Phase-Dependent Influences of Wing Stretch Receptors on Flight Rhythm in the Locust

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SUMMARY AND CONCLUSIONS

1. In restrained locusts we have reexamined the effects on the flight rhythm of stimulating wing stretch receptors. Contrary to earlier reports, we have found a strong phase-dependent influence. Single stimulus trains delivered close to the onset of depressor motor activity reset the flight rhythm. When presented approximately midway between depressor bursts, they had no effect.

2. Stretch-receptor stimulation time-locked to the occurrence of depressor activity caused an increase in the frequency of the flight rhythm that began on the first cycle of stimulus presentation. It also caused a marked prolongation of the duration of flight activity. These effects did not occur when the stimulus trains were delayed relative to depressor activity.

3. Presentation of stimulus trains at a constant rate close to the wingbeat frequency resulted in the entrainment of the flight rhythm to the stimulus train rate. During entrainment, the onset of each stimulus train was close to the onset of depressor activity.

4. Intracellular recordings from flight interneurons revealed that many of those depolarizing in phase with depressor activity received a short-latency excitatory connection from one or both of the forewing stretch receptors. One of these interneurons is presumed to be an element in the rhythm generator, since the application of brief depolarizing currents to the interneuron reset the flight rhythm.

5. We conclude the forewing stretch receptors are important elements in the system generating the normal flight rhythm for the following reasons: a) they can reset the flight rhythm, b) they discharge rhythmically during flight, c) their removal decreases wingbeat frequency, d) when stimulated at the appropriate phase in the flight cycle they elevate the wingbeat frequency, e) their activity can entrain the flight rhythm, and f) they make excitatory connections to at least one interneuron considered to be in the flight oscillator.

6. None of our data support the concept that phasic activity in the stretch receptors has a tonic influence on the flight rhythm generator. We suggest this concept be abandoned for the flight system of the locust. Since there is no evidence in any other rhythmic motor system of phasic sensory input exerting a tonic influence on motor output, this concept must now be regarded as unproved.

INTRODUCTION

There is now considerable evidence that brief (phasic) sensory signals can influence centrally generated motor rhythms (2, 6–9, 13, 23, 24). At present, however, we have very little information on how phasic sensory signals normally act in the control and generation of rhythmic motor activity. It is widely believed that phasic sensory signals are utilized to regulate the frequency and/or magnitude of centrally generated rhythms, but play little role in the temporal ordering of motor activity (5, 17). Strong evidence for this general scheme was first presented for the flight system of the locust (20) and since that time, considerable support has come from studies on a wide variety of rhythmic motor systems in other animals.

One of the surprising observations in the early work on locust flight was that stimulation of wing stretch receptors did not in-
fluence the flight rhythm on a cycle-by-cycle basis (21, 22). Rather, stretch-receptor stimulation led to a slow elevation of the flight frequency (time constant of about 2 s or 25 cycles) independent of the timing of each stimulus train in the flight cycle. This result led Wilson and his colleagues (21, 22) to propose that phasic sensory signals from the wing stretch receptors produced only a tonic excitatory influence onto the central rhythm generator for flight. If this is true, an obvious question is whether phasic sensory signals are used in this manner in other rhythmic motor systems. So far no other clear examples have been reported. This stands in contrast to the numerous reports of rapid phase-dependent influences of phasic sensory signals (2, 3, 6, 8, 13, 74), including two studies on the flight system of the locust in which brief perturbations of wing movements or electrical stimulation of wing muscles were shown to produce phasic effects (12, 19). The receptors responsible for these rapid effects on the flight rhythm have not yet been identified, but the possibility that they include the wing stretch receptors has raised doubts about the proposal that the stretch receptors are without phasic effects on the flight motor output (19).

Therefore, a reexamination of the effects of stretch receptor stimulation on the flight rhythm now seems warranted, particularly in view of the fact that the early results by Wilson and his colleagues have not been confirmed.

In this investigation we have found that stimulation of both forewing stretch receptors close to the onset of depressor activity resets the flight rhythm. Furthermore, correctly timed stimuli caused an immediate increase in wingbeat frequency and significantly prolonged the duration of flight activity. From these and other data we conclude that the stretch receptors are involved in the generation of the flight rhythm on a cycle-by-cycle basis. Our data not only demonstrate phase-dependent influences of the wing stretch receptors on the flight rhythm, but they also refute the only claim that phasic sensory signals can have a purely tonic excitatory effect on a central rhythm generator.

