Proprioceptive Input Patterns Elevator Activity in the Locust Flight System

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SUMMARY AND CONCLUSIONS

1. In the locust, *Locusta migratoria*, the roles of two groups of wing sense organs, hind wing tegulae and wing-hinge stretch receptors, in the generation of the flight motor pattern were investigated. A preparation was employed that allowed the intracellular recording of neural activity in almost intact tethered flying locusts or after selective manipulations of sensory input. The functions of the two sets of receptors were assessed 1) by studying the phases of their discharges in the wingbeat cycle (Fig. 3), 2) by the selective ablation of input from the receptors (Figs. 4–7), and 3) by the selective stimulation of the receptor afferents (Figs. 8–12).

2. Input from the tegulae was found to be responsible for the initiation of elevator activity (Figs. 9 and 10) and for the generation of a distinct initial rapid depolarization (Figs. 4, 5, and 8) characteristic of elevator motor neuron activity in intact locusts (Figs. 1 and 16).

3. Input from the wing-hinge stretch receptors was found to control the duration of elevator depolarizations by the graded suppression of a second late component of the elevator depolarizations as wingbeat frequency increased (Figs. 6, 7, 11, and 12). The characteristics of this late component of elevator activity suggested that it is generated by the same (central nervous) mechanism that produces the elevator depolarizations recorded in deafferented animals (Fig. 2). Apparently this late component contributes to the intact pattern of elevator depolarizations only at lower wingbeat frequencies and is abolished by the action of stretch-receptor input at frequencies above ~ 15 Hz (Figs. 1,

2, and 4). At these high wingbeat frequencies elevator activity is dominated by the rapid depolarizations generated as a result of tegula input.

4. The present study demonstrates 1) that the timing of elevator motor neuron activity is determined by phasic afferent input from tegulae and stretch receptors and 2) that input from the stretch receptors controls the duration of elevator activity in the wingbeat cycle following the wing movement that was responsible for the generation of the receptor discharge.

INTRODUCTION

Recent studies on a variety of locomotory systems have demonstrated that proprioceptive signals play an important role in the patterning of motor activity. For example, phasic proprioceptive signals can entrain centrally generated rhythms for swimming in the dogfish and lamprey (28), walking in cats and crayfish (5, 26), and flight in the locust (24). Furthermore, proprioceptive signals are used for initiating the transition between stance and swing phases during walking in cats, insects, and crayfish (4, 7, 17, 26) and for initiating the onset of elevator activity in the flight system of the locust (19, 32). An important problem now is to determine the mechanisms by which proprioceptive signals exert their actions.

In the flight system of the locust it was first demonstrated more than a decade ago that sensory feedback influences the timing of motor output (29). Since then evidence has accumulated that the different receptor groups associated with the locust's wings and

the wind-sensitive hairs on the head participate in flight-pattern generation (6, 12, 14, 15, 18, 24). This evidence was obtained from reset and entrainment studies investigating the effects of electrical and mechanical stimulation of receptor organs on the electromyographically recorded flight motor pattern. These studies demonstrated, and usually quantified, phase-dependent influences of afferent input on the flight motor pattern, but they gave little information concerning the mechanisms by which sensory feedback might participate in motor-pattern generation. Elucidation of these mechanisms requires knowledge of 1) the activity patterns of receptor afferents in behaving animals, 2) the central pathways from afferents to motor neurons, and 3) the influence of input from different groups of afferents on the pattern of synaptic input to flight motor neurons. The activity patterns of a variety of afferents have now been determined in tethered flying animals (6, 14, 15), and some of the central connections made by these and other afferents have been described (8, 9, 13, 19, 23). What is lacking is a detailed knowledge of the influences different groups of afferents exert on the pattern of synaptic input to motor neurons. Some information on this issue has been obtained by recording the synaptic input to flight motor neurons in intact tethered flying animals (32). This analysis revealed that afferent input is essential for the generation of characteristic aspects of the depolarizations observed in elevator motor neurons of intact locusts.

The aim of the present study was to examine the roles of two sets of wing receptors, the tegulae and the wing stretch receptors, in the production of the rhythmic depolarizations in flight motor neurons. Previous studies have shown that both these groups of receptors have strong effects on flight activity: removal of the hind wing tegulae delays the onset of elevator activity relative to depressor activity (19), and stimulation of wing stretch receptors resets the flight rhythm in a phase-dependent manner (18, 22) and promotes the generation of depressor activity (14). The experiments described in this paper were done in a preparation that allowed the intracellular recording of motor neuronal activity in intact, tethered flying locusts or after selective manipulation of afferent input (tran-

section and/or electrical stimulation of sensory nerves). We provide further evidence that the onset of elevator activity in the intact animal is due to the immediate effect of phasic input from hind wing tegula afferents. In addition we present data showing that afferent input from the stretch receptors serves to control the duration of the elevator activity by a graded suppression of the late portion of the elevator depolarizations.

MATERIALS AND METHODS

Animals

Male and female *Locusta migratoria* from a laboratory culture at the University of Alberta were used for all experiments, their ages ranging between 2 and 4 wk after the imaginal moult. Experiments were carried out at room temperature (ca. 23°C), and no differences were noted with respect to sex.

Preparation

The preparation that we used in the present study allowed intracellular recordings to be made from flight motor neurons in tethered flying locusts that were intact or from animals after surgical removal of sensory afferents or during the electrical stimulation of sensory nerves. The locusts were glued (bee's wax-rosin mixture 1:2), ventral side up, to a steel holder by their thoracic sternites in a manner that allowed their wings to move freely (33). Mounting our animals in an inverted position did not noticeably alter the flight motor pattern with respect to the timing of elevator activity (20, in preparation). In particular, we have been unable to confirm that inverting the animal changes the timing of elevator activity from a phase-constant to a latency-constant pattern as reported by others (27). Flight activity was elicited by short wind puffs directed onto the animal's head. The steel holder left the median portion of the thorax uncovered such that small openings could be cut into the sternal cuticle to expose the meso- or metathoracic ganglion. After the removal or deflection of the tracheal branches covering the ganglia these were supported individually by stainless steel spoons.

Enlargement of the hole in the cuticle and additional removal of fatty tissue provided access to the metathoracic nerve branches that were recorded or stimulated in this study. To gain access to the prothoracic nerves six (Fig. 8A gives an overview over the branches of nerve 1 that are of interest in this study) parts of the prosternum and the soft cuticle connecting pro- and mesosternum had to be removed. This produced a fairly large opening and care had to be taken to avoid any

damage to muscle attachment sites or fine nerve branchings.

Afferent input from the tegulae was removed by excising the whole receptor organs. The tegulae are knob-shaped organs inserted into soft cuticle at the anterior base of each wing and can easily and reliably be extirpated (19). Two different methods were applied to abolish afferent input from the wing stretch receptors [and the closely associated chordotonal organ (21)]. One method entailed excising the organs from outside, starting at their attachment sites in the soft cuticle of the wing bases (16). This was not very reliable. The success of the operation had to be verified by subsequent recordings from the afferent nerve 1 (which reliably picked up stretch-receptor activity), and it always caused some damage to the direct flight muscles, particularly the tergocoxal muscles. Much more reliable and involving less overall damage was the second method that consisted of cutting the meso- and metathoracic nerves 1D. Unfortunately, this procedure also severed all the motor neurons supplying the indirect dorsal longitudinal flight muscles, since these run in the same nerve. Despite the absence of activity in the dorsal longitudinal muscles the characteristics of the elevator depolarizations were similar to those observed when the sensory organs (stretch receptors and chordotonal organs) were selectively removed. This is because the lack of activity in the dorsal longitudinal muscles did not prevent excitation of the hind-wing tegulae during the wing downstroke. In RESULTS we show that it is the input from hind-wing tegulae that is responsible for producing the initial component of the elevator depolarizations. To abolish input from the wing campaniform sensillae, the ventral portions of the costal and subcostal veins that contain these sensillae (10) were cut off the wing surface.

