any qualitative differences in the patterns recorded in intact and deafferented animals does not necessarily mean that the underlying mechanisms are identical in both. It is entirely possible that qualitatively similar patterns could be generated by different mechanisms. Thus EMG studies alone can not provide unambiguous data regarding the role of afferent input in generating motor patterns, although they can certainly pro-

vide useful clues. More information can be gained by electrically or mechanically stimulating peripheral receptors in conjunction with EMG recordings (Pearson et al. 1983; Möhl 1985; Horsmann and Wendler 1985; Wendler 1974). This approach can identify receptors as elements of the flight oscillator but it does not reveal anything about the cellular mechanisms for sensory regulation of the motor output.

The most direct approach for determining the mechanisms that underlie pattern generation is the use of intracellular recording techniques in order to examine synaptic events in central neurons. This is the main approach we have used to study the mechanisms by which sensory input is involved in pattern generation for flight (Wolf and Pearson 1987a, b; 1988). These intracellular studies have revealed a component of synaptic input to elevator motoneurons that is entirely absent in deafferented animals. This observation, and not the change in the EMG pattern, was the most influential in leading us to the conclusion that the timing of elevator activity is determined by different mechanisms in intact and in deafferented animals. Again it could be argued that the change in synaptic input to elevator motoneurons following deafferentation is due to a change in the characteristics of the central oscillator as it slows from about 20 to 12 cycle/s, i.e. the pattern in both situations is generated by the same basic network. However, recently we have presented evidence that the characteristic initial depolarization that is recorded in elevator motoneurons of intact animals (see Fig. 9) is produced as a direct result of phasic input from afferents arising from the hindwing tegulae (Pearson and Wolf 1988; Wolf and Pearson 1987b, 1988). The increase in the D-E interval we have observed following deafferentation reflects the fact that phasic afferent input is no longer available to generate the rapid initial depolarizations in elevator motoneurons. Thus the D-E interval is increased as is the phase of the elevator activity in the depressor cycle. Given the consistency of our results obtained from EMG studies and from intracellular recordings we conclude that the change in the characteristics of the D-E interval following deafferentation does indeed indicate a change in the mechanism establishing the timing of the onset of elevator activity.

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