**Materials and Methods**

Experiments were performed at room temperature on adult male and female locusts, *Locusta migratoria*. Animals were obtained from a long-established colony at the University of Alberta.

The aim of the experiments was twofold: 1) to investigate the effects on the flight rhythm of stimulating the forewing stretch receptors and 2) to determine the connections made by these stretch receptors to identified flight interneurons. The following preparations were used for each of these studies.

**Stimulation of forewing stretch receptors**

A schematic diagram of the preparation is shown in Fig. 1. Following removal of the wings and amputation of all six legs, animals were pinned ventral side up on a cork board. The pins were placed through the cuticle of the first thoracic segment. To allow unrestricted movements of the wing stumps, the animal was raised slightly from the cork board by sliding it up the supporting pins. Flight activity was induced by a wind stimulus directed toward the head from a 1.2-cm inside diameter tube placed 8 cm in front of the animal. The wind stimulus was turned on and off by an electromagnetically activated pneumatic valve, and the wind velocity was controlled by a regulator attached to a compressed air cylinder. Wind velocities could be varied over the range of 0.5–4 m/s, but in most experiments it was kept constant at 2 m/s. The characteristics of flight activity in this preparation are described in the first section.

**RESULTS**

(Figs. 3 and 4). The flight motor activity was monitored by electromyograph (EMG) recordings from either the forewing or hindwing first basalar muscles. These muscles function as direct wing depressors. The EMG electrodes (100-μm copper wire insulated except for the tip) were inserted through the ventral cuticle at the site of muscle attachment (dep forewing and dep hindwing in Fig. 1A.)

The axon from a forewing stretch receptor bifurcates to enter the thoracic ganglia via prothoracic nerve 6 and mesothoracic nerve 1 (Fig. 1B). These are the only afferents to bifurcate in this manner (1, 4). This unique anatomical feature allowed selective activation of the axonal branch entering the mesothoracic ganglion by applying electrical stimuli to prothoracic nerve 6. Both prothoracic nerves 6 were exposed by removing a small flap of cuticle above the pro- to mesothoracic connectives (Fig. 1A). To eliminate any possible effects of antidromic stimulation of motor axons and also any effects of stimulating other afferents, both nerves 6 were cut or crushed close to the prothoracic ganglion. Both nerves 1 were cut distal to the junction with nerve 6. This eliminated afferent input from all wing receptors. The nerves 6 were lifted on the electrodes from the hemolymph and coated with petroleum jelly to
FIG. 1. A: schematic diagram of the preparation used to investigate the effects of stimulating forewing stretch receptors on the flight rhythm. Animals were pinned ventral side up on a cork board following removal of wings and legs. Flight was induced by wind directed toward the head at velocities between 0.5 and 4 m/s. Nerves containing axons of forewing stretch receptors, N6(pro) and N1(meso), were exposed by removing a small piece of ventral cuticle and the underlying trachea. EMG recordings were made from either the forewing or hindwing first basalar muscle, dep forewing and dep hindwing. The insertion of these muscles on the ventral cuticle is recognized by the absence of fine hairs. B: sketch of a forewing stretch receptor showing its axonal bifurcation; one branch projecting to the prothoracic ganglion via nerve 6 (N6) and the other entering the mesothoracic ganglion via nerve 1 (N1). The stretch receptor of each side was selectively stimulated by electrodes placed on both prothoracic nerves 6. These nerves were crushed or cut (arrowheads) close to the prothoracic ganglion to prevent any influence of other sensory axons in these nerves or of antidromic activation of motor axons. When stimulating forewing stretch receptors, both nerves 1 were cut distal to the junction of nerves 6. Nerves 1 were left intact when recording the activity of the stretch receptors during flight activity.

Prevent drying. Electrodes were monopolar 75 \( \mu \)m silver wires with the indifferent placed in the hemolymph either lateral to the prothoracic ganglion or in the abdomen. In all experiments stimuli were applied simultaneously to both the right and the left forewing stretch receptors using two separate stimulators triggered from a common source.