Deafferentation with respect to the wing sense organs was usually achieved by cutting the sensory nerves 1 of the meso- and metathoracic ganglia and the nerves 6 of the pro- and mesothoracic ganglia close to their origins at the respective ganglia. These nerves contain all afferent fibers from the wing region. To stimulate the hind-wing tegula afferents in a deafferented locust, the metathoracic nerves 1C1 were left intact, but the tegula was excised, and all the other branches of nerve 1 were severed close to their origins on nerve 1 or 1C. Stretch-receptor afferents were stimulated in the prothoracic nerve 6 and in these cases the mesothoracic nerve 1 was severed distal to the point where nerve 6 branches off. In the deafferented preparations the nerves supplying the direct flight muscles (nerves 3, 5, and 6) were often also transected, (except for one that provided the reference electromyogram) to reduce vibrations of the ganglia due to flight muscle activity.

Recording techniques

Intracellular recordings from flight motor neurons were obtained with 50–100 M Ω glass micropipettes filled with a solution of 5% Lucifer yellow in distilled water. Most recordings were taken from the large and readily visible motor neuron somata. Except for the smaller-sized action potentials the activity recorded in the somata was similar to that observed in neuropile recordings (32). After the recording of neural activity, the cells were injected with Lucifer dye (5–10 nA hyperpolarizing current for 5–15 min). The ganglia were then removed from the locusts, fixed, dehydrated, and viewed as whole mounts in an epifluorescence microscope for a morphological identification of the stained neurons (11).

Hook electrodes (uninsulated stainless steel or silver wire, 0.1 mm and 0.075 mm in diameter, respectively) were used in a bipolar or in a monopolar configuration. In the latter case, the indifferent electrode was placed in the nearby hemolymph. The nerve segment on the recording electrodes was insulated from the hemolymph with silicone grease. Stretch-receptor activity was recorded from the nerves 6 of the pro- or mesothoracic ganglia that contain a branch of the single fore- and hind-wing stretch-receptor afferent, respectively (Ref. 3, Fig. 11). Tegula activity was monitored by recording from the meso- or metathoracic nerves 1C. The large spikes in these recordings reliably represented the activity in tegula afferents (19). The spikes produced by other afferents from the wing were much smaller and could, to a large part, be abolished by removal of the campaniform sensillae.

Electromyograms (EMGs) were routinely recorded from one of the following depressor muscles: hind-wing subalar (129), hind-wing 1st basalar (127), or, on one occasion, fore-wing 1st basalar (97). Copper wires (diameter 0.1 mm, insulated except for the tips) were inserted into prepared holes in the cuticle. The first basalars were recorded at their sternal attachment sites (20), the subalar from the pleural cuticle. The EMGs recorded from depressor muscles were used primarily for aligning two or more superimposed recordings from elevator motor neurons taken from the same animal. The fact that there is a time lag (ca. 7 ms) between the onset of depressor activity in the hind wings and fore wings (20, 30) did not influence the interpretation of the data. This is because the depolarizations in all elevator motor neurons (fore wing and hind wing) occur almost synchronously (11, 32), the characteristics of depressor depolarizations are relatively little affected by deafferentation (Fig. 1), and because the time lag between hind-wing and fore-wing depressor activity persists after deafferentation (20, 27).

FIG. 1. *A*: depolarization patterns recorded in flight motor neurons of intact (*left*) and deafferented (*right*) locusts. Activity recorded in an elevator motor neuron (fore-wing anterior tergocoxal, 89) of same individual before and after deafferentation is shown in *top* 2 examples, *bottom* examples present depolarizations recorded in a depressor motor neuron (fore wing 1st basalar, 97) of a different animal. Electromyograms (EMGs) shown below each of the intracellular records, in this and all the following figures, were obtained from hind-wing depressor muscles (either the subalar muscle, 129, or the first basalar muscle, 127). *B*: depolarization pattern in elevator motor neurons of intact locusts at low wingbeat frequencies. Four typical wingbeat cycles recorded at successively longer wingbeat periods were selected from a longer flight sequence. Fore-wing anterior tergocoxal motor neuron (89) was recorded, and positions of particular wingbeat cycles in the flight episode are indicated. Note constant initial component and gradual appearance of late depolarization as wingbeat period increased. Calibrations 100 ms, 10 mV.

Stimulation technique

For nerve stimulation experiments, hook electrodes were used only in a bipolar configuration to minimize stimulation artifacts in the intracellular recordings. Stretch-receptor afferents were stimulated by hook electrodes placed bilaterally on the pro- or mesothoracic nerves 6 as described in the section on recording techniques (Fig. 11*A*). This resulted in a selective stimulation of stretch-receptor afferents, since these are the only afferents to send a branch through nerve 6 (3). To avoid coactivation of the dorsal longitudinal muscles (some dorsal longitudinal motor neurons run also via nerve 6) the respective nerves 1D that supply

these muscles as well as the stretch-receptor and chordotonal organs had to be severed (see above). To stimulate the afferents arising from the hind-wing tegulae, hook electrodes were placed bilaterally on the metathoracic branches N1C of nerve 1 (Ref. 19, Fig. 8). Afferents enter nerve 1C not only from the tegula but also from the wing nerve. However, the afferents arising from the tegula are by far the largest diameter fibers running in this nerve (2). This fact allowed a selective activation of tegula afferents by careful adjustment of the stimulus voltage. The threshold voltage for the stimulation of tegula afferents in nerve 1C was determined by noting the occurrence of excitatory

postsynaptic potentials (EPSPs) in elevator motor neurons, and the stimulus voltage for the subsequent experiments was then set to between 1.2 and 1.5 times the threshold value. We have previously shown that this procedure selectively and reliably excites tegula afferents (19).

Electrical stimuli were delivered to the nerves in trains of 4–10 pulses at rates ranging from 250 to 300 Hz. The duration of the single stimulus was 0.1 ms. This pattern of stimulation was intended to mimic as closely as possible the natural bursts occurring in the afferent fibers. The stimulus trains were either triggered with an adjustable delay, by the hind-wing depressor EMG to give a time-locked stimulation at each wingbeat cycle, or they were delivered at a rate of 1–3 Hz to give a pseudorandom stimulation at various phases of the wingbeat cycle. It should be noted that stimulation of afferents associated with either pair of wings influenced the synaptic input to homologous motor neurons supplying fore-wing and hind-wing muscles in a qualitatively similar manner.

Evaluation of data

Data were stored on magnetic tape (Vetter model 3 with FM channels) and run out on a chart recorder (Gould ES1000) at reduced speed for further evaluation. Where individual records are shown in the figures, typical examples were selected for presentation. All the presented data were confirmed by recordings between 9 and 48 different animals.

The magnitude of elevator depolarizations was judged by measuring the area covered by the depolarizations in the voltage-versus-time plots produced by the chart recorder. The area was determined by means of a digitizing tableau (Microplan). The minimum membrane potentials of adjacent flight oscillations were connected, and the resulting line was taken as base line. Action potentials were not taken into account when outlining the depolarizations of the membrane potential. Repeatedly tracing the same depolarizations indicated that this method was fairly accurate. Repeated measurements differed by only 1.4% on average, with the maximum difference being 4.7% ($n = 75$). Additional small systematic errors might have been produced when judging the area of action potentials. Selectively determining the area of the first or second component of the elevator depolarizations was possible only in those cases where the two components were clearly distinguishable (ca. 85% of the depolarizations in an average recording, for examples see Fig. 1B). The border line between the two components was assumed to be the trajectory of the repolarization slope of the rapid elevator depolarizations observed at high wingbeat frequencies.

RESULTS

Patterns of motor neuron activity in intact and deafferented locusts

A detailed comparison of the patterns of motor neuron activity recorded in intact and deafferented tethered flying locusts has been given previously (32). A brief outline of these results (as presented in Fig. 1) is necessary, however, for an assessment of the data provided by the more selective manipulations of sensory input described below. The major results of our previous study (32) were the following. The characteristics of depressor motor neuron depolarizations were relatively little affected by a complete removal of input from the wing sense organs, as was their spike discharge (Fig. 1A, *bottom*). It was for this reason that a depressor EMG was chosen as a reference for flight activity. By contrast, deafferentation caused considerable changes in the pattern of elevator motor neuron activity. The elevator motor neurons were found to form a very homogeneous group of neurons, and thus the following description applies to them all. In intact locusts the elevator depolarizations had steep rising and repolarization slopes and were of short duration (Fig. 1A, *top left*). In the deafferented situation, elevator depolarizations commenced more gradually, and the initial moderate depolarization slope was followed by a fairly steep final depolarization (Fig. 1A, *top right*). Spikes were initiated mainly by this final depolarization and to a variable extent by the preceding moderate rising slope. The repolarization, by contrast, remained almost as steep as in the intact animal. As a consequence, the depolarizations peaked at a later phase with respect to depressor activity (Fig. 16B).