Stimuli were delivered in two ways. The first was to trigger short stimulus trains from the large EMG spikes recorded from the metathoracic first basalar (wing depressor) muscle. In this situation the stimulus trains were time-locked to the motor output. The delay between the occurrence of the depressor spike and the triggering of the stimulus train could be varied, thus allowing the stimulus to be delivered at different points within each flight cycle. The second method of stimulation was simply to deliver the stimulus trains repetitively at a constant rate. This second method of stimulation was used when investigating the ability of the stretch receptors to entrain the flight rhythm. Stimuli were delivered as short trains lasting from 20 to 30 ms at frequencies between 100 and 400 Hz (usually being 350 Hz). Each stimulus pulse was 0.1 ms in duration. The stimulus strength for activation of the stretch receptor axon in nerve 6 was between 0.5 and 1.1 V. In most experiments the strength was set initially at 1.3 V and reduced if noticeable effects were observed at lower voltages. In other experiments the stimulus strength was set to 0.1–0.2 V higher than threshold. Threshold was monitored by placing recording electrodes on both mesothoracic nerves 1 in addition to the stimulating electrodes on the prothoracic nerves 6 (Fig. 2). The results using either procedure for adjusting stimulus strength were identical.
FIG. 2. Selective activation of forewing stretch receptor. A: arrangement of stimulating and recording electrodes on prothoracic nerve 6 (n6) and mesothoracic nerve 1 (n1). B: almost simultaneous occurrence of spikes recorded from the axon branches of the forewing stretch receptor in n6 and n1. These spikes occurred spontaneously and the oscilloscope was triggered off the negative peak in the n1 recording. C: stimulation of nerve 6 with a single current pulse activated the axonal branch of the stretch receptor in n1. Note the similar forms of the n1 spikes in B and C. The initial negative deflection in C is the stimulus artifact. By arranging stimulating and recording electrodes as shown in A, the stimulus intensity could be adjusted to be just threshold for activation of the forewing stretch receptor.

The depressor EMGs, the stimulus trigger signals, and the stimulus pulses were recorded on a chart recorder. Instantaneous frequency plots of each flight sequence were also displayed on a storage oscilloscope and photographed.

The ventral preparation (Fig. 1A) was also used to record the stretch-receptor discharge pattern during flight activity. In this case the mesothoracic nerves 1 were not cut distal to the junction with prothoracic nerves 6, and the electrodes were placed either on prothoracic nerves 6 or mesothoracic nerves 1. When recording from prothoracic nerves 6, these nerves were cut close to the prothoracic ganglion to eliminate efferent activity in depressor motor axons.

Connections of forewing stretch receptors to flight interneurons

The preparation used for determining the connections of the forewing stretch receptors to flight interneurons was an extension of the preparation used to study the activity of flight interneurons (14). Animals were mounted dorsal side up and the thoracic ganglia exposed by removing the gut and overlying muscles. The meso- and metathoracic ganglia were placed on a rigid stainless steel plate and bathed in saline (in mM) (147 NaCl, 10 KCl, 4 CaCl₂, 3 NaOH, 10 N-2-hydroxyethyl-piperazine-N’-2-ethanesulfonic acid (HEPES) buffer). Monopolar stimulating electrodes were placed on prothoracic nerves 6. Insulation was provided by coating the nerves on the electrode with petroleum jelly. Stimulation of prothoracic nerve 6 gave selective activation of the stretch receptor (see previous section). Stimuli were delivered at 1/s while a search was made in the meso- and metathoracic ganglia for interneurons receiving stretch receptor input. Intracellular recording electrodes were filled with lucifer yellow and would have had a resistance of between 40 and 60 MΩ if filled with potassium acetate.

Following impalement of an interneuron receiving stretch receptor input, the animal was induced to generate flight activity by blowing air on the head. This provided the physiological characterization of the interneuron. The anatomical characteristics were established by the injection of lucifer yellow for 5 min with a constant current of about –3 nA. Ganglia were fixed in 4% paraformaldehyde for 30 min, dehydrated, and cleared in methyl salicylate. The interneurons were identified by reference to physiological and anatomical characteristics determined in other studies aimed at specifying these characteristics (14, 15).

RESULTS

Rhythmic wing movements in restrained preparations

Wind at a velocity of 2 m/s directed toward the head of a legless animal restrained ventral side up always initiated vigorous rhythmic movements of the wing stumps. EMG recording from wing elevator and depressor muscles showed a pattern of motor activity similar to that observed in animals flying in a wind tunnel, including hindwing leading forewing activity (Fig. 5). Thus we will refer to the motor pattern generated in our restrained preparations as the flight motor pattern or, more simply, flight activity, but we recognize that this pattern may not be identical in all its features to that occurring in normal flying animals.

In our restrained preparations the initial wingbeat frequency (measured from EMG recordings) was in the range of 14–20 cycles/s (mean = 16; n = 20), and it declined progressively a few seconds after the onset of the wind stimulus (Fig. 3). Rhythmic activity ceased within 10 s of stimulus onset in the majority of animals. In the remainder, rhythmic wing movements were maintained...
FIG. 3. Characteristics of flight activity in a restrained animal fixed ventral side up. Wings and legs removed. Graphs plot wingbeat frequency (cycles per second averaged over every 0.25 s) versus time from the onset of a continuous wind stimulus directed toward the head at a velocities of 1 m/s (crosses), 2 m/s (filled circles), and 2.5 m/s (open circles).

After more than 1 min at a relatively constant frequency (about 12 cycles/s). Cessation of rhythmic activity occurred when the wingbeat frequency declined to about 10 cycles/s. Increasing the wind velocity slowed the decline in wingbeat frequency and prolonged the duration of flight activity, but these were not strong effects (Fig. 3).

The wingbeat frequency and the duration of flight activity were both reduced by severing the two mesothoracic nerves 1 distal to the junction with prothoracic nerve 6 (Fig. 4). This lesion removed all afferent input from the forewings. Increasing the wind velocity resulted in only a slight reversal of these effects.

Activity of forewing stretch receptors

In order to determine the appropriate parameters for electrical stimulation of the stretch receptors, we first recorded stretch-receptor activity during flight activity in restrained preparations. Figure 5 shows an example of the activity recorded from one forewing stretch receptor in such a preparation. The forewing stretch receptors usually discharged in high-frequency bursts (300–400 impulses/s) beginning about 10 ms before depressor motor activity recorded from the first basalar muscle of the metathoracic segment. The number of spikes in each burst was somewhat variable but usually within the
FIG. 5. Burst activity in forewing stretch receptor during rhythmic wing movements in a restrained preparation. Activity in the stretch receptor (large spikes in bottom traces) was recorded from mesothoracic nerve 1 (n1) and the depressor activity monitored by EMG recording from the ipsilateral first basalar muscles of the forewing (m97) and the hindwing (m127). Note that the stretch receptor bursts occurred approximately in phase with activity in the hindwing depressor muscle. When stimulating the stretch receptors during flight activity, the stimulus trains were triggered off spikes recorded in the hindwing depressor muscle. Thus with zero delay between stimulus onset and hindwing depressor spikes, we were able to mimic approximately the pattern of stretch-receptor activity occurring in a restrained animal. Records A and B are from the same flight sequence taken approximately 0.5 and 2 s from the start of flight activity, respectively.

range of 3–6 (Fig. 5). Even so, the minimal discharge rate during a burst was rarely less than 300 impulses/s. The high-frequency bursts we recorded in our preparations are essentially the same as those recorded by Möhl (11) in tethered flying animals but significantly different from those recorded by Wilson and Gettrup (21). Wilson and Gettrup reported the stretch receptors discharging at a much lower frequency or even discharging only a single spike in phase with depressor activity. The difference between Wilson and Gettrup’s results and the results of Möhl (11) and ourselves may have been due to the more extensive dissection used in Wilson and Gettrup’s experiments disrupting the normal mechanical properties of wing movements and, thus, stretch receptor activation.

Stimulation of forewing stretch receptors

Stimulation of both forewing stretch receptors with short-duration high-frequency trains time-locked to depressor motor activity had a profound influence on the flight activity (Figs. 6–9). The most obvious effect was a significant elevation of the wingbeat frequency (Fig. 6), which commenced on the first cycle in which the stimulus train was delivered (Fig. 7). The wingbeat frequency continued to increase over the next few cycles and a relatively stable elevation in frequency was achieved within about 6 cycles of the stimulus sequence onset (Fig. 7). Termination of the stimulation resulted in an immediate reduction of the wingbeat frequency followed by a slow decline paralleling that which would have occurred had the stimulus sequence not been given.