Although, on average, deafferentation slowed the flight rhythm to about half the normal frequency, i.e., from ca. 20 to 10 Hz (30), intact and deafferented locusts actually produced flight motor activity over a considerable range of wingbeat frequencies. In both the intact and deafferented situation, the duration of depressor depolarizations did not change very much with wingbeat frequency, but it was rather the duration of the elevator depolarizations that accounted for changes in wingbeat period. In intact locusts, the initial steeply rising part of the elevator depolarizations remained remarkably constant

throughout the observed frequency range of ~ 8 to 21 Hz, and the duration of the depolarizations was prolonged at lower wingbeat frequencies (Fig. 1*B*). In deafferented locusts, by contrast, it was largely a change in the gradual rising slope of the depolarizations that accounted for changes in wingbeat period (Ref. 32, Fig. 1*A*, top right).

A detailed analysis of the depolarizations recorded from elevator motor neurons in intact animals suggested that at low wingbeat

frequencies they are composed of two independent components: an initial rapid depolarization characteristic of the intact flight pattern and a variable late depolarization with a certain resemblance to the depolarizations occurring in deafferented animals (Figs. 1*B* and 2). In intact locusts the characteristic initial part of the elevator depolarizations was present at all wingbeat frequencies. The late portion of the depolarizations was observed only at frequencies below ~ 15 Hz

FIG. 2. Compound character of elevator depolarizations recorded in intact locusts at low wingbeat frequencies. *A*: record on left shows depolarization in an elevator motor neuron (fore-wing anterior tergocoxal, 89) recorded at a wingbeat frequency (WBF) of ~ 15 Hz in an intact locust. A depolarization recorded in the same individual at a lower wingbeat frequency (ca. 10 Hz) is shown in the middle record. Right record shows a depolarization recorded in the same motor neuron after deafferentation of the animal. This depolarization was selected for an exact match in wingbeat period (as determined from the EMG) with the depolarizations recorded in the intact situation at the lower wingbeat frequency. *B*: rapid short depolarization recorded at high wingbeat frequency is superimposed onto the depolarization recorded in the deafferented animal. Depolarizations were aligned with respect to the 1st spike in the preceding depressor EMG. Above superimposed traces, potential recorded at low wingbeat frequency in intact animal is shown, EMGs being in register with those recorded in the deafferented situation. Note similarity between contour of superimposed records and depolarization recorded in intact locust at ca. 10 Hz. *C*, *D*: records from intact locust at lower wingbeat frequency and from deafferented animal were selected at a slightly higher wingbeat frequency than in *A* and *B* (ca. 12 Hz, recordings from the same animal). In this case the late part of the depolarization recorded in the intact animal at the lower wingbeat frequency was distinctly smaller than the corresponding part of the contour resulting from the superposition. Calibration 100 ms, 10 mV.

FIG. 3. Schematic diagram summarizing timing of tegula and stretch-receptor activity in the flight cycle. *Top* trace shows the depolarizations recorded in an elevator motor neuron (fore-wing anterior tergocoxal, 89) of an intact locust at wingbeat frequencies of ~ 15 Hz (*left*) and 11 Hz (*right*). Next 2 traces present timing of discharges (mean values of onset and termination, see text) in fore- and hind-wing tegula afferents [no single spikes shown since ca. 80 fibers arise from each tegula (2)]. The following 2 traces show timing of fore- and hind-wing stretch-receptor activity. A typical number of spikes is shown (for variability see text), and the standard deviations for the timing of the 1st and last spike in bursts are indicated. EMG is from a hind-wing subalar muscle, 129. SR data were determined from recordings made in 1 individual (50 wingbeat cycles evaluated). Results were confirmed qualitatively in 15 other animals. Calibrations 100 ms, 10 mV. SR, stretch receptor.

FIG. 4. Depolarization patterns in elevator motor neurons after removal of the tegulae. *A*: depolarizations recorded in a fore-wing tergocoxal (89) neuron of an intact animal. *B*: depolarizations recorded in hind-wing tergocoxal (113) motor neuron of another animal after removal of all tegulae. *C*: potentials recorded in fore-wing tergocoxal (89) motor neuron of a deafferented preparation. For each situation, potentials recorded at 3 different wingbeat frequencies are superimposed with respect to the preceding depressor burst in the EMG (1st basalar or subalar). Note that 1) in the intact animal the initial rapid depolarization remains almost unaffected by changes in wingbeat frequency and 2) the appearance of a variable late component that becomes more prominent at longer wingbeat periods. After removal of the tegulae or after complete deafferentation, the rising slope is variable and becomes more gradual toward longer wingbeat periods. Calibrations 100 ms, 10 mV.

FIG. 5. Reversible abolition of tegula discharge by immobilization of the wings and its effect on elevator motor neuron activity. *Top*: recording from metathoracic nerve 1 after lesion of the ipsilateral stretch-receptor and campaniform sensillae afferents. Under these conditions the recording selectively picked up spikes of tegula afferents and action potentials produced by the dorsal longitudinal motor neurons (large spikes in phase with depressors, marked by DL). *Middle*: recording from an elevator motor neuron (fore-wing tergosternal, 83/4). During the first 3 depolarizations, the wings were immobilized in an upstroke position. They were released during the 4th depolarization, and the tegulae resumed their normal discharge pattern during the remaining 3 cycles (smaller spikes, *top*). EMG shows relative timing of elevator and depressor activity, namely the hind-wing 1st basalar (127, depressor, spikes marked by arrows) and tergosternal (113, elevator) muscles. Calibrations 100 ms, 10 mV.

and was variable in both amplitude and duration according to the actual wingbeat frequency. This component became increasingly prominent with decreasing wingbeat frequency and at ~ 10 Hz it resembled the potentials recorded in deafferented locusts, with respect to both amplitude and phase of appearance in the cycle of depressor activity. In Fig. 2A, this is demonstrated by a comparison of two elevator depolarizations produced at the same wingbeat frequency, one before and the other after deafferentation of

the same animal. When the potential recorded in the deafferented situation is superimposed on a typical rapid depolarization that had been recorded in the same individual before deafferentation and at a high wingbeat frequency, the contour outline of the superposition is very similar to the depolarization observed in the intact animal at the lower wingbeat frequency (Fig. 2A, *right*). This superposition procedure gave similar results at all corresponding wingbeat frequencies recorded in the intact and the deaf-

FIG. 6. Depolarization pattern in elevator motor neurons after removal of input from wing stretch receptors. *A*: recordings from hind-wing tergosternal motor neuron (113) after the nerves 1D in meso- and metathorax had been severed to abolish afferent input from stretch receptors (and the associated chordotonal organ). *B*: 3 depolarizations recorded at successively lower wingbeat frequencies in *A* were superimposed with respect to the preceding depressor burst in the EMG. This superposition shows that the initial part of depolarizations remains almost unaffected by changes in wingbeat frequency (note relatively constant latency). Late part of depolarization has a relatively constant repolarization slope remains constant. *C*: recordings in intact locust show, by comparison, that the late part of depolarization decreased in amplitude toward higher wingbeat frequencies (arrows point at repolarization slopes). Calibrations 100 ms, 10 mV.

ferented situations, although the amplitude of the variable late component decreased toward higher wingbeat frequencies until it was apparently absent at frequencies above ca. 15 Hz (Fig. 1B). That is, the late portion of the elevator depolarization recorded in the intact locust still corresponded to the potential observed in the deafferented animal with respect to the phase of appearance but not with respect to amplitude (Fig. 2B).