The second clear effect of stimulation of the stretch receptors was a marked prolongation of the duration of flight activity in response to continuous wind stimulation to the head (Fig. 6). With a wind velocity of 1–2 m/s, the duration of flight activity was usually quite short (2–5 s) in preparations in which all sensory input from the forewings had been removed by cutting nerves 1 (Fig. 4). In these preparations stimulation of the forewing stretch receptors time-locked to depressor activity often prolonged flight activity for periods exceeding 2 min. The longest we observed was 3.5 min. A difficulty we encountered in attempts to determine the maximum prolongation of flight activity produced by stretch receptor activation was a progressive decline in the amplitude of the
FIG. 6. Elevation of wingbeat frequency by stimulation of both forewing stretch receptors. Average wingbeat frequency (cycles per second) was calculated every 0.5 s for four consecutive cycles. On the first trial (open circles, dotted lines) only the wind stimulus to the head was presented. In this trial flight ceased after 4 s. On the second trial (filled circles, solid line) stimulation of both forewing stretch receptors commenced 1.5 s after the onset of flight activity and led to a marked elevation in the wingbeat frequency. Also the duration of the flight activity was prolonged, lasting more than 20 s (only the initial 10 s is shown on the graph). Note the response in the first 1.5 s was almost identical in the two trials. Stimulus parameters: frequency, 350/s; train duration, 20 ms; strength, 0.6 V. Each stimulus train was triggered with zero delay from the spike recorded from the hindwing first basalar muscle. The wind stimulus was applied continuously throughout both trials (velocity, 1.5 m/s).

FIG. 7. Time course of elevation in wingbeat frequency in response to stimulation of both forewing stretch receptors. At the onset of the stimulus sequence (stim ON) a stimulus train was delivered to both stretch receptors every cycle for about 10 cycles. Each stimulus train was time-locked with zero delay to hindwing depressor activity. Stimulus onset was preceded by at least 10 cycles with no stimulus presentation. Data from 19 trials in one animal have been averaged and the instantaneous frequency (reciprocal of cycle time) normalized to the instantaneous frequency of the interval immediately preceding the first stimulus train. The vertical bars give the standard deviation. Note that the frequency increased progressively over the first six cycles of stimulus presentation (see also Fig. 8) and that the first stimulus train caused a rise in frequency. Stimulus parameters: frequency, 400/s; train duration, 30 ms; strength, 1.2 V.

EMG recorded from the depressor muscles. After a few minutes, triggering of the stimulus trains became unreliable and precise time-locking to depressor activity could not be maintained. Thus we have not excluded the possibility that stretch-receptor stimulation may be able to extend the duration of flight activity for periods much longer than a few minutes. Certainly some preparations were still beating their wings vigorously at the time the stimulus triggering became unreliable.

A third observation was that time-locked stretch-receptor stimulation occasionally maintained flight activity following termination of the wind stimulus to the head. In these few cases, cessation of wind stimulus led to a reduction in the wingbeat frequency but not to a value less than that produced by wind stimulation alone.

In summary, stretch-receptor stimulation time-locked to the beginning of depressor activity elevated wingbeat frequency, prolonged the duration of flight activity, and in some cases maintained flight activity follow-
ing termination of the wind stimulus to the head.

The question now is whether these effects are due simply to a tonic excitatory influence on the flight oscillator or whether they depend on the precise timing of stretch-receptor activity in the flight cycle. To answer this question we varied the delay between the onset of depressor activity (spike in the first basalar muscle of the metathoracic segment) and the triggering of the stimulus trains to prothoracic nerves 6. The results clearly demonstrated the necessity for precise timing of stretch-receptor activity. For example, Fig. 8 shows that when the stimulus was delayed 35 ms following depressor activity there was no elevation of wingbeat frequency, whereas a delay of zero led to a pronounced elevation of frequency.

To investigate in more detail the phasic influence of stretch-receptor activity we delivered a single stimulus train every fifth cycle and observed the effect of varying the delay on the flight rhythm. Figure 9 shows that a single stimulus train delivered close to depressor activity shortened only a single cycle and had no influence on the duration of subsequent cycles. Thus the flight rhythm was reset. Stimuli delivered approximately midway between consecutive depressor spikes (delays of 30–40 ms) had little or no influence on the rhythm. These data clearly demonstrate that resetting is a phase-dependent phenomenon. Because of the normal variability in cycle time and the method we used for triggering the stimulus trains, we were unable to determine whether a greater resetting effect would have occurred had the stimulus train been delivered just prior to depressor activity. This might be expected to occur because the stretch receptor normally begins discharging slightly before depressor activity (Fig. 5).

Our observations that stretch receptor activity can reset the flight rhythm and that this resetting depends on the precise timing of stretch-receptor activity within the flight cycle demonstrate that phasic information signaled by the stretch receptors very likely is utilized in the generation of the normal flight rhythm. If this is true, then we would expect that rhythmic stimulation of the stretch receptors could entrain the flight rhythm. To test this prediction we presented stimulus trains at a constant rate following the initiation of flight activity. In this case there was

![Graph showing instantaneous frequency versus time with two delays: 0 ms and 35 ms.](image)

**Fig. 8.** Elevation in wingbeat frequency and prolongation of flight activity depends on the timing of the stimulus presentation in the flight cycle. Records of instantaneous frequency (reciprocal of cycle time) versus time for two trials have been superimposed. In both trials identical stimulus trains were delivered to both forewing stretch receptors beginning at about 2 s following the onset of flight activity. In the first trial there was zero delay between each depressor spike and the onset of the stimulus train. In the second trial the delay was 35 ms. With zero delay there was a marked elevation in wingbeat frequency and a prolongation in the duration of flight activity. These effects did not occur with a 35-ms delay. The profile of flight activity for a stimulus delay of 35 ms was similar to that for wind alone (not shown). Stimulus parameters: frequency, 400/s; train duration, 30 ms; strength, 1.3 V.
no obligatory coupling of the stimulus trains to the motor output. In all six animals tested there was a strong entrainment of the output frequency to the stimulus frequency. An example is shown in Fig. 10. Here the wingbeat frequency was entrained to 14/s on one trial and 17/s on another. Entrainment was usually established within a second of the onset of the stimulus sequence and was only produced when the frequency of the stimulus sequence was higher than the wingbeat frequency at the beginning of stimulation. We did not investigate in detail the range of frequencies over which entrainment could be elicited, but it seems that this range is quite wide. In one animal, for example, we were able to entrain the flight rhythm to any value between 12 and 18 cycles/s.

During entrainment the onset of each stimulus train occurred close to the time of depressor activity (Fig. 10B, C), but this timing did not mimic exactly the events in intact restrained preparations. The difference is that in intact preparations the stretch-receptor bursts began slightly before hindwing depressor activity (Fig. 5), whereas during entrainment they usually began just after hindwing depressor activity (Fig. 10B, C). We are uncertain why there should be this difference. One possibility is that the absence of input from other peripheral receptors (te-
gula and chordotonal organs) alters the normal phasing of stretch-receptor input relative to motor output.

**Connections of stretch receptors to flight interneurons**

In our previous studies on the locust flight system we have identified numerous interneurons involved in the central generation of the flight rhythm and in the patterning of activity in flight motoneurons (14–16). During flight activity these interneurons discharge rhythmically with high-frequency bursts occurring in phase with either elevator or depressor motor activity. To date we have found that 12 of these interneurons receive short-latency excitatory synaptic connections from one or both of the forewing stretch receptors. Two examples are shown in Fig. 11. The EPSPs evoked in these interneurons (Fig. 11C) are typical of those seen in other interneurons. The latency was short and constant, being 2.5–3 ms and 3.5–4 ms in the meso- and metathoracic ganglia, respectively, the duration was about 25 ms, and the amplitude was within the range of 1–4 mV but relatively constant for any one neuron. On a number of occasions we penetrated the axon of the stretch receptor in the mesothoracic or the metathoracic ganglion. These recordings showed that conduction time in the stretch receptor axon accounts for all but 0.5–1 ms of the latency of the excitatory postsynaptic potentials (EPSPs). Thus we conclude that the EPSPs we recorded in interneurons are produced via monosynaptic pathways. All but 1 of the 12 flight interneurons found receiving an excitatory connection from a forewing stretch receptor discharged in phase with depressor motoneurons during flight activity (Fig. 12B). The discharge of the 12th just preceded depressor