In summary, the above results suggest the following conclusions: 1) The depolarizations recorded in elevator motor neurons of intact tethered flying locusts are composed of two independent components: an initial rapid depolarization of relatively constant amplitude and duration and a variable late depolarization that contributes to elevator

activity only at low wingbeat frequencies. 2) The initial component is generated as a consequence of sensory input (since it was observed only in intact animals). 3) The variable late depolarizations observed at low wingbeat frequencies are related to the elevator depolarizations generated in deafferented preparations. The question is raised of what regulates the appearance of the late component of elevator depolarizations with changing wingbeat frequency.

Activity of the tegulae and the wing-hinge stretch receptors in intact tethered flying locusts

If we want to consider the role of afferent input for the generation of certain aspects of the flight pattern it is essential to know the

FIG. 7. Dependence of the magnitude of elevator depolarizations (ordinate) on wingbeat period (abscissa) in intact animals (○) and in animals with stretch-receptor input ablated (●). Area covered by elevator depolarizations (shaded areas in examples at top and bottom, action potentials not considered) was determined for wingbeat periods between 60 and 100 ms. 83 depolarizations in the anterior tergoxal motor neuron, 89, of an intact animal were evaluated and 136 depolarizations in the hind wing tergoxal motor neuron, 113, after stretch-receptor ablation. To avoid errors due to different recording amplitudes, the 2 sets of recordings were selected for having exactly the same amplitude of initial rapid part of depolarizations. Solid line is best fit to data points (not shown) that were obtained by superimposing elevator depolarizations recorded in a deafferented locust at different wingbeat frequencies with initial rapid depolarization observed in that animal at wingbeat frequencies > 15 Hz before deafferentation (same individual that provided the data for the intact situation, 50 superpositions). Process of superposition is described in detail for Fig. 2. SDs are indicated only in 1 direction for each data point. Ordinate in units of voltseconds, Vs.

timing of the afferent discharge in the wing-beat cycle. Thus we shall first describe the pattern of tegula and stretch-receptor activity in the tethered intact preparation.

Spike activity in the fore-wing and hind-wing tegula afferents was observed by recording from the meso- and metathoracic nerves 1C, respectively. The discharge pattern of tegula afferents was remarkably constant and independent of changes in wingbeat frequency. In one animal analyzed in detail, over the frequency range of 12 to 19 Hz, the tegulae of fore wings and hind wings were found to produce spike bursts of 21 ± 4 -ms and 24 ± 3 -ms duration, respectively (mean $\pm$ SD, $n = 50$). These bursts occurred at relatively constant latencies of 23 ± 3 ms and 15 ± 3 ms with respect to the previous hind-wing depressor burst (results summarized in Fig. 3, for example of an original recording see Fig. 5).

A comparison of the pattern of tegula activity with the elevator motor neuron depolarizations (Fig. 3, *top* record) shows that the

activity in the afferents, particularly from the hind-wing tegulae, commenced a few milliseconds before the membrane potential in elevators began to rise and lasted throughout the initial part of the depolarizations.

Spikes of the stretch receptors were observed in hook-electrode recordings from nerve 1 or nerve 1D. The fore-wing stretch receptors were found to discharge several (usually from 3 to 7, with an average of 4) spikes between ca. 2 and 15 ms after the first spike in the depressor EMG. This was true for wingbeat frequencies of 15 Hz or above. At lower wingbeat frequencies (down to 10 Hz), the stretch receptors showed a tendency to produce longer and quite variable spike bursts with up to 14 spikes. These bursts could commence up to 20 ms before depressor activity and could last until 20 ms after it (compare Refs. 14 and 16). The hind-wing stretch receptors produced only very few (3 at the maximum) or, quite often, no spikes at all, particularly at high wingbeat frequencies. If present, the spikes were discharged from

FIG. 8. Stimulation of hind-wing tegula afferents in a locust with excised tegulae restores intact pattern of elevator motor neuron activity. *A*: diagram illustrating the experimental situation. In an animal that had all tegulae removed, the hind-wing tegula afferents in nerve 1C (N1C) were stimulated bilaterally (*middle* traces in *B* and *C*), and the depolarization pattern in an elevator motor neuron (*top* traces, 118) and a depressor EMG (*bottom* traces, 127) were recorded. This diagram also shows the structure of tegula afferents (*left*) and an anterior tergo-coxal motor neuron (*right*) in the metathoracic ganglion. *B*: stimulus trains (for parameters see METHODS) were triggered by EMG spikes and given in the middle of a longer flight sequence (ca. 1.5 s of central portion omitted). *C*: 2 sections of the flight sequence with matching wingbeat periods, one recorded with and the other without stimulation of tegula afferents, are superimposed with respect to the depressor EMG. This illustrates that stimulation of tegula afferents elicited the typical initial part of depolarizations but did not significantly alter the late component. Calibrations 100 ms, 10 mV.

25 ms before to 10 ms after an action potential in the depressor EMG. The data on the stretch receptors are summarized in Fig. 3.

Depending on wingbeat frequency, stretch-receptor activity commenced from ~10 to 40 ms before the rapid onset of the elevator depolarizations (compare motor neuron recording in the *top* trace of Fig. 3). The last spike in a stretch-receptor burst was produced only shortly after or even a few milliseconds before the rise in membrane potential commenced.

Depolarization patterns in elevator motor neurons after selective removal of sensory input

The selective ablation of input from groups of wing receptors may provide evidence for their role in flight-pattern generation. We thus examined the pattern of elevator motor neuron depolarizations after removal of the tegulae or transection of the stretch-receptor afferents.

REMOVAL OF THE TEGULAE. After all four tegulae of the experimental animal had been removed, distinct changes could be observed in the depolarization pattern recorded from elevator motor neurons. The initial rapid depolarizations characteristic of the intact pattern (Fig. 4A) were missing and the rising

slope of the depolarizations was gradual and depended on wingbeat frequency (Fig. 4B). This indicated that the tegulae are involved in the generation of the initial rapid depolarizations characteristic of the intact pattern of elevator activity. With respect to the gradual frequency-dependent rising slope, the elevator depolarizations observed after removal of the tegulae resembled those recorded in deafferented locusts (Fig. 4C). The potentials recorded in the two situations were not identical, however, presumably as a result of input from other wing sense organs that remained present after tegula removal. When compared with the potentials observed in completely deafferented animals, the depolarizations had slightly steeper and shorter-lasting rising slopes and were, on average, shorter in duration.

Discharges of the tegula receptors could also be abolished, in a reversible manner, by immobilizing a locust's wings in the upstroke position during the production of flight motor activity (Fig. 5, *top*). Although this procedure not only altered the activity pattern of the tegulae but of other wing receptors as well, an effect on the initial rapid part of the elevator depolarizations should be expected if the tegulae were responsible for its generation. We consistently observed that the initial portion of the depolarizations was

FIG. 9. Resetting of flight rhythm by stimulation of hind-wing tegula afferents. Simultaneous recordings of hind-wing anterior tergocoxal (elevator, 118, *top*) and 1st basalar (depressor, 127, *middle*) motor neurons are shown. EMG (*bottom*) was recorded from the contralateral 1st basalar. Animal was intact and its hind wing tegula afferents were stimulated bilaterally at random. Artifacts caused by stimulus trains are apparent in all 3 traces and further marked by arrows (stim). Each stimulus train elicited a separate elevator depolarization and terminated (2nd and 3rd stimulus train) or suppressed (1st train) depressor activity. Calibrations 100 ms, 10 mV.