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**FIG. 11**. Identified flight interneurons receiving an excitatory synaptic connection from a forewing stretch receptor. A and B: drawings of the structure of the interneurons in the mesothoracic ganglion. C: EPSPs evoked in these interneurons by stimulation of the stretch receptor ipsilateral to the somata: top, interneuron shown in A; bottom, interneuron shown in B. Interneurons shown in A and B discharge rhythmically in phase with depressor motoneurons during flight activity and make excitatory (D) and inhibitory (E) connections to hindwing depressor and elevator motoneurons, respectively. D and E: top traces, spikes in interneurons; bottom traces, postsynaptic potentials (PSPs) in motoneurons. Vertical calibration is for PSPs in C–E.
activity. Thus excitatory connections from the forewing stretch receptors appear to be selectively distributed to depressor interneurons. This corresponds to the previously described selective excitatory connections of stretch receptors to depressor motoneurons (4). It should be noted, however, that not all interneurons active in phase with depressors and not all depressor motoneurons were observed to receive input from the forewing stretch receptors.

Of particular interest in this investigation was our finding that stretch receptors make excitatory connections to identified interneurons considered to be elements in the central rhythm generator. This was to be expected since stretch-receptor activity can reset the rhythm (Fig. 9). A drawing of the structure of one of the interneurons is shown in Fig. 12A. Previously we have shown the passage of depolarizing currents into interneurons with this structure resets the flight rhythm (15) or decreases the flight frequency (14, 16). An example of the resetting effect is shown in Fig. 12B. Although the finding of stretch receptor input to this interneuron shows the stretch receptors have access to the central rhythm generator, it does not explain the results we described in the previous section. For example, depolarization of the interneuron increases cycle time (Fig. 12B), whereas stretch receptor stimulation shortens cycle time (Fig. 9). To determine the cellular basis for the phase-dependent influences of stretch receptor activity on the flight rhythm requires a much more extensive analysis of stretch receptor connections to interneurons and a far greater knowledge of the cellular mechanisms involved in the generation of the flight rhythm.

**DISCUSSION**

The main new finding of this investigation was a phase-dependent influence of forewing stretch receptors on the flight rhythm in *L. migratoria*. This influence results in an elevation of wingbeat frequency and a marked prolongation in the duration of flight activity. These findings indicate that the stretch receptors are important elements in the flight rhythm generator. In this discussion we first review our evidence for a phase-dependent influence of stretch-receptor activity on the flight rhythm and discuss possible reasons why this was not detected in earlier investigations. We then present our views on why the stretch receptors should be regarded as integral elements of the flight rhythm generator.

**Phase-dependent influence of stretch receptors on flight rhythm**

Our conclusion that stretch-receptor activity can influence the flight rhythm in a phase-dependent manner comes from two observations. The first is that a single stimulus train resets the flight rhythm only when de-
livered close to the onset of depressor activity (Fig. 9), and the second is that the timing of stretch-receptor stimulation within the flight cycle was critical in causing the elevation of wingbeat frequency and a prolongation in the duration of flight activity (Fig. 8). Thus, for the stretch receptors to influence the flight rhythm, their activity must be appropriately timed within the flight cycle. The period over which the stretch receptors exert their maximum influence on the flight rhythm was not determined precisely in these experiments, but it was clearly over a range close to the onset of depressor activity. This corresponds to the timing of stimulation during entrainment of the flight rhythm (Fig. 10) and also to the timing of stretch-receptor activity during flight activity in restrained (Fig. 5) and tethered animals (11). From these three independent observations we conclude that the forewing stretch receptors normally influence the flight rhythm close to the onset of depressor activity and that this influence can only be exerted at this time. Presumably related to this fact is our finding that the stretch receptors selectively make excitatory connections to interneurons that are active in phase with depressors.

In their initial studies on the wing stretch receptors, Wilson and Gettrup (21) were unable to detect any phase-dependent influence of stretch-receptor stimulation on the motor output, nor were they able to entrain the output frequency by repetitive stimulation of the stretch receptors. There are a number of possible reasons why they failed to elicit these phenomena. The first is that a quite different procedure was used in setting up the preparation. Wilson and Gettrup bisected the thorax and removed the entire thoracic musculature on one side of the animal to expose nerve 1Bb innervating the fore- and hindwing stretch receptors on the other side. The effects of such a radical dissection on the physiological state of the nervous system are unknown, but it is quite conceivable that it depresses reflexes and so prevents the stretch receptors from phasically influencing the flight rhythm. The second reason is that Wilson and Gettrup stimulated nerves 1Bb in both the meso- and metathoracic segments on one side of the animal. Nerve 1Bb in each segment contains the axon of the stretch receptor as well as axons from a number of other wing receptors. Thus, there is the possibility that activation of other afferents masked the phase-dependent effects of the stretch receptors. Furthermore, it is conceivable that the phase-dependent influences are not elicited by stimulation of stretch receptors in adjacent ipsilateral segments. Finally, Wilson and Gettrup’s failure to observe phase-dependent effects may have been due to the low stimulus frequency they used (200 Hz, which is about half that normally used in our experiments). We doubt that this is the reason, however, for we have found qualitatively similar effects to those described in this paper with train frequencies as low as 100/s.

In summary, we have demonstrated phase-dependent influences of stretch-receptor activity on the flight rhythm. We cannot be certain about why earlier (21, 22) studies failed to detect these influences but the most obvious possibilities are 1) a depressed physiological state of the preparations due to extensive dissection, 2) stimulation of the stretch receptors at sites that may have resulted in the activation of other receptors and so masked the phasic effects, and 3) stimulation of the stretch receptors in different segments rather than the same segment. Because of the positive results obtained in this investigation we did not attempt to assess which of these three possibilities is more likely.

Stretch receptors as rhythm generator

Studies on deafferented preparations leave no doubt that rhythmic motor activity can be generated in the absence of sensory input (14, 20). However there are at least two important differences in the output patterns observed in intact and deafferented preparations. The first is that deafferentation leads to a reduction in the wingbeat frequency to about one-half normal and the second is that it significantly reduces the duration of flight activity in response to a constant wind stimulus. Thus, sensory input is clearly involved in the maintenance of a high wingbeat frequency and in the maintenance of flight activity for long periods of time. Previously it has been shown that the wing stretch receptors serve these functions (20, 21). However, these receptors have never been regarded as
elements in the rhythm generator. Rather, it has been generally accepted that they simply provide a tonic excitatory influence onto the flight rhythm generator. The results of the present investigation seriously question the validity of this view. First, the phase-dependent effects of stretch receptor input (Figs. 8 and 9) demonstrate that these receptors are doing more than simply providing a tonic excitatory influence on the flight oscillator and second, the resetting of the flight rhythm by stretch receptor stimulation (Fig. 9) suggests that the stretch receptors are elements in the rhythm-generating network. In fact the stretch receptors fulfill every criterion normally used for identifying elements in rhythm-generating systems (18); they discharge rhythmically in phase with the flight motor output, they alter the motor output when removed, and they reset the flight rhythm in a phase-dependent manner. Thus, from a conceptual viewpoint there are no reasons for not regarding the stretch receptors as elements within the rhythm-generating system. This position is strengthened by our finding of connections from the stretch receptors to interneurons, presumed to be central elements in the rhythm generator (Fig. 12).

It could be argued, however, that the stretch receptors are not part of the flight rhythm generator because their removal does not prevent the generation of a rhythmic motor pattern resembling the pattern in normal-flying animals. We feel this is not a valid reason for excluding them from the system generating the normal flight rhythm simply because not all neurons in rhythm generators are necessary for the generation of the rhythm. For example, in the pyloric system of the lobster stomatogastric ganglion no single neuron is essential for the generation of rhythmic activity, but removal of single neurons can influence the phasing and timing of motor activity (10). Unfortunately we do not know the extent to which deafferentation leads to a restructuring of activity in different motoneurons during a single flight cycle. If significant reorganization of the motor pattern does occur, this would further indicate that the rhythm generator for flight includes peripheral elements.

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