FIG. 10. Resetting characteristics for stimulation of hind-wing tegula afferents (deafferented animal, 279 stimulus presentations evaluated). Abscissa gives phase of stimulus presentation [1st stimulus in 5-pulse train, with respect to 1st spikes in EMG (127) bursts]. For values of stimulus phase between 0 and 1, the wingbeat cycle during which the stimulus had been presented was affected in its duration and thus considered as the "perturbed period" for evaluation. In some cases, however, it was the duration of the subsequent wingbeat period that was affected. Negative values of stimulus phase were assigned in these cases. On the ordinates 3 parameters are represented: 1) Phase shift (top graph), negative values mean that the spike in the depressor EMG that terminated the perturbed wingbeat period was advanced with respect to previous flight rhythm, positive values mark a delay. Phase shift was calculated as follows: average duration of 3 wingbeat cycles before stimulus presentation was subtracted from duration of perturbed period, and the result was divided by this average duration: (perturbed period-average period)/average period. 2) middle curve (o) presents the delay between stimulus presentation, and the first spike in the subsequent depressor burst divided by the average duration of 3 wingbeat cycles before stimulus presentation to correct for variations in wingbeat frequency. 3) Bottom curve (•) gives average duration of 3 wingbeat periods before stim-

not generated when the wings were held in the upstroke position and the tegulae remained inactive (Fig. 5, middle).

REMOVAL OF INPUT FROM THE WING STRETCH RECEPTORS. After removal of input from the wing stretch receptors (and the closely associated chordotonal organ) the initial rapid depolarizations were still generated in elevator motor neurons (Fig. 6). At low wingbeat frequencies the elevator depolarizations resembled closely those seen in the intact animal, except that the late depolarizations were generally larger (of about the same amplitude as the initial rapid component) (Fig. 6A, right). At higher frequencies, however, the late component was not reduced in amplitude as reported for the intact situation (Figs. 1B, 2, and 6C) but rather appeared to merge into the initial rapid depolarization with more or less constant amplitude (Fig. 6A, middle).

If there actually were a merging of the initial rapid and the late components without the late portion being suppressed, the area covered by the depolarization as a whole should be larger than if a suppression occurred. Moreover, with no suppression occurring, it might be possible to approximate the outcome of the merging process by a simple superposition of the initial rapid depolarization recorded at high wingbeat frequencies with the gradually developing depolarizations observed in the deafferented situation (as shown in Fig. 2).

An analysis of the area covered by the compound elevator depolarizations and of its dependency on the wingbeat frequency corroborated the above interpretation (Fig. 7). The depolarizations recorded in locusts with severed stretch-receptor afferents (*filled circles*) were consistently just slightly larger than would be expected from a superposition of the initial rapid depolarization and the potentials recorded in elevator motor

ulus presentation, minus duration of the period after the perturbed wingbeat cycle divided by average duration. A value of 0 indicates that the stimulus did effect a change in wingbeat frequency and that a phase shift was not compensated during subsequent wingbeat periods. To relate values of stimulus phase to flight motor activity, typical discharges of wing depressor and elevator muscles are drawn under the diagram. For each data point SDs are indicated.

neurons of the deafferented animal at the respective wingbeat frequencies (*solid line*). The fact that the observed depolarizations were slightly larger than predicted from a superposition indicated that in the zone of overlap both depolarizations summated to a certain extent. Furthermore, the close correspondence between the data represented by the *solid line* and the *filled circles* indicated that after stretch-receptor removal the two components were merged without any sign of a suppression of the late depolarization.

The depolarizations recorded in intact locusts (*open circles*), on the other hand, had a distinctly smaller size than the depolarizations recorded after removal of stretch-receptor input at corresponding wingbeat frequencies. They were also consistently smaller than predicted from the superposition of the rapid and the late depolarizations. This latter fact is consistent with the idea that the late

depolarizations are suppressed toward high wingbeat frequencies in intact animals rather than being merged into the rapid depolarizations without change in amplitude, as after removal of stretch-receptor input.

Selective stimulation of sensory afferents

Ablation experiments as described in the previous section have one general shortcoming. If sensory feedback participates in flight-pattern generation then the ablation of one sensory system may alter flight performance and hence feedback from the remaining sense organs. Thus any changes in motor pattern cannot directly be attributed to the absence of input from the ablated receptor. If, after the removal of one group of receptors, it is possible to restore the intact pattern of activity by an appropriate stimulation of their afferent fibers, however, this provides important additional evidence for the func-

FIG. 11. Stimulation of stretch-receptor afferents at the normal phase of their activity suppresses the late component of elevator motor neuron depolarizations. *A*: diagram illustrating the experimental situation. Both fore-wing stretch receptors were activated by stimulating their branches in prothoracic nerves 6 (stim). Diagram also shows structure of fore-wing stretch receptor in the thoracic ganglia (*left*) and the structure of a tergosternal motor neuron in the metathoracic ganglion (*right*). *B*: recording from hind-wing tergosternal (*top*, 113) when fore-wing stretch-receptor afferents were stimulated bilaterally (*middle*). Input from fore-wing stretch-receptor organs had been abolished by cutting the mesothoracic nerves 1D. Stimulus trains were triggered by depressor EMG (*bottom*, 129) and delivered during the middle of a longer flight episode (about 1 s of central portion omitted). Stimulation of stretch-receptor afferents reduced the late part of elevator depolarizations and sped up flight rhythm. *C*: input from all 4 stretch-reafferents had been abolished (nerves 1D cut). Apart from this, the experimental situation was the same as described for *B* (hind-wing anterior tergocoxal, 118). *D*: effect of fore-wing stretch-receptor stimulation after removal of all tegulae. Other aspects as described for *B* (hind-wing tergosternal, 133). In this case stimulation of stretch-receptor afferents sped up flight rhythm and shortened, but did not abolish, elevator depolarizations. Calibrations 100 ms, 10 mV.

tion of the respective receptors. It is also necessary to investigate the relative contributions of each receptor group and possible interactions.

STIMULATION OF TEGULA AFFERENTS AT THE PHYSIOLOGICAL PHASE OF THEIR ACTIVITY. As described above, the characteristic rapid elevator depolarizations were missing after removal of the tegulae. Bilateral stimulation of the hind-wing tegula afferents at the normal phase of their discharge in the wingbeat cycle restored the pattern of elevator activity typically recorded in intact locusts (Fig. 8*B*). In other words, stimulation of the tegula afferents led to the generation of the initial rapid depolarizations. These were superimposed onto the rising slope of the depolarizations recorded after tegula removal and the typical compound elevator depolarizations could be observed (Fig. 8*C*). Stimulation of hind-wing tegula afferents also produced the rapid depolarizations in completely deafferented locusts (compare Fig. 15*C*).

RANDOM STIMULATION OF TEGULA AFFERENTS. The above results indicated that input from the tegulae is involved in the initiation of elevator activity. Further support for this came from experiments in which the hind-wing tegula afferents were stimulated randomly at various phases of the wingbeat cycle in locusts with excised tegulae. Under these circumstances, stimulation of the tegula afferents completely reset the flight rhythm [type zero reset, (31)] by initiating a (rapid) depolarization in elevator motor neurons and terminating any ongoing depressor activity, regardless of the phase of the stimulus (Fig. 9). This is illustrated by the shape of the reset characteristics in Fig. 10 as calculated from the depressor EMG. Three graphs are shown. The upper one represents the phase-response curve. The average slope of this curve is exactly one, which is characteristic of a complete reset, i.e., every stimulus train initiated an independent new flight cycle (by contrast, a slope of zero would indicate that the stimulus has no effect on the flight rhythm). The *middle* curve shows that the interval (in phase units to correct for variations in wingbeat frequency) between stimulus presentation and the subsequent burst of depressor activity is almost constant, regardless of the stimulus phase. This is just

what one would expect for a complete reset. The interval is slightly longer if the stimulus is given during a depressor depolarization, indicating that it takes a little longer to initiate an elevator burst at this phase of the wingbeat cycle than at any other. The lower graph shows that the wingbeat cycle after the period that had been disturbed by the stimulus is as long as the periods had been before the stimulus presentation, thus demonstrating that the reset is not just a transient one being compensated for later in the flight sequence. These reset curves were almost identical in animals that either had the tegulae excised or were completely deafferented.

STIMULATION OF STRETCH-RECEPTOR AFFERENTS AT THE PHYSIOLOGICAL PHASE OF THEIR ACTIVITY. Bilateral stimulation of stretch-receptor afferents at about the normal phase of their activity sped up the flight rhythm (the nerves containing the stretch-receptor afferents had been severed distal to the stimulation site to avoid coactivation of dorsal longitudinal motor neurons, see METHODS). Intracellular recordings revealed that this acceleration of the flight rhythm was due to a suppression of the late component

FIG. 12. Negative correlation between stretch-receptor discharge and magnitude of the late component of elevator depolarizations in an intact tethered flying locust. Discharges of both fore-wing stretch receptors and depolarizations in the hind-wing anterior tergo-coxal motor neuron (118) were recorded. Ordinate presents area covered by late component of elevator depolarizations (illustrated in bottom left inset). Abscissa gives sum of spikes discharged by 2 fore-wing stretch receptors during time interval from 25 to 0 ms before spike initiation in elevators. This time interval corresponds approximately to time in wingbeat cycle where stretch-receptor stimulation exerted its strongest effect according to data presented in Fig. 13. SDs are indicated for each data point. Ordinate in units of voltseconds, Vs.

of the elevator depolarizations typically recorded at low wingbeat frequencies (Fig. 11*B*; compare also Fig. 1*B* and Fig. 6*C*). This suppression resulted in a depolarization pattern similar to that observed in intact locusts at high wingbeat frequencies. An almost complete suppression of the late component of the elevator depolarizations was usually achieved only after the stimulus had been given over three to five successive wingbeat cycles.

The stimulation of stretch-receptor afferents suppressed the late component of elevator depolarizations in two other experimental situations. 1) In animals that had all four nerves 1*D* severed, i.e., input from fore- and

hind-wing stretch receptors and chordotonal organs removed (Fig. 11*C*) (as reported in the previous section, the late depolarizations were apparently present at all wingbeat frequencies in these animals, see Figs. 6 and 7). 2) If the hind-wing stretch-receptor afferents were stimulated (instead of the afferents arising from the fore wing, as described above). The stimulation of either pair of receptors had the same effect on the late component. This is noteworthy, since the discharge patterns of the two differed considerably in the intact tethered flying animal (Fig. 3) and because the two pairs do not make identical connections with thoracic neurons (23). Similar influences on elevator motor neurons

FIG. 13. Differential effect of fore-wing stretch-receptor stimulation on initial and late components of elevator motor neuron depolarizations. Experimental situation was similar to that described for Fig. 11*B* except that stimulus trains were delivered at a constant rate of 2 Hz. This resulted in the presentation of 1 stimulus train at different phases in about every 5th wingbeat cycle. Effect of stimulus on elevator depolarizations was noted for cycle in which it was presented and for 2 subsequent wingbeat cycles (as defined by depressor EMG; negative values of phase were assigned in latter cases, since stimulus preceded evaluated wingbeat cycle). Abscissa thus spans 3 wingbeat cycles, phase 0 corresponding to the depressor discharge (1st spike in EMG) at beginning of wingbeat period during which stimulus was presented. Elevator depolarization preceding wingbeat period of stimulus presentation was taken as a reference (100%) for determining stimulus-related effects in the following 3 cycles. This was necessary because wingbeat frequencies in evaluated flight episodes ranged from ca. 9 to 13 Hz, and area covered by depolarizations depended strongly on wingbeat frequency (see Fig. 7). 3 parameters are plotted: areas of initial rapid (○) and late (●, top graph) components of elevator depolarizations and, in the bottom graph, phase shift as defined in Fig. 10. SDs are indicated for each data point.

cannot be explained by connections to flight motor neurons, despite the fact that the two sets of receptors project into the meso- and metathoracic ganglia.

It is evident from these results that input from the wing stretch receptors functions to suppress the late portion of the elevator depolarizations. Further support for this view comes from an analysis of the relationship between stretch-receptor activity and the prominence of subsequent elevator depolarizations in undisturbed tethered flying locusts. Figure 12 shows that in the intact animal the size of the late component of the elevator depolarizations is negatively correlated to the number of stretch-receptor spikes discharged during a certain time interval (25 ms, see figure legend) preceding the elevator depolarization.

It is important to note that in locusts that had all tegulae removed or that were completely deafferented, stimulation of stretch-receptor afferents was not able to abolish elevator depolarizations (Fig. 11*D*; see also Fig. 15). Stimulation of the afferents considerably reduced the duration of the depolarizations and consequently sped up the flight rhythm. A complete suppression of elevator depolarizations was never observed, however.

RANDOM STIMULATION OF STRETCH-RECEPTOR AFFERENTS. Having established that input from the stretch-receptor organs has an effect on the late portion of the elevator depolarizations, the question is raised of whether the initial rapid component might also be affected, provided there is an appropriate timing of the afferent input. To investigate this problem, hind- or fore-wing stretch-receptor afferents of an intact locust were stimulated bilaterally at random phases of the wingbeat cycle. This gave the following results (Fig. 13). 1) A stimulus train was effective in suppressing the appearance of the late component of the elevator depolarizations only when delivered at certain phases of the wingbeat cycle. The phases at which stimulus trains were effective ranged from ca. +0.4 to -0.4 with respect to the hind-wing depressor EMG (Fig. 13, *filled circles* in *top* coordinate system). 2) A single stimulus train delivered at the appropriate phase of the wingbeat cycle was usually not sufficient to completely suppress the appearance of the late component in the subsequent elevator depolarization (compare Fig. 11*B*). This is reflected by the fact that the minimum value of the *middle* curve in Fig. 13 is larger than zero. In cases where the late component was

FIG. 14. Generation of late component of elevator motor neuron depolarizations is negatively correlated to preceding activity of fore wing stretch receptors during episodes with an unstable flight pattern. Intracellular recording (3rd trace) was obtained from the hind wing tergosternal (113). A hook-electrode recording from prothoracic nerve 6 (2nd trace) monitored spikes of dorsal longitudinal motor neurons (large action potentials marked by DL) and of fore-wing stretch-receptor afferent (2nd largest class of spikes). Spikes of stretch receptor, as defined by their amplitude in the hook-electrode recording, are presented in isolation in the *top* trace. EMG was obtained from fore-wing 1st basalar muscle (97). Note negative correlation between number of spikes in stretch-receptor discharge and size of subsequent elevator depolarization. Calibrations 100 ms, 10 mV. SR, stretch receptor.

not very pronounced in the elevator depolarizations preceding the stimulus presentation, however, a complete suppression could be observed. 3) The initial rapid elevator depolarizations were not affected by the stimulation of stretch-receptor afferents, regardless of the stimulus phase (Fig. 13, *open circles*). Together with the observation that stretch-receptor ablation did not influence the occurrence or amplitude of these rapid depolarizations (see Fig. 6), this suggests that these depolarizations are not directly dependent on stretch-receptor activity. 4) The flight rhythm was reset by the stimulation of forewing stretch-receptor afferents at phases between ca. -0.3 and 0.6 (Fig. 13, *bottom*). The reset effect was small when compared with the effect of stimulating tegula afferents (maximal phase shift of -0.1). Its magnitude was not directly related to the reduction in the size of the late component of elevator depolarizations. One probable reason is that these late depolarizations could show considerable variations in amplitude without corresponding changes in the overall duration of the elevator depolarizations (see Fig. 1*B* for example). Furthermore, input from the stretch-receptor afferents not only influences the activity of elevator motor neurons but also affects the depolarization pattern of depressors to a certain extent (8, 14). These influences will contribute to the shape of the reset characteristic.

The phase-response curve shown in Fig. 13 demonstrates that only a stretch-receptor

discharge preceding the elevator depolarization is effective in suppressing the late component. A stretch-receptor discharge is produced by an upward movement of the wings (14, 16), but it evidently does not exert its effect on the ongoing period but rather on the subsequent cycle of elevator activity, which then produces the next upward movement. This delayed action may explain the occurrence of an unstable flight pattern that was frequently observed in tethered flying animals (Fig. 14). For periods lasting from a few hundred milliseconds to some seconds, the elevator depolarizations often alternated between two extremes—the late component of the depolarizations being either small or missing, or large and well developed. One possible interpretation of this phenomenon is as follows. An elevator depolarization with a large late component (generating many elevator spikes) produces a pronounced long-lasting wing upstroke. This in turn strongly excites the stretch receptors and the intense stretch-receptor burst suppresses the late component in the subsequent elevator depolarization. Thus the next upstroke is weaker, as is the stretch-receptor discharge, leading to the generation of a pronounced late depolarization and so on.

Combined stimulation of afferents from stretch receptors and tegulae

Considering the results reported in the previous sections we expected that a combined stimulation of tegula and stretch-re-

FIG. 15. Combined stimulation of tegula and stretch-receptor afferents in a deafferented preparation results in a pattern of elevator depolarizations resembling that observed in intact animals. In a deafferented locust, the fore wing anterior tergo-coxal motor neuron (*top*, 89) was recorded, and the afferents of hindwing tegulae and fore wing stretch receptors were stimulated (*middle*, compare Figs. 8*A* and 11*A*). Stimulus trains to tegula (downward-pointing pulses) and stretch-receptor afferents (upward-pointing pulses) were triggered by depressor EMG (*bottom*, 127). *A*: typical elevator depolarizations as observed in deafferented animal. *B*: stimulating stretch-receptor afferents reduced duration of these potentials in the course of several wingbeat cycles (compare to *A*, see also Fig. 11*D*). Additional stimulation of tegula afferents elicited rapid elevator depolarizations and led to an abolition of late depolarizations. The latter were immediately restored, however, when EMG failed to trigger the stimulus trains during 1 wingbeat cycle. *C*: when stretch-receptor afferents were activated fewer times, the late component of elevator depolarizations remained present (different preparation). Calibrations 100 ms, 10 mV.

ceptor afferents in a deafferented locust should restore a depolarization pattern in elevator motor neurons similar to that recorded in intact animals. Figures 15 and 16 show that this is, in fact, the case. Fore-wing stretch receptor and hind-wing tegula afferents were stimulated at approximately their normal phases of activity (compare Fig. 3). Figure 15*A* shows the pattern of elevator activity recorded after deafferentation. Stimulation of the stretch-receptor afferents shortened these typical late depolarizations (Fig. 15*B*, note small elevator muscle potentials in the EMG). Additional stimulation of the teg-

ula afferents elicited the rapid depolarizations characteristic of the intact pattern of elevator activity and caused a dropout of the remainder of the late depolarizations. The trigger circuit missed one depressor spike in the EMG and did not provide the stimulus trains to stretch-receptor and tegula afferents during this particular wingbeat cycle. In this period the initial rapid depolarization was missing, and the late depolarization reappeared. If the stimulation of the stretch-receptor afferents was missing or weak, the stimuli delivered to the tegula afferents elicited the initial rapid component but did not necessarily abolish the late depolarizations (Fig. 15*C*).

Figure 16 provides a comparison of the elevator depolarizations recorded in intact and deafferented individuals (Fig. 16*B*) and the depolarizations occurring when tegula and stretch receptor were stimulated in a deafferented animal (Fig. 16*A*). The depolarizations are scaled with respect to the wingbeat period. This representation demonstrates the similarities between the elevator depolarizations recorded in intact locusts and in deafferented animals during application of the stimuli.

FIG. 16. Comparison of time courses of elevator depolarizations in intact locusts and in deafferented animals with and without stimulation of tegula and stretch-receptor afferents. *A*: two elevator depolarizations (fore wing anterior tergocoxal 89) recorded during the experiment described in Fig. 15 are superimposed. One depolarization was recorded in the deafferented animal while stimulating the tegula and stretch-receptor afferents (stim), the other without such stimulation (deaff). Depolarization recorded in deafferented animal was photographically reduced to normalize wingbeat periods as determined in depressor EMGs (bottom, 127). This allows comparison of time courses of elevator depolarizations in the period of depressor activity. Photographic reduction of cycle period (using a photocopier) also reduced amplitude of depolarization. This accounts for small amplitude of the record from deafferented animal in this figure. *B*: Same procedure was performed for potentials recorded in elevator motor neuron of a different animal before (intact) and after (deaff) deafferentation. A comparison of both diagrams shows that elevator depolarizations elicited in a deafferented locust by a combined stimulation of tegula and stretch-receptor afferents (Fig. 15) are very similar to those recorded in an intact animal at high wingbeat frequencies.

DISCUSSION

From the above data the following picture emerges, regarding the control of elevator activity by tegula and stretch-receptor input. 1) The elevator potentials recorded in intact tethered flying locusts are composed of two independent components: an initial rapid depolarization and a variable late component. The late component is present only at low wingbeat frequencies. 2) The initial rapid depolarizations are generated as a result of tegula input. 3) The late depolarizations are suppressed at high wingbeat frequencies by the action of stretch-receptor input. 4) The variable late depolarizations recorded in intact animals may be generated by the same (central nervous) mechanism as the elevator depolarizations observed in deafferented preparations. We will now consider the evidence supporting and contradicting these conclusions.

Two components of elevator depolarizations

There is considerable evidence that the elevator depolarizations recorded in intact lo-

culsts at low wingbeat frequencies are composed of two independent superimposed components. First, the two components have characteristic features that distinguish them. The initial rapid depolarizations showed a remarkably constant appearance with respect to rising slope, amplitude, and duration (Fig. 4A). Although merged together with the late components, the initial depolarizations were usually distinctly separated from the late depolarizations by a shoulder or even a small gap or hyperpolarization (see Fig. 1B for examples and Ref. 32, Fig. 8). This allowed an unequivocal determination of their duration and amplitude. The late depolarizations, by contrast, were variable in both amplitude and duration, ranging from a small shoulder in the repolarization phase of the initial rapid depolarization to a full-sized depolarization with an amplitude similar to that of the initial component (Fig. 1B). A second argument for the existence of two independent components is the differential effect that tegula and stretch-receptor stimulation had on the two types of depolarizations. Stimulation of stretch-receptor afferents selectively affected the late component of the elevator depolarizations and had no effect on the initial rapid depolarizations (Fig. 13). Stimulation of the tegula afferents, on the other hand, selectively promoted the appearance of the initial depolarizations, provided stretch-receptor input was absent or weak (Figs. 8 and 15C; see below).

An analysis of activity in flight interneurons further supported the idea of two independent components constituting elevator depolarizations. In many flight interneurons that were depolarized during the phase of elevator activity, two separate bursts of spike discharge were observed—an initial burst, often with high spike frequency, and a late burst of variable duration and often with a lower rate of spike discharge (unpublished observations). Other interneurons discharged only during the initial portion of elevator activity and then only in intact animals. These were the interneurons reported to make direct connections to elevator motor neurons and to receive strong input from the hind-wing tegulae (19). All this is compelling evidence in favor of the conclusion that the elevator depolarizations in intact locusts are

in fact composed of two independent components.

A consistent finding in intact animals was that at wingbeat frequencies of <15 cycles/s the cycle period was related to the duration of the second component (Figs. 4A, 6, A and B, 7, and 11). Since there was little change in the duration of the first component or in the duration of the depressor depolarizations as cycle time increased, we conclude that the slowing of the flight rhythm below 15 cycles/s is associated almost entirely with the progressive increase in the duration of the second component of the elevator depolarization. We have not yet established what causes the slowing of the flight rhythm and hence the appearance of the second component in intact tethered animals.

Tegula input initiates elevator activity

The following observations support the conclusion that tegula input is responsible for the generation of the rapid elevator depolarizations and initiates elevator activity in intact animals. First, ablation of the tegulae abolished the initial rapid elevator depolarizations (Fig. 4). Second, a temporary abolition of the tegula discharges also abolished this initial portion of elevator activity (Fig. 5). Third, in animals with excised tegulae, stimulation of tegula afferents at the appropriate phase of the wingbeat cycle restored these rapid depolarizations (Fig. 8).

In a previous study we have investigated central nervous projections of tegula afferents that might form the neuronal substrates for the described effects (19). The main finding in that study was that hind-wing tegula afferents make strong monosynaptic excitatory connections to the ipsilateral metathoracic elevator motor neurons and indirect (probably disynaptic) excitatory connections to the mesothoracic and contralateral metathoracic elevator motor neurons. In addition, interneurons known to inhibit elevator motor neurons were found to be inhibited by input from the hind-wing tegulae. Depressor motor neurons were found to receive polysynaptically mediated IPSPs in response to a stimulation of the tegula afferents, and interneurons known to excite the depressor motor neurons were inhibited by input from the tegulae. This pattern of central nervous connections of the tegula afferents is consistent with their role in the initiation of elevator

depolarizations and the termination of ongoing depressor activity as reported in the present study (Fig. 9).

Despite the strong evidence that tegula input is necessary for the initiation of the elevator depolarizations in intact locusts, the mechanism of its action is not completely understood. In particular, it is not clear whether input from the tegulae produces its effect via a reflex in which the magnitude of the elevator depolarizations is directly related to the magnitude of the afferent input. Alternatively, tegula input might trigger a burst-generating system that is responsible for the remarkably constant features of the initial rapid elevator depolarizations. Experiments in which tegula input was temporarily abolished by immobilizing the wings in an elevated position suggested the latter to be more likely (Fig. 5). In addition, preliminary experiments indicated that the stimulation of hind-wing tegula afferents in quiescent locusts can elicit elevator motor neuron depolarizations in an all-or-none manner.

Equivalence of the elevator depolarizations in deafferented locusts and the late component of elevator depolarizations in intact locusts

The fact that the late depolarizations observed in elevator motor neurons of intact locusts are equivalent to the gradually rising depolarizations recorded in deafferented animals is suggested by the following observations. First, both depolarizations share characteristic features, such as the phase of occurrence with respect to depressor activity, the steep repolarization slope, the average amplitude, and the fact that the depolarizations peak late in the cycle of depressor discharge (indicating a gradual rising slope for the late component). These common features are most clearly demonstrated by the superpositions presented in Fig. 2. Second, the experiments involving ablation and stimulation of stretch-receptor afferents (Figs. 6, 7, 11, and 12) suggested that the late component of the elevator depolarizations is generated autonomously by the central nervous system in the absence of stretch-receptor input, as are the elevator depolarizations in deafferented animals. The data presented in Fig. 7 are of particular interest in this context. They show that the late component of the elevator depo-

larizations produced after abolition of stretch-receptor input can be successfully approximated for purposes of quantitative evaluation by the potentials recorded in deafferented locusts. This emphasizes the similarity between both depolarizations.

There is one finding that might indicate that the late depolarizations generated in intact animals and those produced after removal of the tegulae or complete deafferentation are of a different nature. In intact locusts, input from stretch-receptor afferents was able to suppress the appearance of the late depolarizations completely (Figs. 1B and 11). After removal of the tegulae or complete deafferentation, however, the elevator depolarizations were shortened but never completely abolished by the stimulation of stretch-receptor afferents (Figs. 11 and 15). A possible explanation is that either tegula input or the presence of the initial rapid depolarization is a prerequisite for stretch-receptor input to take full effect in suppressing the late depolarizations.

Stretch-receptor input controls the duration of elevator depolarizations

The following observations support the conclusion that stretch-receptor input is involved in controlling the duration of elevator depolarizations, apparently by a suppression of their late component. First, ablation of stretch-receptor afferents promoted the appearance of these late depolarizations (Fig. 6). Second, stimulation of stretch-receptor afferents reduced and often abolished the late components of elevator depolarizations (Fig. 11) provided the stimuli were delivered at phases close to the natural phase of stretch-receptor discharge (Fig. 13). Third, in undisturbed tethered flying locusts the number of stretch-receptor spikes discharged within a certain time interval preceding the elevator depolarization was negatively correlated to the prominence of the late component of the elevator depolarizations (Figs. 12, 14).

The term "suppression" as used to describe the effect of stretch-receptor input on elevator activity throughout this paper has to be interpreted in a very general sense considering that the underlying mechanism is not yet known. For instance, the action of stretch-receptor input might consist of a promotion of the transition from elevator to de-

pressor activity. It should be noted, however, that the late component of the elevator depolarizations appears to be actually suppressed toward higher wingbeat frequencies rather than being merged into the initial rapid depolarization. This is indicated by the changes in the shape of the late components with increasing wingbeat frequency (Fig. 1B) and by the observation that full-amplitude late components were present at all wingbeat frequencies after the removal of stretch-receptor input (Fig. 6). A comparison of the area covered by the elevator depolarizations under both conditions and at various wingbeat frequencies further supported this view (see RESULTS and Fig. 7).

As already outlined in the RESULTS this effect of stretch-receptor input on the elevator depolarizations appears to be mediated via a feedforward mechanism. It is well established (14, 16) that the receptor discharge is produced by the wing upstroke resulting from the preceding elevator depolarization. However, the effect of the stretch-receptor discharge is apparent only in the subsequent elevator depolarization (phase-response curve in Fig. 13). As a possible consequence of this delayed action an instability was often observed in the flight pattern, as evident in the alternating presence and absence of the late component in sequences of elevator depolarizations (see RESULTS and Fig. 14).

The neuronal mechanisms underlying the described action of stretch-receptor input are completely unknown. There apparently exists a delay between the receptor discharge and the suppression of the late component of the elevator depolarizations (compare data in Figs. 3, 11, and 14). This delay is in the range of ca. 25–35 ms, depending on the wingbeat frequency and phase of receptor discharge. It cannot be explained by the presently known projections of stretch-receptor afferents. Burrows (8) demonstrated that stretch-receptor afferents produced short-latency inhibitory postsynaptic potentials (IPSPs) in elevator motor neurons. These IPSPs are very small and they could only explain a short-latency effect of stretch-receptor input. Reye and Pearson (22, 23, 24) reported that the discharge patterns of many flight interneurons were changed dramatically by phasic stimulation of stretch-receptor afferents in deafferented preparations.

These results do not provide any clues for the explanation of the above results, however.

Although we examined the effect of stretch-receptor input on the activity of elevator motor neurons, our results regarding the timing of stretch-receptor activity may also have a bearing on current ideas concerning the control of depressor activity by stretch-receptor input. In our preparation the discharges of the stretch receptors occurred during and shortly after depressor motor neuron activity in flight episodes characterized by high wingbeat frequency (Fig. 3). According to this, timing input from these receptors cannot influence, and in particular cannot advance, the spikes produced by the depressor neurons in the present wingbeat cycle, as it was proposed by Möhl (14). Such an effect could at best be imagined for the low wingbeat frequencies around 10 Hz (see Fig. 3), a frequency range, however, that was not investigated by Möhl.

General considerations

Although the roles of tegulae and stretch receptors in flight-pattern generation might appear quite clear-cut based on the results of this study, the situation is certainly much more complicated. First, so far we have considered only the generation of the depolarization patterns in elevator motor neurons. Although the changes associated with deafferentation were much smaller in the depressor than in the elevator motor neurons (32), the former will undoubtedly be influenced by sensory input (8, 14). Second, we have, as yet, examined the roles of only two sets of wing sense organs. There are at least three more groups of wing receptors and many receptors elsewhere on the body, some of which have already been shown to influence the generation of the flight pattern (see INTRODUCTION). Third, even for the tegulae and the stretch receptors the mechanism of their action is not yet understood, although, in the case of the tegulae, important data concerning their central nervous projections and interneuronal connectivity are known and are in agreement with the present results (19).

Regardless of the unsolved problems mentioned above, it is clear from the present investigation that sensory input plays an even

more important role in locust flight-pattern generation than previously envisaged [e.g., (1)]. Apparently, the generation of the initial rapid depolarizations that dominate the pattern of elevator activity in intact tethered flying locusts at high wingbeat frequencies depends on afferent input from the tegulae. Presumably, the late components that are observed at lower wingbeat frequencies are produced by the same (central nervous) mechanism as the depolarizations that constitute elevator activity in deafferented preparations. These depolarizations appear to contribute to the intact pattern of elevator activity only at wingbeat frequencies below ~ 15 Hz and seem to be entirely suppressed as a result of wing stretch-receptor input at higher values of frequency. Thus, at high wingbeat frequencies, the elevator activity characterizing the flight pattern of the deafferented locust appears to be actually replaced by activity generated in response to

sensory feedback. This is not to say that the wing sense organs are in any way more important than the central nervous elements of the flight oscillator. Rather, both central and peripheral elements appear to be inextricably interwoven in the generation of the intact flight pattern, and any attempt to make a fundamental distinction between them would not be meaningful.

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